



Toxicological profile for Glycerol

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Propane-1,2,3-triol (PubChem)

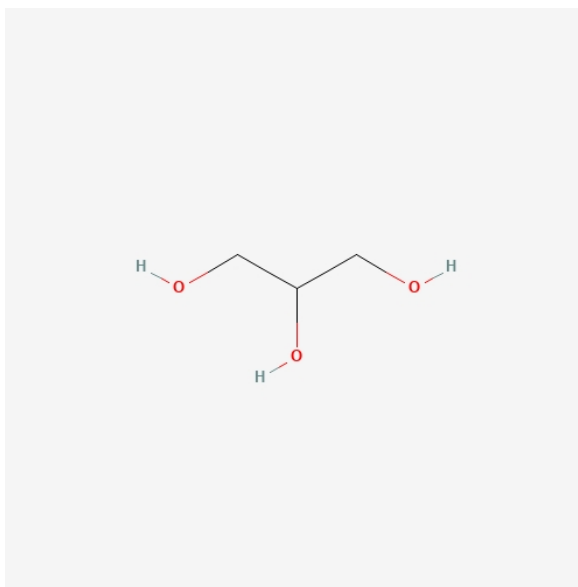
1.2. Synonyms

glycerol; glycerin; Glycyl alcohol; Trihydroxypropane; Glyceritol; Propanetriol; Osmoglyn; Glysantin; Grocolene; Glyrol; Glycerinum; Ophthalgan; Vitrosupos; Dagrax; Moon; Glycerolum; Optim; Star; Bulbold; Cristal; UNII-PDC6A3C0OX; EINECS 200-289-5; di-o-tolylphenylphosphine; . Glyceol; . Cognis G; . 1,2,3-propanetriol; . 1 2 3-Trihydroxypropane; . UNII-F92TF92VBF; . 2-hydroxypropane-1,3-diol; . 2-propanol,1,3-dihydroxi-; . UNII-522DM106CR; . AKOS000120102; (PubChem)

1.3. Molecular formula

$C_3H_8O_3$ (PubChem)

1.4. Structural Formula



(PubChem)1.5. Molecular weight (g/mol)

1.5. Molecular weight (g/mol)

92.09 (PubChem)

1.6. CAS registration number

56-81-5

1.7. Properties

1.7.1. Melting point

17.2-20°C (ChemSpider; EPISuite, 2017; Finnish Environment Institute)

1.7.2. Boiling point

290°C (ChemSpider; EPISuite, 2017); 182-290°C at 20 mmHg (335-476°C at 760 mmHg) or 20°C at 182 mmHg (61°C at 760 mmHg) (ChemSpider); 300°C (SCHEER, 2021)

1.7.3. Solubility

Miscible (ChemSpider; PubChem); 1e+006 mg/L at 25°C (EPISuite, 2017; PubChem); 5.296 x 10⁶ mg/L at 25°C, ≥100 mg/mL at 20°C, 10.86 M (920 g/L) (PubChem)

1.7.4. pKa

0.07E-13; 14.4 (EPISuite, 2017)

1.7.5. Flashpoint

160°C (ChemSpider); 177°C (open cup) or 176°C (closed cup) (PubChem)

1.7.6. Flammability limits (vol/vol%)

Explosive limits: 2.6–11.3% (vol. in air) (PubChem)

1.7.7. (Auto)ignition temperature

739 deg F [393°C] or 698 °F [370°C] (PubChem)

1.7.8. Decomposition temperature

290°C (ChemSpider)

1.7.9. Stability

Stable. Incompatible with perchloric acid, lead oxide, acetic anhydride, nitrobenzene, chlorine, peroxides, strong acids, strong bases. Combustible (ChemSpider). Mixtures of glycerin with water, ethanol (95%) and propylene glycol are chemically stable. Glycerin may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20°C (PubChem); Risk of explosion in contact with: chlorine, strong oxidizing agents, hydrogen peroxide, halogens, potassium permanganate, sodium peroxide, nitriles, peroxy compounds, perchloric acid/lead oxide, perchloryl nitrile, perchlorates, nitric acid/sulfuric acid, silver perchlorate/impact. The substance can react dangerously with: aniline/nitrobenzene, calcium hypochlorite, chromium trioxide, acetic anhydride + phosphorous oxychloride, fluorine/lead oxide, sodium hydride, phosphorus oxides, phosphorus halides, hydrides (GESTIS)

1.7.10. Vapor pressure

0.000106 hPa at 25°C (calculated); 0.01 Pa [7.5E-5 mmHg] at 25°C (PubChem); 0.000168 mmHg at 25°C (EPISuite, 2017); 0.13 Pa [0.001 mmHg] (SCHEER, 2021)

1.7.11. log Kow

-1.76 (EPISuite, 2017); -1.88 (ChemSpider)

2. General information

2. General information

2.1. Exposure

Type of end use	% of production volume (approx.)	Specific applications
Pharmaceuticals	<10	Excipient and formulation aid.
Chemical intermediate, nitration , and esters	15	Chemical synthesis
Cosmetic and Toiletries	20	Cosmetics including fragrances, bath and hair preparations, eye makeup, soaps and skin care preparations.
Resins, polyols and polyurethanes	20	Intermediate and monomer
Industrial Fluids	<10	Antifreezes, lubricants and hydraulic fluids
Tobacco	<10	Humectant
Cellulose films	<10	Intermediate
Food	<10	Food additive
Other chemical uses	<10	

As taken from OECD SIDS, Glycerol (56-81-5).

“Glycerine is used in foods and beverages as a humectant, solvent and sweetener, and may help preserve foods. It is used as a filler in commercially prepared low-fat foods (e.g. cookies), and as a thickening agent in liqueurs..... Glycerine-containing hyperhydration solutions are regularly used by athletes....Glycerine is also used in medical, pharmaceutical and personal care preparations, mainly as a means of providing lubrication and as a humectant. It is found in cough syrups, elixirs, toothpaste, skin care products, shaving cream and hair care products” (EFSA, 2010).

Estimated intake from flavouring use: 24.435 mg/kg bw/day.

As taken from Burdock, 2010.

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for glycerol (FEMA no. 2525) as a flavouring substance:

Baked goods	18/1300	Hard candy	22/980
Beverages, non-alcoholic	0.7/570	Jams and jellies	42/54
Beverages, alcoholic	20000/20000	Meat products	1/40
Chewing gum	17/6000	Milk products	84/84
Confections and frostings	70/23000	Processed fruits	0.02/0.07
Fats and oils	0.2/0.2	Seasonings and flavors	400000/400000

Frozen dairy	0.3/500	Snack foods	22/24
Gelatins and puddings	26/360	Soft candy	2/4
Gravies	0.2/0.2	Sweet sauces	74/74

As taken from Cohen SM et al. 2020. Food Technology 74(3), 44-65.

“Electronic cigarettes (e-cigarettes or electronic nicotine delivery systems [ENDS]) are electrically powered devices generally similar in appearance to a cigarette that deliver a propylene glycol and/or glycerol mist to the airway of users when drawing on the mouthpiece....” As taken from Bullen C et al. 2013. BMC Public Health 13, 210. PubMed, 2014

“Electronic cigarette consumption ('vaping') is marketed as an alternative to conventional tobacco smoking. Technically, a mixture of chemicals containing carrier liquids, flavors, and optionally nicotine is vaporized and inhaled. The present study aims at the determination of the release of volatile organic compounds (VOC) and (ultra)fine particles (FP/UFP) from an e-cigarette under near-to-real-use conditions in an 8-m(3) emission test chamber. Furthermore, the inhaled mixture is analyzed in small chambers. An increase in FP/UFP and VOC could be determined after the use of the e-cigarette. Prominent components in the gas-phase are 1,2-propanediol, 1,2,3-propanetriol, diacetyl, flavorings, and traces of nicotine. As a consequence, 'passive vaping' must be expected from the consumption of e-cigarettes. Furthermore, the inhaled aerosol undergoes changes in the human lung that is assumed to be attributed to deposition and evaporation. PRACTICAL IMPLICATIONS: The consumption of e-cigarettes marks a new source for chemical and aerosol exposure in the indoor environment. To evaluate the impact of e-cigarettes on indoor air quality and to estimate the possible effect of passive vaping, information about the chemical characteristics of the released vapor is needed.” As taken from Schripp T et al. 2013. Indoor Air 23(1), 25-31. PubMed, 2014

“The development of electronic cigarettes (e-cigs) has the potential to offer a less harmful alternative for tobacco users. This clinical study was designed to characterize e-cig users' exposure to nicotine, and to investigate the acute effects of e-cigs on the hemodynamic measurements (blood pressure and heart rate) in comparison with the effects of regular smoking. Five e-cigs and one Marlboro® cigarette were randomized for twenty-three participants under two exposure scenarios from Day 1 to Day 11: half-hour controlled administration and one hour ad lib use....The combination of glycerin and propylene glycol as the vehicle facilitated delivery of more nicotine than glycerin alone....” As taken from Yan XS & D’Ruiz C. 2014. Regul. Toxicol. Pharmacol. 71(1), 24-34. PubMed, 2014

“....This study investigated and characterised the impact of 'vaping' (using electronic cigarettes) on indoor environments under controlled conditions using a 30m(3) emission chamber. The study determined the composition of e-cigarette mainstream vapour in terms of propylene glycol, glycerol, carbonyls and nicotine emissions using a smoking machine with adapted smoking parameters. Two different base recipes for refill liquids, with three different amounts of nicotine each, were tested using two models of e-cigarettes. Refill liquids were analysed on their content of propylene glycol, glycerol, nicotine and qualitatively on their principal flavourings. Possible health effects of e-cigarette use are not discussed in this work. Electronic cigarettes tested in this study proved to be sources for propylene glycol, glycerol, nicotine, carbonyls and aerosol particulates. The extent of exposure differs significantly for active and passive 'vapers' (users of electronic cigarettes). Extrapolating from the average amounts of propylene glycol and glycerol condensed on the smoking machine filter pad to the resulting lung-concentration, estimated lungconcentrations of 160 and 220mgm(-3) for propylene glycol and glycerol were obtained, respectively....In chamber studies, peak concentrations of 2200µgm(-3) for propylene glycol, 136µgm(-3) for glycerol and 0.6µgm(-3) for nicotine were reached....” As taken from Geiss O et al. 2014. Int. J. Hyg. Environ. Health 218(1), 169-80. PubMed, 2014

Glycerin (CAS RN 56-81-5) is listed as an ingredient (at given concentrations, where specified) in hobby/craft (15-19%), auto (0-40%, includes "discontinued" products), home maintenance (1-60%), inside the home (0-60%, includes "old" products), personal care (0-100%), commercial/institutional (1-100%, includes "old" products), landscaping/yard (5-10%), pesticides (4.77-10%) and pet care (0.0119-55%, includes "old" products) products by the CPID.

Used as a denaturant, hair conditioning, humectant, oral care, perfuming, skin conditioning, skin protecting, solvent and viscosity controlling ingredient in cosmetics in the EU. As taken from CosIng.

Glycerol is listed as a fragrance ingredient by IFRA.

Glycerol is number 10 in the US EPA's list of top 20 chemicals used in children's products (top uses: personal care products) (US EPA, 2014).

Estimated intake from its use as a flavouring is 17 and 220 mg/day in Europe and the USA, respectively (JECFA, 2001).

Probable	Routes	of	Human	Exposure:
According to the 2006 TSCA Inventory Update Reporting data, the number of persons reasonably likely to be exposed in the industrial manufacturing, processing, and use of glycerin is 1000 or greater; the data may be greatly underestimated(1). [(1) US EPA; Inventory Update Reporting (IUR). Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Washington, DC: U.S. Environmental Protection Agency. Available from, as of Jul 12, 2011: http://cfpub.epa.gov/iursearch/index.cfm **PEER REVIEWED**				

As taken from HSDB, 2012.

"The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). In 2014, glycerin was the third most frequently reported ingredient in the VCRP database (after water and fragrance). Glycerin was reported to be used in 15,654 cosmetic products; 10,046 are leave-on products, 5441 are rinse-off products, and 167 products are diluted for the bath. These uses include 862 products for use near the eye, 160 lipsticks, 369 hair dyes and colors, 1259 bath soaps and detergents, 7756 skin care products, and 244 suntan preparations (Table 2). Glycerin is reported to be used in 125 baby products. Two uses for anhydrous glycerin were reported in the VCRP; these uses were merged with the glycerin uses.

A survey was conducted of industry by the Personal Care Products Council (Council) of the maximum use concentrations for this ingredient. Glycerin is reported to be used at up to 79.2% in leave-on products, 99.4% in rinse-off products, and 47.9% in products diluted for the bath. It is used at up to 21% in baby products, 40.6% in eye lotions, 25% in perfumes, 47.3% in hair grooming aids, 68.6% in oral hygiene products, 99.4% in skin cleaning products, and 17.9% in suntan preparations.

Glycerin was reported to be used in aerosol/spray products that include: hair sprays at concentrations at up to 30% (in propellant spray products at concentrations up to 10% and in pump spray products up to 30%), pump deodorants at up to 4%, face and neck products at up to 10%, body and hand products at up to 5%, moisturizing products at up to 3.3%, and suntan products at up to 10% (in propellant spray products at up to 6% and in pump spray products up to 10%). These propellant/pump spray products could possibly be inhaled. In practice, 95%-99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays. Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be

deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) in any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.”

As taken from CIR, 2015.

“Background: Although dustiness and viscosity are potential determinants of dermal exposure, their effect on exposure is poorly understood. The goal of this study was to investigate the effect of dustiness and viscosity on dermal exposure by each of three dermal exposure pathways (deposition, surface contact, and immersion). METHODS: The hands of four volunteers were exposed to non-toxic substances: particulate with varying dustiness (calcium acetate, zinc oxide, and Epsom salt) and liquids of varying viscosity (three glycerol/water solutions containing 20, 50, or 85% glycerol) by each pathway. Dermal exposure was measured by a systematic wipe of the entire hand. Calcium acetate, zinc oxide, and Epsom salts were analysed on wipes by inductively coupled plasma/atomic emission spectrometry and glycerol was measured by gas chromatography with a flame ionization detector. The relationship between exposure and either dustiness or viscosity was examined using either parametric (analysis of variance) or non-parametric (Kruskal-Wallis) tests. RESULTS: Both viscosity and dustiness appeared to have an effect on dermal exposure. Increasing viscosity lead to higher exposures by the immersion pathway ($P < 0.001$) but lower exposures by the deposition pathway (although this relationship was not statistically significant: $P = 0.19$). Viscosity had no apparent effect on exposure from surface contact. Dustiness did not affect transfer of particulate to the skin by immersion ($P = 0.403$) but it did affect exposure by the surface transfer and deposition pathways. The dustiest substance (calcium acetate) transferred to skin more readily following contact with contaminated surfaces than zinc oxide or Epsom salts ($P = 0.016$). For the deposition pathway, the highest exposures were seen for the dustiest substance (calcium acetate) but statistical analysis was not conducted as 67% of measurements were below detection limits. CONCLUSION: The results suggest that both viscosity and dustiness can affect dermal exposure. They also show that the determinants of dermal exposure can be different for each of the dermal exposure pathways.” As taken from Gorman Ng M et al. 2013. Ann. Occup. Hyg. 57(5), 627-39. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23316077>

“The aim was to describe the exposure to excipients among neonates hospitalised in the neonatal intensive care unit (NICU) of a public hospital in Brasilia, Brazil. This was a retrospective study based on medicines that were prescribed electronically to neonates (≤ 28 days) who were admitted to the NICU of a hospital in Brasilia between January 1 and March 31, 2012. Excipients were identified from the medicine package leaflets and were classified according to toxicity. Seventy-nine infants received a total of 1,303 prescriptions comprising 77 formulations and 70 active drugs. Eighty-six excipients were identified, of which, 9 were harmful excipients (HE) and 48 were potentially harmful excipients (PHE). Almost all the neonates (98.7 %) were exposed to at least one HE and PHE. Preterm neonates ($n = 64$; 1,502 neonate days) presented high risk of exposure to polysorbate 80 (3.26/100 neonate days), sodium hydroxide (3.39), PG (3.19) and propylparaben (3.06). Full-term neonates ($n = 15$; 289 neonate days) presented risks in relation to phenol (4.84), ethanol (3.8) and sodium citrate (3.46). CONCLUSION: Neonates in NICUs in Brazil are exposed to a wide variety of HE and PHE with unpredictable results. Safer alternatives are needed, as well as further studies on the subject.” As taken from Souza S Jr. 2014. Eur. J. Pediatr. 173(7), 935-45. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24500397>

The European Food Safety Authority (EFSA) noted in 2017 that “glycerol (E 422) can be produced by a variety of methods and ... many of them lead to the presence or formation of contaminants, which are of toxicological concern”.

“The highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined non-brand-loyal exposure scenario”.

“The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use is consumption of a beverage. Therefore, the Panel calculated the volume of flavoured drinks required to be consumed in order to exceed the acute bolus exposure (125 mg/kg bw per hour) calculated by the Panel to be the minimum dose required to have a therapeutic effect. When considering the available data, the Panel noted that infants and toddlers can be exposed to more than 125 mg glycerol/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink.”

As taken from EFSA, 2017.

Glycerol (CAS RN 56-81-5) is used as a cryoprotectant, emollient, flavour enhancer, hair-conditioning agent, humectant, lubricant, plasticizer, preservative antimicrobial, skin-conditioning agent - humectant, solvent, sweetening agent, tonicity agent, viscosity increasing agent, water-miscible cosolvent and wetting agent in non-medicinal natural health products. In addition, “glycerinum” (proper name: glycerol) is listed as a homeopathic substance (Health Canada, 2021).

“Secondhand electronic cigarette (e-cigarette) aerosol (SHA) might impair indoor air quality and expose bystanders. This study aims to investigate exposure to SHA in controlled conditions of enclosed settings simulating real-world scenario. An experiment was performed in a car and in a room, in which SHA was generated during a 30-minute ad libitum use of an e-cigarette. The experiment was replicated on five consecutive days in each setting. We measured PM_{2.5}, airborne nicotine concentrations, and biomarkers of exposure to SHA, such as nicotine metabolites, tobacco-specific nitrosamines, propylene glycol, and glycerol in bystanders' saliva samples before, during, and after the exposure period. Self-reported health symptoms related to exposure to SHA were also recorded. The results showed that the highest median PM_{2.5} concentration was recorded during the exposure period, being 21 µg/m³ in the room setting and 16 µg/m³ in the car setting-about twofold increase compared to the baseline. Most concentrations of the airborne nicotine and all biomarkers were below the limit of quantification in both settings. Bystanders in both settings experienced some short-term irritation symptoms, expressed as dry throat, nose, eyes, and phlegm. In conclusion, short-term use of an e-cigarette in confined spaces increased indoor PM_{2.5} level and caused some irritation symptoms in bystanders.” As taken from Amalia B et al. 2021. Indoor Air. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33905602/>

“To assess potential exposure of non-users to exhaled constituents from pod and cartridge electronic nicotine delivery systems (ENDS) products, an environmental clinical study was conducted with (n = 43) healthy adult smokers. Room air concentrations of 34 selected constituents (nicotine, propylene glycol, glycerol, 15 carbonyls, 12 volatile organic compounds, and 4 trace metals) and particle number concentration (0.3 to 25 µm) were compared from use of two ENDS products and conventional cigarettes using room ventilations representative of a residential, an office or a hospitality setting over a 4-h. exposure period. Products used were JUUL ENDS, Virginia Tobacco flavor (Group I), VUSE Solo, Original flavor (Group II) (5.0 and 4.8% nicotine by weight, respectively) and subjects' own conventional cigarettes (Group III). Cumulative 4-h room air sampling and particle counting were performed during prescribed (Groups I and II) and ad libitum product use (all Groups). Conventional cigarette use resulted in significantly more constituents detected and higher 4-h cumulative constituent concentrations compared to use of the ENDS products tested, except for the predominant ENDS ingredients, propylene glycol and glycerol. Use of conventional cigarettes also resulted in greater total particle number concentration than either prescribed or ad libitum use of either of the ENDS used in this study.” As taken from Oldham MJ et al. 2021. Sci. Rep. 11(1), 1736. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33462299/>

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
glycerol	3088559915	98.38	3798780177	100.00
Total ion chromatogram	3139418495	100	3798780177	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004) J. Anal. Appl. Pyrolysis 71, 223–311.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Glycerol CAS 56-81-5	70,000	99.5	Glycerol (99.8) 2 Unidentified Products (0.2)	35,000 70

“When 30g (in portions) of glycerol on silica were heated at 700°C until the organic material had disappeared in an air flow of 1.5l/min, 60% remained unchanged in the pyrolysate. The remaining 40% contained 14 pyrolysis products, including PAHs, quinines and phenols (Kröller 1965)”.

“When a small sample of glycerol was heated from room temperature to 900°C in the presence of air over period of 100 seconds, the individual compounds identified were water, acrolein, glycolaldehyde and glycerol (a substantial amount remaining unpyrolysed). (Philip Morris USA Internal Report 2001)”.

“Acrolein formation from the pyrolysis of glycerol has been reported in other studies (Hurd 1929; Lawrie 1929; Stein et al. 1983). The latter of these studies identified CO, acetaldehyde and acrolein as the products of decomposition of glycerol in steam, conducted in a laminar flow reactor at 650-700°C. Acetaldehyde and acrolein decomposed further to ethylene, methane and hydrogen (Stein et al.1983)”.

“Following studies involving pyrolysis, humectants, specifically the cigarette additive glycerol, had associated cigarette smoke constituents including acrolein, formaldehyde and acetaldehyde (Wynder et al, 1967)”.

In a pyrolysis study, 100% of glycerol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

On a cigarette basis, addition of glycerin significantly increased the amount of tar and water in the smoke while decreasing the amount of nicotine. Glycerin is a humectant and is expected to add water to the tobacco and smoke and it also transfers to the smoke itself. The net affect of addition of glycerin is therefore to dilute the smoke with water and glycerin. As a result, most of the smoke constituents decrease when measured on a cigarette basis. On a tar basis, nicotine was significantly decreased at 10% and 15% glycerin while water was increased at all addition levels. Since the cigarettes were manufactured to an approximate constant tobacco weight, as glycerin was added, the amount of tobacco was decreased. The reduction in nicotine in the smoke on a tar basis is therefore thought to be simply due to the reduction in the amount of tobacco in the cigarette. Addition of 10% or 15% glycerin resulted in a statistically significant increase in acrolein and a decrease in acetaldehyde, propionaldehyde, aromatic amines, nitrogen oxides, tobacco

specific nitrosamines, and phenols. Addition of glycerin at the 5% level also reduced many smoke constituent yields, but there was no increase in acrolein. (Carmines E.L. and Gaworski C.L.; 2005)

“OBJECTIVES: To investigate how the two main electronic (e-) cigarette solvents-propylene glycol (PG) and glycerol (GL)-modulate the formation of toxic volatile carbonyl compounds under precisely controlled temperatures in the absence of nicotine and flavor additives. METHODS: PG, GL, PG:GL = 1:1 (wt/wt) mixture, and two commercial e-cigarette liquids were vaporized in a stainless steel, tubular reactor in flowing air ranging up to 318°C to simulate e-cigarette vaping. Aerosols were collected and analyzed to quantify the amount of volatile carbonyls produced with each of the five e-liquids. RESULTS: Significant amounts of formaldehyde and acetaldehyde were detected at reactor temperatures $\geq 215^{\circ}\text{C}$ for both PG and GL. Acrolein was observed only in e-liquids containing GL when reactor temperatures exceeded 270°C . At 318°C , 2.03 ± 0.80 μg of formaldehyde, 2.35 ± 0.87 μg of acetaldehyde, and a trace amount of acetone were generated per milligram of PG; at the same temperature, 21.1 ± 3.80 μg of formaldehyde, 2.40 ± 0.99 μg of acetaldehyde, and 0.80 ± 0.50 μg of acrolein were detected per milligram of GL. CONCLUSIONS: We developed a device-independent test method to investigate carbonyl emissions from different e-cigarette liquids under precisely controlled temperatures. PG and GL were identified to be the main sources of toxic carbonyl compounds from e-cigarette use. GL produced much more formaldehyde than PG. Besides formaldehyde and acetaldehyde, measurable amounts of acrolein were also detected at $\geq 270^{\circ}\text{C}$ but only when GL was present in the e-liquid. At 215°C , the estimated daily exposure to formaldehyde from e-cigarettes, exceeded United States Environmental Protection Agency (USEPA) and California Office of Environmental Health Hazard Assessment (OEHHA) acceptable limits, which emphasized the need to further examine the potential cancer and non-cancer health risks associated with e-cigarette use.” As taken from Wang P et al. 2017. PLoS One 12(1), e0169811. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28076380>

“The flash pyrolysis of glycerin was investigated by the use of isotopic labeling with ^{13}C , in conjunction with GC/MS analysis of the products. The formation of acetaldehyde and acrolein was shown to occur by unimolecular reaction. Acetaldehyde was found to be produced by at least two competing mechanisms. Mechanism “A” delivered C-2 of glycerin to the carbonyl group of acetaldehyde, whereas mechanism “B” delivered C-2 of glycerin to the methyl group of acetaldehyde. Formaldehyde was exclusively derived from C-1 or C-3 by either mechanism. The partition between the two mechanistic paths was found to be influenced by the presence of potassium salts and acids, but not by the presence of benzoyl peroxide or galvanoxyl. Mechanism “A” is postulated to be a concerted cyclic version of the Grob Fragmentation, proceeding through an intermediate which is hydrogen-bonded between the 1- and 3-hydroxyl groups to simultaneously generate enol-acetaldehyde, formaldehyde and H_2O . Mechanism “B” as favored by the presence of alkali is postulated to involve hydrogen-bonding between adjacent hydroxyl groups and to be an alkaline version of the pinacol rearrangement followed by retro-aldol fragmentation of the intermediary 3-oxopropoxide anion. These mechanistic classes are both fundamentally important, not only for their effect on glycerin, but for being able to provide numerous means of initial pyrolytic carbon-carbon bond breakage along carbohydrate carbon-chains, given the numerous 1,2,3-triol interactions that are possible. Further nomenclature is introduced to refine the distinctions among isotopomers and isotopologs (ipsomer, ipsolog, naturalomer, “nominal” isotopolog, ubiquilog), as extensions of a concept previously adopted by IUPAC.”

Paine JB et al. (2007) Carbohydrate pyrolysis mechanisms from isotopic labeling: Part 1: The pyrolysis of glycerin: Discovery of competing fragmentation mechanisms affording acetaldehyde and formaldehyde and the implications for carbohydrate pyrolysis.

2.3. Ingredient(s) from which it originates

“Obtained from oils and fats as a by-product in the manufacture of soaps and fatty acids; synthesized from propylene; also production from sugars by fermentation”

Reported to occur naturally in cocoa, apple, cider, beer, sour cherries, peach and wine.

As taken from Burdock, 2010.

Natural

Pollution

Sources:

Glycerin was reported present in Cork Oak, *Quercus suber* L.(1). [(1) Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Glycerin. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Jul 12, 2011: <http://www.ars-grin.gov/duke/> **PEER REVIEWED**

Glycerol occurs in combined form in all animal and vegetable fats and oils. Glycerol is rarely found in the free state in those fats but is usually present as a triglyceride combined with such fatty acids as stearic, oleic, palmitic, and lauric acids, and these are generally mixtures or combinations of glycerides of several fatty acids. Such oil as coconut, palm kernel, cottonseed, soybean, and olive oil yield larger amounts of glycerol than do such animal fats as lard and tallow. Glycerol also occurs naturally as triglycerides in all animal and vegetable cells in the form of lipids such as lecithin and cephalins. [Morrison LR; Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2011). New York, NY: John Wiley & Sons; Glycerol. Online Posting Date: 4 Dec 2000] **PEER REVIEWED**

As taken from HSDB, 2012.

Occurs in avocado seeds.

As taken from Khan IA and Abourashed EA, 2010.

3. Status in legislation and other official guidance

Occupational Exposure Standards:

OSHA Standards:

Permissible Exposure Limit: Table Z-1 8-hr Time Weighted Avg: 15 mg/cu m. /Mist, total dust/ [29 CFR 1910.1000 (USDOL); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Permissible Exposure Limit: Table Z-1 8-hr Time Weighted Avg: 5 mg/cu m. /Mist, respirable fraction/ [29 CFR 1910.1000 (USDOL); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Vacated 1989 OSHA PEL TWA 10 mg/cu m is still enforced in some states. /Mist, total/ [NIOSH. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 97-140. Washington, D.C. U.S. Government Printing Office, 1997., p. 365] **QC REVIEWED**

Vacated 1989 OSHA PEL TWA 5 mg/cu m is still enforced in some states. /Mist, resp/ [NIOSH. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 97-140. Washington, D.C. U.S. Government Printing Office, 1997., p. 365] **QC REVIEWED**

NIOSH Recommendations:

NIOSH concluded that the documentation cited by OSHA was inadequate to support the proposed PEL (as an 8 hour TWA) of 10 mg/cu m for glycerine (mist). [NIOSH. NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM. Department of Health & Human Services, Centers

for Disease Prevention & Control. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2005-151 (2005)] **PEER REVIEWED**

FIFRA Requirements:

Residues resulting from the use of the following substances as either an inert or an active ingredient in a pesticide chemical formulation, including antimicrobial pesticide chemicals, are exempted from the requirement of a tolerance under FFDCA section 408, if such use is in accordance with good agricultural or manufacturing practices. Glycerin is included on this list. [40 CFR 950(e) (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

As the federal pesticide law FIFRA directs, EPA is conducting a comprehensive review of older pesticides to consider their health and environmental effects and make decisions about their future use. Under this pesticide reregistration program, EPA examines health and safety data for pesticide active ingredients initially registered before November 1, 1984, and determines whether they are eligible for reregistration. In addition, all pesticides must meet the new safety standard of the Food Quality Protection Act of 1996. Pesticides for which EPA had not issued Registration Standards prior to the effective date of FIFRA, as amended in 1988, were divided into three lists based upon their potential for human exposure and other factors, with List B containing pesticides of greater concern and List D pesticides of less concern. Glycerol is found on List D. Case No: 4044; Case Status: No products containing the pesticide are actively registered ... The case /is characterized/ as "cancelled." Under FIFRA, pesticide producers may voluntarily cancel their registered products. EPA also may cancel pesticide registrations if registrants fail to pay required fees or make/meet certain reregistration commitments, or if EPA reaches findings of unreasonable adverse effects.; Active ingredient (AI): Glycerol; AI Status: The active ingredient is no longer contained in any registered pesticide products ... "cancelled." [USEPA/OPP; Status of Pesticides in Registration, Reregistration and Special Review p.313 (Spring, 1998) EPA 738-R-98-002] **QC REVIEWED**

Atmospheric Standards:

This action promulgates standards of performance for equipment leaks of Volatile Organic Compounds (VOC) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOC, considering costs, non air quality health and environmental impact and energy requirements. Glycerol is produced, as an intermediate or a final product, by process units covered under this subpart. [40 CFR 60.489 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

FDA Requirements:

Substances migrating to food from paper and paperboard products used in food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act: Glycerin is included on this list. [21 CFR 182.90 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 20, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

GLYCERIN used as a multiple purpose GRAS food substance in food for human consumption is generally recognized as safe when used in accordance with good manufacturing practice. [21 CFR 182.1320 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

GLYCERIN used as a general purpose food additive in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding

practice. [21 CFR 582.1320 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2011: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

As taken from HSDB, 2012.

ACGIH: The TLV value previously assigned to glycerin mist has been withdrawn “due to insufficient data relevant to human occupational exposure”

OSHA TWA: Glycerin mist 15 mg/m³ (total dust); 5 mg/m³ (respirable fraction)

DFG MAKs TWA: 200 mg/m³ (Inhalable fraction of the aerosol) (Category: Substances for which local irritant effects determine the MAK value, also respiratory allergens. Excursion factor 2, Duration 15 min, average value (in certain cases, a momentary value (concentration that should not be exceeded at any time) can also be established), Number per shift 4, Interval 1 hour.)

As taken from ACGIH, 2021.

OCCUPATIONAL EXPOSURE LIMITS

OEL-BELGIUM: TWA 10 mg/m³, MAR2002

OEL-FINLAND: TWA 20 mg/m³, NOV2011

OEL-FRANCE: VME 10 mg/m³, FEB2006

OEL-GERMANY: MAK 50 mg/m³, inhal, 2011

OEL-KOREA: TWA 10 mg/m³ (mist), 2006

OEL-MEXICO: TWA 10 mg/m³ (inhalable), 2004

OEL-THE NETHERLANDS: MAC-TGG 10 mg/m³, 2003

OEL-NEW ZEALAND: TWA 10 mg/m³ (mist), JAN2002

OEL-PERU: TWA 10 mg/m³, JUL2005

OEL-SWITZERLAND: MAK-W 50 mg/m³, KZG-W 100 mg/m³, inhal, JAN2011

OEL-UNITED KINGDOM: TWA 10 mg/m³, OCT2007

OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN check ACGIH TLV;

OEL IN SINGAPORE, VIETNAM check ACGIH TLV

As taken from RTECS, 2019.

Glycerin is included on the FDA's list of “Substances Added to Food (formerly EAFUS) as an anticaking agent or free-flow agent, drying agent, emulsifier or emulsifier salt, humectant, masticatory substance, nutritive sweetener, solvent or vehicle, stabilizer or thickener and texturizer. It is generally recognised as safe (GRAS) under 21 CFR sections 182.1320 (glycerin) and 182.90 (Substances migrating to food from paper and paperboard products) and is also covered under 21 CFR sections 169.175 (Vanilla extract), 172.811 (Glyceryl tristearate), 175.300 (Resinous and polymeric coatings), 175.320 (Resinous and polymeric coatings for polyolefin films), 176.210 (Defoaming agents used in the manufacture of paper and paperboard), 177.1390 (Laminate structures for use at temperatures of 250 deg. F and above), 177.2420 (Polyester resins, cross-linked), 177.2800 (Textiles and textile fibers) and 178.3500 (Glycerin, synthetic).

As taken from FDA, 2022, 2023

“The U.S. Pharmacopeia-National Formulary (USP-NF) standards state that the amount of any individual impurity in glycerin cannot exceed 0.1%, and that the total for all impurities, including diethylene glycol and ethylene glycol, must not exceed 1%.”

As taken from CIR, 2015.

Glycerin is approved for use in over-the-counter drugs, such as anorectal drug products, dermal protectants (up to 45%), in ophthalmic drug products (up to 1%), and in oral health care products [21 CFR 346.14 (Protectant active ingredients), 21 CFR 347.10 (Skin protectant active ingredients), 21 CFR 349.12 (Ophthalmic demulcents), 27 CFR 21.58 (Formula No. 31-A)]:

It is also included under 21 CFR 172.866 (Synthetic glycerin produced by the hydrogenolysis of carbohydrate), 40 CFR 180.1250 (C8, C10, and C12 fatty acid monoesters of glycerol and propylene glycol; exemption from the requirement of a tolerance), 40 CFR 180.910 (Inert ingredients used pre- and post-harvest; exemptions from the requirement of a tolerance), 40 CFR 180.920 (Inert ingredients used pre-harvest; exemptions from the requirement of a tolerance) and 40 CFR 180.930 (Inert ingredients applied to animals; exemptions from the requirement of a tolerance).

As taken from CIR, 2015.

1,2,3-Propanetriol is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950e (Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2023b).

Workplace exposure limits for glycerol mist of 10 mg/m³ per 8-hr TWA reference period have been set by the UK Health and Safety Executive (HSE, 2020).

JECFA (2001) maintained its earlier ADI of “not specified”, concluding that glycerol as a flavouring was of no safety concern at the estimated levels of intake (at the time) of 17 and 220 mg/day in Europe and the USA, respectively.

Glycerol is approved for use as a food contact additive in plastics under Commission Regulation (EU) No 10/2011 with no restrictions other than the generic overall migration limit of 60 mg/kg food.

As taken from EFSA, 2012.

“The CEF Panel concluded that the substances: glycerin, ... do not raise a safety concern when used in oxygen absorbers in sachets, patches or cards, placed in the headspace of the packaging or when used in direct contact with food, excluding liquid food or foods that have an external aqueous liquid phase on the surface such as sliced fruits and fresh meat.”

As taken from EFSA, 2013a.

A programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Commission Regulation (EU) No 257/2010 of 25 March 2010. The deadline for the submission of data for glycerol (E 422) is 30 November 2013 (EFSA, 2013b).

Glycerol (E 422) is authorised for use as a food additive in the EU under legislation (EU) no. 1129/2011 and also, as a Group I, Additive, under legislation (EU) nos 438/2013 and 2015/647, 2015/1832 and 2018/1497.

As taken from European Commission, 2011, 2019.

There is a REACH dossier on glycerol (ECHA, undated).

Glycerol (CAS RN 56-81-5) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Drinking Water Risk Based Concentration (RBC) for glycerine: 400 mg/l (based on a repeat-dose toxicology study (US EPA, 2013).

Glycerol is included on the US EPA list of Safer Chemical Ingredients with functional use: solvents (US EPA, 2023a).

FDA Maximum (Recommended) Daily Dose:

Name	Value
ActivityCategory_MCASE_mg	Low
ActivityCategory_MRDD_mmol	Low
ChemicalReplicateCount	1
Dose_MRDD_mg	999 mg/kg-bw/day
Dose_MRDD_mmol	10.9 mmol/kg-bw/day
Endpoint	Maximum Recommended Daily Dose
LOGINV_MRDD_mmol	-1.04 mmol
N100_MRDD_mmol	0.1 mmol
Note_FDAMDD	form of drug administered not known
Species	human
StudyType	Clinical Reports
TherapeuticCategory	Diagnostic aid

Glycerine (CAS RN 56-81-5) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR List (Chemical Data Reporting Rule) and the 2020 CDR Partial Exempt lists.

US EPA 2020 CDR List. US EPA 2020 CDR Partial Exempt List. US EPA TSCA inventory.

“There is no need for a numerical ADI for glycerol (E 422). The Panel concluded that there is no safety concern regarding the use of glycerol (E 422) as a food additive according to Annex II and III (Part 1, 2, 3, 4 and 5) for the general population at the refined exposure assessment for the reported uses of glycerol as a food additive.”

As taken from EFSA, 2017.

Glycerol has been designated as GRAS (generally recognized as safe) for use in food by FEMA (FEMA no. 2525) (Hall RL and Oser BL, 1965).

1,2,3-Propanetriol (CAS RN 56-81-5) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and has been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach”. As taken from AICIS, 2012.

Glycerin is included on the US FDA’s list of inactive ingredients for approved drug products. It is permitted for use as ingredients in various products, at the following maximum potencies per unit dose and maximum daily exposures:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)

GLYCERIN	AURICULAR (OTIC)	SOLUTION	56815	PDC6A3C00X	63.64%w/v	
GLYCERIN	AURICULAR (OTIC)	SOLUTION/DROPS	56815	PDC6A3C00X		12mg
GLYCERIN	AURICULAR (OTIC)	SUSPENSION	56815	PDC6A3C00X	0.05%w/w	
GLYCERIN	BUCCAL	GUM, CHEWING	56815	PDC6A3C00X	28.8mg	
GLYCERIN	BUCCAL	MOUTHWASH	56815	PDC6A3C00X	100mg/1ml	
GLYCERIN	BUCCAL	SYSTEM	56815	PDC6A3C00X	66mg	
GLYCERIN	DENTAL	PASTE	56815	PDC6A3C00X	20%w/w	
GLYCERIN	DENTAL	PASTE, DENTIFRICE	56815	PDC6A3C00X	18%w/w	
GLYCERIN	DENTAL	SOLUTION	56815	PDC6A3C00X	15%w/v	
GLYCERIN	INTRADERMAL	INJECTION	56815	PDC6A3C00X	1.6%w/v	
GLYCERIN	INTRAMUSCULAR	INJECTION	56815	PDC6A3C00X		450mg
GLYCERIN	INTRAMUSCULAR	INJECTION, SOLUTION	56815	PDC6A3C00X	15%w/v	
GLYCERIN	INTRAVENOUS	EMULSION	56815	PDC6A3C00X	2.2%w/v	
GLYCERIN	INTRAVENOUS	INJECTION	56815	PDC6A3C00X		550mg
GLYCERIN	INTRAVENOUS	INJECTION, EMULSION	56815	PDC6A3C00X		15000mg
GLYCERIN	INTRAVENOUS	INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION	56815	PDC6A3C00X	225mg	
GLYCERIN	INTRAVENOUS	INJECTION, SOLUTION	56815	PDC6A3C00X	15%w/v	
GLYCERIN	IONTOPHORESIS	SYSTEM	56815	PDC6A3C00X	168.1mg	
GLYCERIN	NASAL	DROPS	56815	PDC6A3C00X	10mg/1ml	
GLYCERIN	NASAL	SOLUTION	56815	PDC6A3C00X	25mg/1ml	
GLYCERIN	NASAL	SPRAY	56815	PDC6A3C00X	22.3mg/1ml	
GLYCERIN	NASAL	SPRAY, METERED	56815	PDC6A3C00X		13mg
GLYCERIN	OPHTHALMIC	EMULSION	56815	PDC6A3C00X	2.3%w/v	
GLYCERIN	OPHTHALMIC	GEL	56815	PDC6A3C00X	0.88%w/w	
GLYCERIN	OPHTHALMIC	SOLUTION	56815	PDC6A3C00X	6%w/v	
GLYCERIN	OPHTHALMIC	SOLUTION/DROPS	56815	PDC6A3C00X	2.25%w/v	
GLYCERIN	OPHTHALMIC	SUSPENSION	56815	PDC6A3C00X	2.5%w/v	
GLYCERIN	OPHTHALMIC	SUSPENSION/DROPS	56815	PDC6A3C00X	2.5%w/v	
GLYCERIN	ORAL	CAPSULE	56815	PDC6A3C00X		3487mg
GLYCERIN	ORAL	CAPSULE, COATED	56815	PDC6A3C00X	14.21mg	
GLYCERIN	ORAL	CAPSULE, EXTENDED	56815	PDC6A3C00X	132.32mg	

		RELEASE				
GLYCERIN	ORAL	CAPSULE, GELATIN COATED	56815	PDC6A3C00X	3.37mg	
GLYCERIN	ORAL	CAPSULE, LIQUID FILLED	56815	PDC6A3C00X		185mg
GLYCERIN	ORAL	CONCENTRATE	56815	PDC6A3C00X		1800mg
GLYCERIN	ORAL	DROPS	56815	PDC6A3C00X	300mg/1ml	
GLYCERIN	ORAL	ELIXIR	56815	PDC6A3C00X	500mg/1ml	
GLYCERIN	ORAL	LIQUID	56815	PDC6A3C00X		7200mg
GLYCERIN	ORAL	PASTE	56815	PDC6A3C00X		3822mg
GLYCERIN	ORAL	SOLUTION	56815	PDC6A3C00X		32400mg
GLYCERIN	ORAL	SOLUTION, CONCENTRATE	56815	PDC6A3C00X	200mg/1ml	
GLYCERIN	ORAL	SUSPENSION	56815	PDC6A3C00X		26208mg
GLYCERIN	ORAL	SUSPENSION, EXTENDED RELEASE	56815	PDC6A3C00X		2000mg
GLYCERIN	ORAL	SUSPENSION/ DROPS	56815	PDC6A3C00X		750mg
GLYCERIN	ORAL	SYRUP	56815	PDC6A3C00X		4500mg
GLYCERIN	ORAL	TABLET	56815	PDC6A3C00X		42mg
GLYCERIN	ORAL	TABLET, CHEWABLE	56815	PDC6A3C00X		12mg
GLYCERIN	ORAL	TABLET, COATED	56815	PDC6A3C00X	1mg	
GLYCERIN	ORAL	TABLET, EXTENDED RELEASE	56815	PDC6A3C00X		7mg
GLYCERIN	ORAL	TABLET, FILM COATED	56815	PDC6A3C00X	1.55mg	
GLYCERIN	PERIODONTAL	TABLET	56815	PDC6A3C00X	0.53mg	
GLYCERIN	RECTAL	SUPPOSITORY	56815	PDC6A3C00X	128mg	
GLYCERIN	RESPIRATORY (INHALATION)	SOLUTION	56815	PDC6A3C00X	5%w/w	
GLYCERIN	SUBCUTANEOUS	INJECTION	56815	PDC6A3C00X		450mg
GLYCERIN	SUBCUTANEOUS	INJECTION, SOLUTION	56815	PDC6A3C00X	15%w/v	
GLYCERIN	SUBCUTANEOUS	INJECTION, SUSPENSION	56815	PDC6A3C00X	1.6%w/v	
GLYCERIN	TOPICAL	AEROSOL	56815	PDC6A3C00X	3%w/w	
GLYCERIN	TOPICAL	AEROSOL, FOAM	56815	PDC6A3C00X	2.11%w/w	
GLYCERIN	TOPICAL	CLOTH	56815	PDC6A3C00X	0.05mg/mg	
GLYCERIN	TOPICAL	CREAM	56815	PDC6A3C00X		1720mg

GLYCERIN	TOPICAL	CREAM, AUGMENTED	56815	PDC6A3C00X		55mg
GLYCERIN	TOPICAL	GEL	56815	PDC6A3C00X		500mg
GLYCERIN	TOPICAL	JELLY	56815	PDC6A3C00X	NA	
GLYCERIN	TOPICAL	LIQUID	56815	PDC6A3C00X	2.5%w/w	
GLYCERIN	TOPICAL	LOTION	56815	PDC6A3C00X		3990mg
GLYCERIN	TOPICAL	OINTMENT	56815	PDC6A3C00X	NA	
GLYCERIN	TOPICAL	SHAMPOO	56815	PDC6A3C00X	5%w/w	
GLYCERIN	TOPICAL	SOAP	56815	PDC6A3C00X	0.76%w/w	
GLYCERIN	TOPICAL	SOLUTION	56815	PDC6A3C00X		1971mg
GLYCERIN	TOPICAL	SPONGE	56815	PDC6A3C00X	2%w/w	
GLYCERIN	TOPICAL	SUSPENSION	56815	PDC6A3C00X	0.6%w/v	
GLYCERIN	TOPICAL	SYSTEM	56815	PDC6A3C00X		13020mg
GLYCERIN	TRANSDERMAL	FILM, EXTENDED RELEASE	56815	PDC6A3C00X	306.2mg	
GLYCERIN	TRANSDERMAL	GEL	56815	PDC6A3C00X		13mg
GLYCERIN	VAGINAL	CREAM	56815	PDC6A3C00X	5%w/w	
GLYCERIN	VAGINAL	DOUCHE	56815	PDC6A3C00X	1%w/w	
GLYCERIN	VAGINAL	JELLY	56815	PDC6A3C00X	NA	
GLYCERIN	VAGINAL	SUPPOSITORY	56815	PDC6A3C00X	227.9mg	
GLYCERIN POLYMER SOLUTION I-137	ORAL	TABLET		NA	0.5mg	
GLYCERIN POLYMER SOLUTION IM-137	ORAL	TABLET		NA	NA	
SORBITOL- GLYCERIN BLEND	ORAL	CAPSULE		NA		2767mg

As taken from FDA, 2023b.

Glycerol is listed by the US EPA Office of Pesticide Programs (undated) and was first registered as a conventional chemical pesticide on 9 December 2003.

“The presence of this ingredient on the List of Prohibited and Restricted Cosmetic Ingredients (the Cosmetic Ingredient Hotlist) indicates that there are potentially significant safety issues. This ingredient must be used in accordance with the restrictions set out on the Hotlist when included in topical natural health products, unless additional evidence for safety is submitted. Reference: <http://www.hc-sc.gc.ca/cps-spc/cosmet-person/hot-list-critique/hotlist-liste-eng.php>”

“Classified as an NHP [natural health product] under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations”.

General:

For oral use, other than when present as part of the capsule shell, Glycerol shall contain no more than 0.1% diethylene glycol and related compounds.”

As taken from Health Canada, 2021

Synonyms:	GLYCERIN
Chemical Names:	1,2,3-PROPANETRIOL; GLYCEROL; TRIHYDROXYPROPANE
CAS number:	56-81-5
JECFA number:	909
INS:	422
FEMA number:	2525
Functional Class:	• Flavouring Agent • FLAVOURING_AGENT • Food Additives • CARRIER_SOLVENT • Food Additives • EMULSIFIER • Food Additives • HUMECTANT • Food Additives • THICKENER
Evaluation year:	2002
ADI:	NOT SPECIFIED (1976)
Comments:	Evaluation not finalized, pending definition of "flavouring agent"
Meeting:	20
Specs Code:	N
Report:	TRS 913-JECFA 59/112
Tox Monograph:	FAS 48-JECFA 57/333 (2001)
Specification:	COMPENDIUM ADDENDUM 12/FNP 52 Add. 12/67 (METALS LIMITS) (2004); FAO JECFA Monographs 1 vol.2/109
Previous Years:	2001, TRS 909-JECFA 57/98, COMPENDIUM ADDENDUM 9/FNP 52 Add.9/148. EVALUATION AS A FLAVOURING AGENT NOT FINALIZED AT THE FIFTY-SEVENTH MEETING (2001); FURTHER INFORMATION REQUIRED TO DETERMINE WHETHER THIS SUBSTANCE IS IN CURRENT USE AS A FLAVOURING AGENT

As taken from WHO, 2021

Glycerin (CAS RN 56-81-5) is included on the FDA's list of Food Contact Substances and listed in 21 CFR Parts: 172.811, 175.300, 175.320, 176.210, 177.1390, 177.2420, 177.2800, 178.3500, 182.1320, 182.90, 169.175.

FDA (2023c)

1,2,3-Propanetriol (glycerol) is listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'; listed as food contact material (Directive 2007/42/EC) and listed as food contact material (Regulation (EU) 2011/10).

As taken from EFSA (2022)

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Glycerol is phosphorylated to alpha-glycerophosphate by glycerol kinase predominantly in the liver (80-90%) and kidneys (10-20%) and incorporated in the standard metabolic pathways to form glucose and glycogen. Glycerol kinase is also found in intestinal mucosa, brown adipose tissue,

lymphatic tissue, lung and pancreas. Glycerol may also be combined with free fatty acids in the liver to form triglycerides (lipogenesis) which are distributed to the adipose tissues. The turnover rate is directly proportional to plasma glycerol levels. [United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of July 14, 2009: <http://www.chem.unep.ch/irptc/sids/OECDsids/sidspub.html> **PEER REVIEWED**

Glycerol is endogenous in the human body. It enters the glycolytic pathway after its conversion in the liver to glycerol-3-phosphate by glycerol kinase. Glycerol-3-phosphate is then oxidized by glycerol-3-phosphate dehydrogenase to yield dihydroxyacetone phosphate, which is then isomerized to glyceraldehyde-3-phosphate, eventually yielding pyruvic acid. [WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of July 14, 2011: <http://www.inchem.org/pages/jecfa.html> **PEER REVIEWED**

Glycerol esters are hydrolyzed to glycerol and the corresponding carboxylic acids. The hydrolysis is catalysed by intestinal lipase, which attacks the ester bonds at carbons 1 and 3. The ester bond at carbon 2 is more resistant to hydrolysis, possibly because of its stereochemistry and steric hindrance. The beta-monoglyceride can, however, spontaneously isomerise to the alpha-form (3-acylglycerol), permitting further hydrolysis to yield glycerol. [WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of July 14, 2011: <http://www.inchem.org/pages/jecfa.html> **PEER REVIEWED**

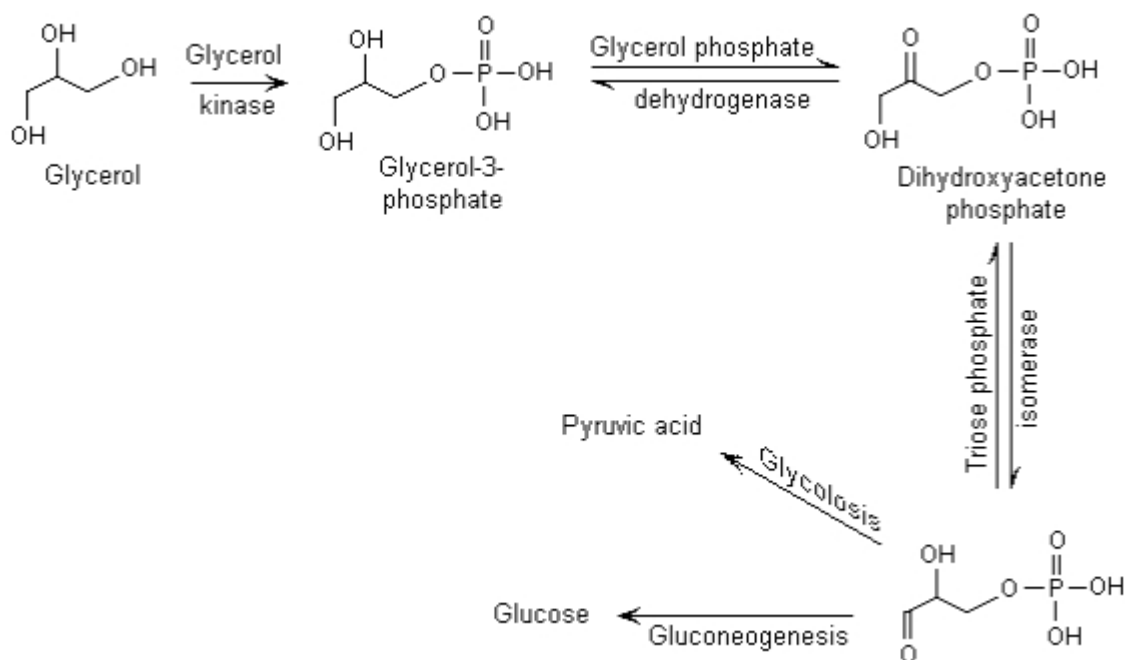
Glycerol, pyruvic acid, and lactic acid are endogenous in humans. Glycerol and pyruvic acid are metabolized completely and are not excreted. ... Glycerol is metabolized via the glycolytic pathway after it has been converted in the liver to glycerol-3-phosphate. [WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of July 14, 2011: <http://www.inchem.org/pages/jecfa.html> **PEER REVIEWED**

Glycerol metabolism is regulated by the enzymes glycerol kinase, (cytosolic) NAD⁺-dependent G3P dehydrogenase and (mitochondrial) FAD-linked G3P dehydrogenase. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

Biotransformation: hepatic; Onset of action: reduction in intraocular pressure - within 10 min; Time to peak effect: reduction in intraocular pressure and vitreous volume - 60 to 90 min; Duration of action: reduction in intraocular pressure - approximately 5 hr. [US Pharmacopeial Convention; US Pharmacopeia Dispensing Information (USP DI); Drug Information for the Health Care Professional 12th ed, V.I p.1455 (1992)] **PEER REVIEWED**

As taken from HSDB, 2012.

"Glycerol and pyruvic acid are metabolized completely and are not excreted" (JECFA, 2001).



As taken from Bassan et al. 2012.

“Obesity and secondary development of type 2 diabetes (T2D) are major health care problems throughout the developed world. Accumulating evidence suggest that glycerol metabolism contributes to the pathophysiology of obesity and T2D. Glycerol is a small molecule that serves as an important intermediate between carbohydrate and lipid metabolism. It is stored primarily in adipose tissue as the backbone of triglyceride (TG) and during states of metabolic stress, such as fasting and diabetes, it is released for metabolism in other tissues. In the liver, glycerol serves as a gluconeogenic precursor and it is used for the esterification of free fatty acid into TGs. Aquaporin 7 (AQP7) in adipose tissue and AQP9 in the liver are transmembrane proteins that belong to the subset of AQPs called aquaglyceroporins. AQP7 facilitates the efflux of glycerol from adipose tissue and AQP7 deficiency has been linked to TG accumulation in adipose tissue and adult onset obesity. On the other hand, AQP9 expressed in liver facilitates the hepatic uptake of glycerol and thereby the availability of glycerol for de novo synthesis of glucose and TG that both are involved in the pathophysiology of diabetes. The aim of this review was to summarize the current knowledge on the role of the two glycerol channels in controlling glycerol metabolism in adipose tissue and liver.” As taken from Lebeck J. 2014. J. Mol. Endocrinol. 52(2), R165-78. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24463099>

“Glycerol is prohibited as a masking agent by the World Anti-Doping Agency and a urinary threshold has recently been recommended. However, little is known about urinary glycerol excretion after exercise, when (1) exogenous glycerol is metabolized increasingly and (2) endogenous glycerol levels are elevated. The purpose of the placebo-controlled cross-over study was to determine the effects of pre-exercise glycerol administration on glycerol metabolism, urinary excretion, and selected blood parameters. After administration of glycerol (G; 1.0 g/kg body weight (BW) + 25 ml fluid/kg BW) or placebo (P; 25 ml fluid/kg), 14 cyclists exercised 90 min at 60% VO₂max . Samples were taken at 0 h (before administration), 2.5 h (before exercise), 4 h (after exercise) and 6.5 h and additional urine samples were collected until 24 h. Exercise increased endogenous plasma glycerol (0.51 ± 0.21 mmol/l) but peak concentrations were much higher in G (2.5 h: 15.6 ± 7.8 mmol/l). Urinary glycerol increased rapidly (58,428 ± 71,084 µg/ml after 2.5 h) and was significantly higher than in P until 13.6 ± 0.9 h (p < 0.01). In comparison with placebo administration, G caused significantly greater changes in plasma volume and haemoglobin concentrations after 2.5 h. BW and urine production were significantly different between P and G

after 2.5 h and post-exercise. Despite exercise-induced increases in endogenous glycerol in the control group, urinary excretion remained well below the previously recommended threshold. In addition, exercise-related glycerol degradation did not appear to negatively affect the detection of exogenously administered glycerol." As taken from Koehler K et al. 2014. *Drug Test Anal.* 6(3), 202-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23359436>

"The objective of this study was to evaluate the effect of three sources of glycerine (crude glycerine from soya bean oil - CGSO, mixed crude glycerine from frying oil and lard--MCG, and a semipurified glycerine from soya bean oil--SPGSO) in four concentrations in the diet (17.5, 35.0, 52.5 and 70.0 g of each type of glycerine per kg of feed) on the levels of plasmatic glycerol and the activity of the hepatic enzyme glycerol kinase in broilers of 22-35 days old (experiment I) and 33-42 days old (experiment II). The highest ($p < 0.05$) plasmatic glycerol level was detected in broilers fed diet containing CGSO. Independent of the source, increasing the concentration of glycerine led to a linear increase ($p < 0.05$) in the plasmatic glycerol concentration. In experiment I, all the diets containing glycerine resulted in increased concentrations of plasmatic glycerol, in relation to the control diet without glycerine. However, in experiment II, only the diet containing 17.5 g of CGSO per kg of feed and the diets formulated with any of the three types of glycerine in the inclusion concentrations of 35, 52.5 and 70 g/kg of feed resulted in higher plasmatic glycerol levels than those observed in the control broilers. The source of glycerine influenced the glycerol kinase activity only in experiment II, where the use of CGSO in the diet increased the enzyme activity. For both experiments, the glycerol kinase activity increased with the inclusion of glycerine in the diet. In conclusion, based on the absence of saturation of the glycerol kinase activity for the three glycerine sources and for both rearing periods evaluated, the broilers can metabolize the glycerol (at the level of the phosphorylation of the glycerol to glycerol-3-phosphate in the liver) present in the glycerine when the diet is supplemented with up to 70 g of glycerine per kg of feed." As taken from Bernardino VM et al. 2014. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 98(2), 328-37. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23692650>

Glycerol occurs naturally in fats (e.g. triglycerides, phospholipids) and is also an endogenous metabolite (Tao et al., 1983). After ingestion of triglyceride, hydrolysis in the gastrointestinal tract yields glycerol and fatty acids which are then absorbed by the mucosa. Glycerol is also released by adipose tissue lipolysis; the catabolic process leading to the breakdown of triglycerides stored in fat cells. Glycerol may then be metabolised through a number of pathways (Lin, 1977; Brisson et al., 2001). Glycerol may be phosphorylated by glycerol kinase to glycerol phosphate followed by oxidation to dihydroxyacetone phosphate via the enzyme glycerol phosphate dehydrogenase (Tao et al., 1983; Brisson et al., 2001). Dihydroxyacetone phosphate can participate in glycolysis and be further oxidised to carbon dioxide (oxidised as an energy substrate) or gluconeogenesis after formation of the intermediate glyceraldehyde 3-phosphate (Tao et al., 1983; Brisson et al., 2001). Exogenous glycerol also participates in lipogenesis (e.g. formation of triglycerides). When rats were i.p. injected with radiolabelled glycerol, radioactivity was found in lipids (Gidez and Karnovsky, 1954). With respect to glycerol utilisation, the liver accounts for up to at least 75% of the total body glycerol-utilising capacity and the kidney accounts for up to 25% (Lin, 1977; Tao et al., 1983). At physiological serum concentrations up to 1 mM glycerol (92 mg/L), uptake into hepatocytes follows first order kinetics (Tao et al., 1983). Gidez and Karnovsky (1954) examined the relationship between the amount of glycerol oxidized compared to the amount administered. At dose levels above 30 mg/rat (about 150 mg/kg bw), an approximately constant proportion of the administered glycerol appeared to be oxidised and exhaled as $^{14}\text{CO}_2$. At lower concentrations, the proportion converted to CO_2 was higher.

"Glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract, it is then distributed into the total body water space and primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as carbon dioxide, with only minor

amounts excreted via urine or faeces. The Panel noted that glycerol concentrations in blood higher than 92 mg/ L would result in renal elimination of glycerol in rats and humans.”

As taken from EFSA, 2017.

“Acrolein is a highly reactive electrophile causing toxic effects, such as DNA and protein adduction, oxidative stress, endoplasmic reticulum stress, immune dysfunction, and membrane damage. This Opinion/Hypothesis provides an overview of endogenous and exogenous acrolein sources, acrolein's mode of action, and its metabolic fate. Recent reports underpin the finding that gut microbial glycerol metabolism leading to the formation of reuterin is an additional source of endogenous acrolein. Reuterin is an antimicrobial multicomponent system consisting of 3-hydroxypropionaldehyde, its dimer and hydrate, and also acrolein. The major conclusion is that gut microbes can metabolize glycerol to reuterin and that this transformation occurs in vivo. Given the known toxicity of acrolein, the observation that acrolein is formed in the gut necessitates further investigations on functional relevance for gut microbiota and the host.” As taken from Zhang J et al. 2018. MBio. 9(1), e01947-17. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29339426>

“Gluconeogenesis (GNG) is de novo production of glucose from endogenous carbon sources. Although it is a commonly studied pathway, particularly in disease, there is a lack of consensus about substrate preference. Moreover, primary hepatocytes are the current gold standard for in vitro liver studies, but no direct comparison of substrate preference at physiological fasting concentrations has been performed. We show that mouse primary hepatocytes prefer glycerol to pyruvate/lactate in glucose production assays and ¹³C isotope tracing studies at the high concentrations commonly used in the literature, as well as at more relevant fasting, physiological concentrations. In addition, when glycerol, pyruvate/lactate, and glutamine are all present, glycerol is responsible for over 75% of all glucose carbons labeled. We also found that glycerol can induce a rate-limiting enzyme of GNG, glucose-6-phosphatase. Lastly, we suggest that glycerol is a better substrate than pyruvate to test in vivo production of glucose in fasting mice. In conclusion, glycerol is the major carbon source for GNG in vitro and in vivo and should be compared with other substrates when studying GNG in the context of metabolic disease states.” As taken from Kalemba KM et al. 2019. J. Biol. Chem. 294(48), 18017-18028. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31645433/>

“Evidence shows that oral glycerol supplementation during the early stages of rat liver cancer reduces the growth of preneoplastic lesions. Besides, human hepatocellular carcinoma (HCC) cells display decreased expression of glycerol channel aquaporin 9 (AQP9) and also diminished glycerol-3-phosphate (G3P) content. According to this, we analyzed glycerol metabolism during the initial stages of rat liver carcinogenesis. Wistar rats were subjected to a 2-phase model of hepatocarcinogenesis (initiated-promoted, IP group) or left untreated (control, C group). Different features of glycerol metabolism were compared between both groups. IP animals showed increased plasma free glycerol levels and liver AQP9 protein expression. Also, IP rats showed increased glycerol kinase (GK) and glycerol-3-phosphate dehydrogenase (GPDH) hepatic activities. Gluconeogenesis from glycerol both in vivo and in isolated perfused liver was higher in rats having liver preneoplasia. Nevertheless, preneoplastic foci notably reduced AQP9 and GK protein expressions, displaying a reduced ability to import glycerol and to convert it into G3P, as a way to preserve preneoplastic hepatocytes from the deleterious effect of G3P. In conclusion, the metabolic shift that takes place in the initial stages of liver cancer development comprises an increased hepatic utilization of glycerol for gluconeogenesis. Enhanced glucose production from glycerol is mostly carried out by the surrounding non-preneoplastic tissue and can be used as an energy source for the early transformed liver cells.” As taken from Lorenzetti F et al. 2020. Biochimie 170, 88-93. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31923480/>

“Most orally administered glycerin is incorporated into body fat or brought into glycolysis or gluconeogenesis pathways (principally in the liver) via glycerokinase-catalyzed phosphorylation and oxidation to dihydroxyacetone phosphate, with ultimate conversion to carbon dioxide and water

in the former pathway, and glucose and glycogen synthesis in the latter. Glycerin can also combine with free fatty acids to form triglycerides. Approximately 80% of glycerin metabolism takes place in the liver and approximately 10% to 20% in the kidney. The metabolism of glycerin to carbohydrate produces 4.3 cal/g glycerin. Most of an oral dose of glycerin is metabolized within 2.5 hours."

As taken from CIR (2019).

4.2. Absorption, distribution and excretion

Data from studies in humans and animals indicate glycerol is rapidly absorbed in the intestine and the stomach, distributed over the extracellular space and excreted. [United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of July 14, 2009: <http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html> **PEER REVIEWED**

After hydrolysis of glycerol esters in the intestine, glycerol is readily absorbed. [WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of July 14, 2011: <http://www.inchem.org/pages/jecfa.html> **PEER REVIEWED**

Following rectal administration, glycerin and sorbitol are poorly absorbed; colonic evacuation of glycerin rectal suppositories or enemas occurs within 15-60 minutes, while colonic evacuation of oral sorbitol occurs within 24-48 hours. [American Society of Health-System Pharmacists 2011; Drug Information 2011. Bethesda, MD. 2011] **PEER REVIEWED**

Following absorption from GI tract, glycerin is distributed throughout the blood. Although glycerin glycerin generally does not appear in ocular fluids, it may enter the orbital sac when the eye is inflamed, with a consequent decrease in osmotic effect. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

Most of an orally administered dose ... is incorporated into body fat ... Approx 7-14% of ... dose ... is excreted unchanged in the urine ... /within 2.5 hr after administration/. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

Following oral administration, glycerin is rapidly absorbed from the GI tract, and peak serum concn occur with 60-90 min. incr intraocular pressure begins to decline with 10-30 min following oral administration of glycerin. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

Elimination: renal; about 7 to 14% of a dose may be excreted unchanged within 2.5 hr. [US Pharmacopeial Convention; US Pharmacopeia Dispensing Information (USP DI); Drug Information for the Health Care Professional 12th ed, V.I p.1455 (1992)] **PEER REVIEWED**

Elimination half-life of glycerin is about 30-40 min. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

As taken from HSDB, 2012.

"Glycerol and pyruvic acid are metabolized completely and are not excreted" (JECFA, 2001).

"Urinary glycerol excretion is associated with plasma glycerol concentrations $> 0.327 \pm 0.190$ mmol/L."

As taken from CIR, 2015.

"The objective of this study was to evaluate the effect of three sources of glycerine (crude glycerine from soya bean oil - CGSO, mixed crude glycerine from frying oil and lard--MCG, and a semipurified glycerine from soya bean oil--SPGSO) in four concentrations in the diet (17.5, 35.0, 52.5 and 70.0 g of each type of glycerine per kg of feed) on the levels of plasmatic glycerol and the activity of the hepatic enzyme glycerol kinase in broilers of 22-35 days old (experiment I) and 33-42 days old (experiment II). The highest ($p < 0.05$) plasmatic glycerol level was detected in broilers fed diet containing CGSO. Independent of the source, increasing the concentration of glycerine led to a linear increase ($p < 0.05$) in the plasmatic glycerol concentration. In experiment I, all the diets containing glycerine resulted in increased concentrations of plasmatic glycerol, in relation to the control diet without glycerine. However, in experiment II, only the diet containing 17.5 g of CGSO per kg of feed and the diets formulated with any of the three types of glycerine in the inclusion concentrations of 35, 52.5 and 70 g/kg of feed resulted in higher plasmatic glycerol levels than those observed in the control broilers. The source of glycerine influenced the glycerol kinase activity only in experiment II, where the use of CGSO in the diet increased the enzyme activity. For both experiments, the glycerol kinase activity increased with the inclusion of glycerine in the diet. In conclusion, based on the absence of saturation of the glycerol kinase activity for the three glycerine sources and for both rearing periods evaluated, the broilers can metabolize the glycerol (at the level of the phosphorylation of the glycerol to glycerol-3-phosphate in the liver) present in the glycerine when the diet is supplemented with up to 70 g of glycerine per kg of feed." As taken from Bernardino VM et al. 2014. J. Anim. Physiol. Anim. Nutr. (Berl.) 98(2), 328-37. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23692650>

"Glycerol is prohibited as a masking agent by the World Anti-Doping Agency and a urinary threshold has recently been recommended. However, little is known about urinary glycerol excretion after exercise, when (1) exogenous glycerol is metabolized increasingly and (2) endogenous glycerol levels are elevated. The purpose of the placebo-controlled cross-over study was to determine the effects of pre-exercise glycerol administration on glycerol metabolism, urinary excretion, and selected blood parameters. After administration of glycerol (G; 1.0 g/kg body weight (BW) + 25 ml fluid/kg BW) or placebo (P; 25 ml fluid/kg), 14 cyclists exercised 90 min at 60% VO_{2max} . Samples were taken at 0 h (before administration), 2.5 h (before exercise), 4 h (after exercise) and 6.5 h and additional urine samples were collected until 24 h. Exercise increased endogenous plasma glycerol (0.51 ± 0.21 mmol/l) but peak concentrations were much higher in G (2.5 h: 15.6 ± 7.8 mmol/l). Urinary glycerol increased rapidly ($58,428 \pm 71,084$ μ g/ml after 2.5 h) and was significantly higher than in P until 13.6 ± 0.9 h ($p < 0.01$). In comparison with placebo administration, G caused significantly greater changes in plasma volume and haemoglobin concentrations after 2.5 h. BW and urine production were significantly different between P and G after 2.5 h and post-exercise. Despite exercise-induced increases in endogenous glycerol in the control group, urinary excretion remained well below the previously recommended threshold. In addition, exercise-related glycerol degradation did not appear to negatively affect the detection of exogenously administered glycerol." As taken from Koehler K et al. 2014. Drug Test Anal. 6(3), 202-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23359436>

Hober and Hober (1937) examined the absorption of unlabelled glycerol from the ligated intestine in anaesthetised rats (no further details provided). After washing, the intestinal lumen was filled with 4 mL 100 mM glycerol solution. After an exposure period of 25 min, the lumen was emptied by rinsing with Ringer's solution and residual glycerol in the washing fluid was determined analytically. The absorption varied between 70 and 89%. Similar experiments with ligated rat stomach (Herting et al., 1956) revealed lower absorption (10–35%) after an exposure period of 6 h. Rapid absorption of radiolabelled glycerol in rats after oral administration was demonstrated by Gidez and Karnovsky (1954). Experiments with ^{14}C -labelled glycerol were performed in 24 h fasted male Wistar rats (number of rats employed was not reported). Rats were administered 60 or 112 mg/ rat by either gavage or intravenous (i.v.) injection and exhaled $^{14}CO_2$ was measured every 15 min for 60 min and then every 20–30 min for up to 240 min. No significant differences were observed between the two routes of administration in terms of exhaled $^{14}CO_2$. Peak levels of $^{14}CO_2$ were reached 50–

80 min after administration, suggesting rapid absorption and metabolism after oral administration. The mean endogenous glycerol level in whole blood of untreated rats ($n = 200$) was reported to be 0.254 mM (23 mg/L) (Ackerman et al., 1975). Kato et al. (2005) examined the absorption of glycerol using a closed loop of the rat small intestine in situ. The absorption of glycerol was saturable and was inhibited by Na⁺-free conditions, suggesting the involvement of a Na⁺-dependent carrier-mediated transport system. Similar results were presented by Yuasa et al. (2003).

In a clinical study performed by McCurdy et al. (1966; see also section 3.2.7), five volunteers (no further details) consumed a 50% solution of unlabelled glycerol in water at a dose level of 1,000–1,270 mg/kg bw after overnight dehydration and administration of pitressin tannate (to further inhibit diuresis). The mean pretreatment glycerol concentration in serum was 0.51 mM (47 mg/L). The serum glycerol levels were measured every 20 min after ingestion for a total of 140 min. Twenty minutes after ingestion, the mean serum concentration increased to 7.7 mM (710 mg/L) and reached a maximum of 16.2 mM (1,490 mg/L) 80 min after the treatment. Thereafter, the serum level declined to 13.3 mM at 140 min. Glycerol appeared in urine and the amount accounted for 7.5–13.9% of the ingested dose (urine collected for 2.5 h after ingestion, from 6 volunteers). Although no conclusions can be drawn on the total absorption, the authors of this study stated that the data indicated rapid absorption of glycerol after ingestion.

Pelkonen et al. (1967) determined the glycerol serum level in 10 volunteers (pretreatment level 0.1 mM or 9.2 mg/L) every 15 min for 60 min after oral treatment with 70 mg glycerol/kg bw (as 5% aqueous solution of unlabelled glycerol). The maximum serum concentration of glycerol occurred 15 min after ingestion, 0.4 mM (37 mg/L). The serum level declined thereafter to pretreatment levels by 1 h.

In a study in rats, it was shown that there was a net synthesis of blood glucose and liver glycogen as a result of ¹⁴C-glycerol administration (88–89 mg/rat; intraperitoneal (i.p.)). Fifteen minutes after administration, 70% of newly formed blood glucose was derived directly from glycerol and 30 min after treatment this value increased to 100%. After 30 minutes, 39% of the newly formed liver glycogen was derived from the administered ¹⁴C-glycerol. This value decreased during the following 1–6 h after application and fell to 15% at 6 h (Gidez and Karnovsky, 1954). In further experiments, the incorporation of ¹⁴C-glycerol into liver lipids was also reported (Gidez and Karnovsky, 1954). At a dose of 1 mM ¹⁴C-labelled glycerol (92 mg/rats; i.p.), most radioactivity was found in liver lipids (5.9% of applied dose) 6 h after application with only minor amounts found in other organs (e.g. 0.26% in the brain and 0.16% in the kidney).

In male Sprague–Dawley rats ($n = 4$ per group) most of the absorbed glycerol was metabolized and exhaled as CO₂ (Michael and Coots, 1971). Within 51 h after gavage of 14–20 mg/kg bw ¹⁴C-labelled glycerol, 73.3% of recovered radioactivity was exhaled as ¹⁴CO₂, 5.2% excreted in urine and 2% in faeces or gastrointestinal contents and 19.5% in the carcass. At glycerol concentrations in the blood up to 1 mM, glycerol excretion in the urine was reported to be undetectable. At higher glycerol levels, a renal excretion of glycerol occurs (Ackerman et al., 1975; Lin, 1977). In male SIV rats, higher glycerol levels in blood were induced by i.v. infusion (Ackerman et al. (1975). Excretion of glycerol via the urine occurred but the interindividual variance of this effect was substantial. At a steady state blood concentration of 1.9 mM (infusion of glycerol at a rate of 500 mg/kg bw per hour; $n = 20$), an average and standard error of the mean of $3.7 \pm 3.5\%$, respectively, of the applied glycerol was eliminated in urine. The infusion of 700 mg/kg bw per hour ($n = 4$) resulted in a range of steady state blood levels between 2 and 40 mM, and the range of renal excretion of glycerol varied between 3.5% and 23% without a clear relationship to the blood level.

The excretion of glycerol via urine was reported to occur in humans at high dose levels (McCurdy et al., 1966). In six volunteers (no further details), glycerol appeared in urine and accounted for 7.5–13.9% of applied dose (urine collected for 2.5 h after ingestion) after ingestion of 1,000–1,270 mg/kg bw unlabelled glycerol (no further data). The disappearance of serum glycerol in humans was analysed in individuals that were injected i.v. with 2,400 mg unlabelled glycerol over a period of

2 min (Pelkonen et al., 1967). The serum levels of glycerol were determined over the subsequent 75–90 min. The removal rates of glycerol in four volunteers varied between 1.35 and 2.21 $\text{Imol/kg bw per minute}$ (mean 1.7 $\text{Imol/kg bw per minute}$). The half-life (at equilibration; to = 10 min after injection) was 10.7–12.3 min (mean 11.7 min). A twofold increase in mean half-life and removal rate were measured in similar trials with 8 diabetics (Pelkonen et al., 1967).

It was shown in volunteers (9 lean and 13 obese subjects, no data about sex and age) that the removal of glycerol from the human blood after infusion varied in proportion to the glycerol plasma concentrations (Bortz et al., 1972). A basal turnover rate of approximately 100 Imol/minute was reported in adult volunteers. At plasma concentrations up to 0.45 mM (41.4 mg/L), the turnover rate increased to 700 Imol/minute in obese subjects, and to a maximum of 400 Imol/minute in lean subjects (0.22 mM glycerol level in plasma). In an intravenous glycerol tolerance test, the turnover rate was calculated as 1.5 $\text{Imol/kg bw per minute}$.

Overall, the Panel considered that glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract; distributed into the total body water space and is primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as CO_2 , with only minor amounts excreted via urine or faeces. The serum concentration of free glycerol in humans has been reported to be normally between 0.46 and 18.5 mg/L (Lin, 1977; Tao et al., 1983). The Panel noted that at glycerol concentrations in blood up to 92 mg/L , no glycerol was detected in the urine but higher levels result in renal elimination of glycerol in rats (Ackerman et al., 1975) and humans (McCurdy et al., 1966).

As taken from EFSA, 2017.

“A study analysing the exhaled breath of EC [electronic cigarette] users reported that of chemicals inhaled, only 6% of nicotine, 8% of PG and 16% of glycerine was exhaled.”

As taken from Public Health England, 2018.

“The study objectives were to demonstrate that glycerol, when topically applied from a roll-on antiperspirant formulation, can be delivered directly to human skin *ex vivo* and the axillary stratum corneum (SC) *in vivo*, and to assess whether it improves the quality of the axillary skin barrier. *Ex vivo* human skin absorption of glycerol was measured following application of a roll-on antiperspirant formulation containing 4% $^{13}\text{C}_3$ -glycerol. Skin distribution of $^{13}\text{C}_3$ -glycerol over 24 h was assessed using gas chromatography-mass spectrometry. *In vivo* axillary SC penetration was measured by confocal Raman spectroscopy and multivariate curve-resolution software 1 h after topical application of a roll-on antiperspirant formulation containing 8% deuterated glycerol (d_5 -glycerol). A clinical study was conducted to determine the efficacy of a roll-on antiperspirant formulation containing 4% glycerol in reducing shaving-induced visual irritation and in increasing axillary-skin hydration. *Ex vivo* skin absorption studies indicated that the formulation delivered $^{13}\text{C}_3$ -glycerol into the SC at all timepoints over the 24-h period. *In vivo* Raman measurements (1 h after application) demonstrated that d_5 -glycerol was detectable to a depth of at least 10 μm in the axillary SC. Application of 4% glycerol from a roll-on antiperspirant formulation to the axilla was associated with significantly less visible irritation and greater skin hydration than observed with the control (glycerol-free) product. These studies demonstrate that glycerol, incorporated in a roll-on antiperspirant formulation, is delivered directly and rapidly to all depths of the axillary SC, and results in improvements in visible irritation and hydration in the axilla.” As taken from Evans RL et al. 2017. Arch. Dermatol. Res. 309(9), 739-748. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28889318>

“Glycerin is rapidly absorbed from the intestine and the stomach, distributed throughout the extracellular space, and excreted renally. Glycerin is phosphorylated to α -glycerophosphate by glycerol kinase, predominantly in the liver (80%-90%) and kidneys (10%-20%), and incorporated via the standard metabolic pathways to form glucose (gluconeogenesis) and glycogen. Glycerin

kinase is also found in intestinal mucosa, brown adipose tissue, lymphatic tissue, lung, and pancreas. Glycerin may also combine with free fatty acids in the liver to form triglycerides (lipogenesis) that can be distributed to adipose tissues. The glycerin turnover rate is directly proportional to plasma glycerin levels.¹⁹ Free glycerin is naturally present in human plasma. Normal serum levels in adult humans range from 0.05 to 0.1 mmol/L. (...)

Orally administered glycerin is rapidly absorbed from the gastrointestinal tract, and peak serum concentrations occur within 60 to 90 minutes. Glycerin is distributed throughout the blood. Glycerin generally does not appear in ocular fluids; however, it may enter the orbital sac when the eye is inflamed. It is not known if glycerin is distributed into milk. The elimination half-life of glycerin is approximately 30 to 45 minutes. Approximately 7% to 14% of an oral dose of glycerin is excreted unchanged in urine during this time. Acute ingestion of glycerin (1 mL/kg in water) in human male patients led to an increase in plasma glycerides. In human female patients, the oral administration of glycerin (1 mL/kg in water) resulted in no change in plasma glyceride concentrations. When glycerin (1 mL/kg/d in 3 doses) was orally administered for 42 days, increased serum glyceride concentrations were observed in both sexes; however, the increase was greater in men."

As taken from CIR (2019).

4.3. Interactions

CONCOMITANT ADMIN OF CARBONIC ANHYDRASE INHIBITORS OR TOPICAL MIOTICS MAY PROLONG THE EFFECTS OF GLYCERIN [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

As taken from HSDB, 2012.

"BACKGROUND: Glycerol in alcohol-based hand rubs has positive effects on skin condition and user acceptability, but, to the authors' knowledge, its effect on the bactericidal effect of alcohol-based pre-operative hand rubs has not been reported. AIM: To investigate the impact of glycerol on the reduction of resident hand flora by ethanol 80% (w/w), isopropanol 75% (w/w) and n-propanol 60% (V/V). METHODS: In three series of in-vivo laboratory tests on volunteers hands, the efficacy of each of three alcohol-based formulations was tested concurrently with and without added glycerol 1.45% (V/V) according to European Norm EN 12791 for testing pre-operative hand rubs. Formulations were allotted at random to 24 volunteers to rub on to their hands for 3 min. Viable counts from fingertip samples were compared with the respective pretreatment counts immediately after treatment and 3 h later. FINDINGS: The 3-h bactericidal effects of the three pure alcoholic formulations were significantly greater than those of formulations containing glycerol ($P < 0.01$). With ethanol, this was also true for the immediate effect. CONCLUSION: Glycerol 1.45% (V/V) inhibits the bactericidal efficacy of alcohol-based surgical hand rubs, especially sustained efficacy." As taken from Suchomel M et al. 2013. J. Hosp. Infect. 83(4), 284-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23433581?dopt=AbstractPlus>

"Bacterial cellulose (BC) is a highly pure form of cellulose, produced in the form of a swollen membrane by several bacteria that demonstrated to be able to modulate the skin release of model drugs. In the present study, the skin irritation potential of BC was evaluated in human subjects. BC membranes with and without glycerin (acting as plasticizer) were tested....BC with glycerin provided a skin moisturizing effect statistically higher than the negative control ($p=0.044$), which was not observed for BC alone....Besides modifying the mechanical properties, the inclusion of glycerin results in a skin moisturizing effect which could be clinically relevant for the treatment for skin diseases characterized by dryness, such as psoriasis and atopic dermatitis." As taken from Almeida IF et al. 2014. Eur. J. Pharm. Biopharm. 86(3), 332-6. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23973717>

“BACKGROUND: Rhabdomyolysis (RM)-induced acute renal failure (ARF) accounts for about 10-40% of all cases of ARF. AIM: The present study investigated the possible protective effect of two nitric oxides (NO)-releasing third generation β -blockers, carvedilol (Carv) and nebivolol (Nebi), against RM-mimicking glycerol (Gly)-induced ARF in rats. MATERIAL AND METHODS: After 24 h dehydration, rats received a single dose of 50% Gly (8 ml/kg, im). They were treated with vehicle, Carv (2.5 mg/kg/day, po) or Nebi (10 mg/kg, po) for 3 successive days starting from an hour prior to Gly injection. Evaluation of blood pressure and locomotor activity was performed during the experiment. 72 h following Gly administration, total protein in the urine, serum levels of creatinine, blood urea nitrogen, sodium and potassium as well as the renal contents of malondialdehyde, reduced glutathione and NO were assessed, together with a histopathological examination of renal tissues. RESULTS: Carv and Nebi attenuated Gly-induced renal dysfunction and histopathological alterations. They decreased the Gly-induced oxidative stress and increased renal NO concentration. Restoration of normal blood pressure and improvement of locomotor activity were also observed. CONCLUSION: The results clearly demonstrate protective effects of Carv and Nebi against renal damage involved in RM-induced ARF and suggest a role of their antioxidant and NO-releasing properties.” As taken from Atwa A et al. 2016. Open Access Maced. J. Med. Sci. 4(3), 329-336. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27703551>

“OBJECTIVE: Contrast media (CM) are a major cause of nephropathy in high-risk patients. The aim of this study was to examine the effects of carnitine (CAR) in advanced nephrotoxicity due to CM administration in rats with glycerol-induced renal functional disorder. METHODS: 40 rats were divided randomly into five groups (n = 8): (1) healthy group; (2) glycerol only (GLY); (3) glycerol and CM (GLY + CM); (4) glycerol, CM and 200 mg kg⁻¹ carnitine (CAR200, Carnitene®; Sigma-tau/Santa Farma, Istanbul, Turkey); and (5) glycerol, CM and 400 mg kg⁻¹ carnitine (CAR400). Kidney injury was induced with a single-dose, intramuscular injection of 10 ml kg⁻¹ body weight (b.w.) of GLY. CAR was administered intraperitoneally. CM (8 ml kg⁻¹ b.w. iohexol, Omnipaque™; Opakim Medical Products, Istanbul, Turkey) was infused via the tail vein to the rats in Groups 3-5. RESULTS: L-carnitine administration significantly decreased serum creatinine and blood urea nitrogen levels. Superoxide dismutase and glutathione activity increased significantly in the treatment groups compared with the nephrotoxic groups. CAR400 significantly reduced malondialdehyde levels to healthy levels. In the treatment groups, tumour necrosis factor (TNF)- α , transforming growth factor 1 β , interleukin 1 β and caspase-3 gene expression decreased compared with the nephrotoxic groups. TNF- α and nuclear factor kappa-beta (NF- κ B) protein expression increased after CM and CAR administration reduced both TNF- α and NF- κ B expressions. Histopathologically, hyaline and haemorrhagic casts and necrosis in proximal tubules increased in the nephrotoxicity groups and decreased in the CAR groups. CONCLUSION: The results reveal that L-carnitine protects the oxidant/antioxidant balance and decreases proinflammatory cytokines and apoptosis in CM-induced nephrotoxicity in rats with underlying pathology. ADVANCES IN KNOWLEDGE: Depending on the underlying kidney pathologies, the incidence of CM-induced nephropathy (CIN) increases. Therefore, this is the best model to represent clinically observed CIN.” As taken from Kunak CS et al. 2016. Br. J. Radiol. 89(1058), 20140724. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26562095>

“Contrast media (CM) is known to have nephrotoxic adverse effects. Epigallocatechin-3-gallate (EGCG) is the most abundant and active catechin in green tea, and has strong antioxidant and anti-inflammatory properties. This study investigated whether EGCG can reduce contrast-induced nephrotoxicity (CIN), alone or with glycerol (GLY)-induced renal damage, and to understand its mechanisms of protection against toxicity, using models of GLY and CIN in rats. The rats were separated into eight groups (n = 6 in each), as follows: Healthy, GLY, CM, GLY + CM, CM + EGCG 50 mg/kg (po), GLY + CM + EGCG 50 mg/kg (po), CM + EGCG 100 mg/kg (po), and GLY + CM + EGCG 100 mg/kg (po). Both doses of EGCG protected against CM-induced renal dysfunction, as measured by serum creatinine and blood urea nitrogen (BUN). In addition, EGCG treatment markedly improved CIN-induced oxidative stress, and resulted in a significant down-regulatory effect on tumor necrosis factor (TNF)- α and nuclear factor (NF)- κ B mRNA expression.

Moreover, histopathological analysis showed that EGCG also attenuated CM-induced kidney damage. Considering the potential clinical use of CM and the numerous health benefits of EGCG, this study showed the protective role of multi-dose EGCG treatment on CIN and GLY-aggravated CIN through different mechanisms.” As taken from Palabiyik SS et al. 2017. Ren. Fail. 39(1), 314-322. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28100100>

“High concentrations of cryoprotective agents are required for cryopreservation techniques such as vitrification. Glycerol is a common cryoprotective agent used in cryopreservation protocols but this agent is toxic at high concentrations. This work is an attempt to mitigate the toxic effects of high concentrations of glycerol on intact chondrocytes in human knee articular cartilage from total knee arthroplasty patients by simultaneous exposure to glycerol and a variety of additive compounds. The resulting cell viability in the cartilage samples as measured by membrane integrity staining showed that, in at least one concentration or in combination, all of the tested additive compounds (tetramethylpyrazine, ascorbic acid, chondroitin sulphate, glucosamine sulphate) were able to reduce the deleterious effects of glycerol exposure when examination of membrane integrity took place on a delayed time frame. The use of additive compounds to reduce cryoprotectant toxicity in articular cartilage may help improve cell recovery after cryopreservation.” As taken from Hahn J et al. 2017. Cryobiology 75, 68-74. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28192075>

5. Toxicity

5.1. Single dose toxicity

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Human	1428 mg/kg	Behavioral - headache - nausea or vomiting	34ZIAG "Toxicology of Drugs and Chemicals," Deichmann, W.B., New York, Academic Press, Inc., 1969 Volume(issue)/page/year: - ,288,1969
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	12600 mg/kg	Behavioral - general anesthetic Behavioral - muscle weakness Liver - other changes	FEPR7 Federation Proceedings, Federation of American Societies for Experimental Biology. (Bethesda, MD) V.1-46, 1942-87. Volume(issue)/page/year: 4,142,1945
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - rat	4420 mg/kg	Behavioral - toxic psychosis Cardiac - other changes Kidney/Ureter/Bladder - other changes	RCOCB8 Research Communications in Chemical Pathology and Pharmacology. (PJD Pub. Ltd., P.O. Box 966, Westbury, NY 11590) V.1-1970- Volume(issue)/page/year: 56,125,1987
LD50 - Lethal dose, 50	Subcutaneous	Rodent - rat	100 mg/kg	Details of toxic effects not reported other than lethal dose value	NIIRDN Drugs in Japan (Ethical Drugs). (Yakugyo Jiho Co., Ltd., Tokyo,

percent kill					Japan) Volume(issue)/page/year: 6,215,1982
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - rat	5566 mg/kg	Details of toxic effects not reported other than lethal dose value	ARZNAD Arzneimittel-Forschung. Drug Research. (Editio Cantor Verlag, Postfach 1255, W-7960 Aulendorf, Fed. Rep. Ger.) V.1- 1951- Volume(issue)/page/year: 26,1581,1976
LDLo - Lowest published lethal dose	Intramuscular	Rodent - rat	10 mg/kg	Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Kidney/Ureter/Bladder - other changes in urine composition	NPRNAY Nephron. (S. Karger Pub., Inc., 79 Fifth Ave., New York, NY 10003) V.1- 1964- Volume(issue)/page/year: 20,47,1978
LD50 - Lethal dose, 50 percent kill	Oral	Rodent mouse -	4090 mg/kg	Details of toxic effects not reported other than lethal dose value	FRZKAP Farmatsevtichnii Zhurnal (Kiev). (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.3- 1930- Volume(issue)/page/year: (6),56,1977
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent mouse -	8700 mg/kg	Behavioral - altered sleep time (including change in righting reflex)	ARZNAD Arzneimittel-Forschung. Drug Research. (Editio Cantor Verlag, Postfach 1255, W-7960 Aulendorf, Fed. Rep. Ger.) V.1- 1951- Volume(issue)/page/year: 28,1579,1978
LD50 - Lethal dose, 50 percent kill	Subcutaneous	Rodent mouse -	91 mg/kg	Details of toxic effects not reported other than lethal dose value	NIIRDN Drugs in Japan (Ethical Drugs). (Yakugyo Jiho Co., Ltd., Tokyo, Japan) Volume(issue)/page/year: 6,215,1982
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent mouse -	4250 mg/kg	Details of toxic effects not reported other than lethal dose value	JAPMA8 Journal of the American Pharmaceutical Association, Scientific Edition. (Washington, DC) V.29-49, 1940-60. For publisher information, see JPMSAE. Volume(issue)/page/year: 39,583,1950
LD50 - Lethal dose, 50 percent kill	Oral	Rodent rabbit -	27 gm/kg	Details of toxic effects not reported other than lethal dose value	DMDJAP Delaware State Medical Journal. (Wilmington, DE) V.1-32(1), 1929-60. For publisher information, see

					DSMJAA. Volume(issue)/page/year: 31,276,1959
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent rabbit -	53 gm/kg	Details of toxic effects not reported other than lethal dose value	NIIRDN Drugs in Japan (Ethical Drugs). (Yakugyo Jiho Co., Ltd., Tokyo, Japan) Volume(issue)/page/year: 6,215,1982
LD50 - Lethal dose, 50 percent kill	Oral	Rodent guinea pig -	7750 mg/kg	Details of toxic effects not reported other than lethal dose value	JHTAB Journal of Industrial Hygiene and Toxicology. (Cambridge, MA) V.18-31, 1936-49. For publisher information, see AEHLAU. Volume(issue)/page/year: 23,259,1941
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	5000 mg/kg	Kidney/Ureter/Bladder - renal function tests depressed	EKFAE9 Eksperimental'naya i Klinicheskaya Farmakologiya. Experimental and Clinical Pharmacology. (Mezhdunarodnaya Kniga, ul. B. Yakimanka, 39, 117049 Moscow, Russia) V.55- 1992- Volume(issue)/page/year: 55,21,1992
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	8 mL/kg	Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	TXCYAC Toxicology. (Elsevier Scientific Pub. Ireland, Ltd., POB 85, Limerick, Ireland) V.1- 1973- Volume(issue)/page/year: 201,143,2004
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	12600 mg/kg	Details of toxic effects not reported other than lethal dose value	ENTOX* Encyclopedia of Toxicology: Reference Book, Elsevier, 2005 Volume(issue)/page/year: - ,449,2005
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	4 mL/kg	Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Kidney/Ureter/Bladder - urine volume	PHMCAA Pharmacologist. (American Soc. for Pharmacology and Experimental Therapeutics, 9650 Rockville Pike, Bethesda, MD 20014) V.1- 1959-

				decreased Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - catalases	Volume(issue)/page/year: 67,41,2003
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	4000 mg/kg	Kidney/Ureter/Bladder - other changes Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,710,2008
LDLo - Lowest published lethal dose	Intramuscular	Rodent - rat	10 mL/kg	Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - multiple enzyme effects	CEXPB9 Clinical and Experimental Pharmacology and Physiology. (Blackwell Scientific Publications, (Australia) Pty Ltd., 107 Barry St., Carlton, Vic. 3053, Australia) V.1- 1974- Volume(issue)/page/year: 33,119,2006
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	4 mL/kg	Vascular - contraction (isolated tissues) Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Kidney/Ureter/Bladder - other changes	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10- Volume(issue)/page/year: 322,461,2007
TDLo - Lowest published toxic dose	Oral	Human - man	3.88 gm/kg	Brain and Coverings - changes in surface EEG Behavioral - excitement Gastrointestinal - hypermotility, diarrhea	JTCTDW Journal of Toxicology, Clinical Toxicology. (Marcel Dekker, 270 Madison Ave., New York, NY 10016) V.19- 1982- Volume(issue)/page/year: 47,312,2009
TDLo - Lowest published toxic dose	Intramuscular	Rodent - rat	5 mL/kg	Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 50,2622,2012

As taken from RTECS, 2019.

Studies in Animals

Species	LD ₅₀ (mg/kg bw)	Reference
ORAL		
Rat	27,200	Hine1953
Rat	>25,300	Bartsch 1976
Rat	>24,000	Clark 1979
Rat	26,000	Anderson 1950
Rat	27,500	Smyth 1941
Rat	>25,000	Tao 1983
Rat	58,400,	Bornmann 1955, Loeser 1954
Rat	>5000, 15750, 27500, 26250-28750	Janssen and de Rooy
Rat	>10000, 12600	Anon, 1945
Mouse	23,000	Hine1953
Mouse	37,950	Bartsch, 1976
Mouse	4,250	Anon, 1977
Mouse	26,000	Anderson, 1950
Mouse	38,000	From Bartsch 1976
Mouse	>38,000	Tao 1983
Mouse	25,888, 37,763	Bornmann 1955, Loeser 1954
Mouse	12,500	Latven, 1939
Mouse	22400, 38000, 31250, 28000	Janssen and de Rooy
Rabbit	27,000	Anon, 1959
Guinea pig	10,000	Hine, 1953
Guinea pig	7,750	Smyth 1941, Sonntag
DERMAL		
Rabbit	>18,700 mg/kg bw	Hine 1953

LD50 values to mammals in non-oral exposure:

8982 mg/kg (ipr-mus)

8300 mg/kg (ipr, rat)

6199 mg/kg (ivn, mus)

5566 ivn-rat

53 ivn-rbt

91 scu-mus

100 scu-rat

LD50 values to mammals in oral exposure, mg/kg :

7750 orl-gpg

4090 orl-mus

12600 orl-rat

As taken from Finnish Environment Institute.

Dermal

No deaths were observed in a group of 6 rabbits after occlusive dermal application for 8 hours of synthetic or natural glycerol at 18,700 mg/kg bw. (Hine 1953).

Oral

In a study with limited reporting, twelve female rats received 27,260 mg natural or synthetic glycerol/kg bw by gavage (Jansson and de Rooy). Cageside observations included muscle spasms and convulsions and survivors appeared normal within 2.5 h of dosing. The number of deaths was not reported. Macroscopic examination of decedents and survivors showed hyperaemia of the pylorus, small intestine and cerebral meninges (3 animals), congestion of the lungs and pale spleen. For this study an LD50 value of 27,200 mg/kg bw was reported (Hine 1953). In other studies, with limited reporting, LD50s of >25,300 and >24, 000 mg/kg were derived for Sprague-Dawley rats (Bartsch 1976) and female Fischer 344 rats (Clark 1979), respectively.

Hine (1953) also investigated the acute toxicity of synthetic or natural glycerol in mice and guinea pigs. Again the reporting was limited, however both species showed similar clinical signs (tremor and convulsions) and macroscopic findings (hyperaemia of pylorus and small intestine, pale spleen, lung congestion). The LD50s for mice and guinea pigs were reported to be 23, 000 and 10, 000 mg/kg, respectively.

A number of acute oral toxicity LD50 values for the rat (range from >5000 to 58400 mg/kg) and the mouse (4,250 to 38,000 mg/kg) are reported in the scientific literature (see table 3.1.2), although where values are very similar, it is not always clear whether or not these are from independent studies. Original reports for several secondary reported LD50 values were not available. The LD50 values reported are consistent with the range of values found in the available literature except in one case, where an oral LD50 value of 4250 mg/kg was reported for the mouse (Anon. 1977).

A number of studies conducted in several species on the acute oral and dermal toxicity of glycerol are included in table 3.1.2. Although the additional studies in table 3.1.2 are poorly reported, the LD50 values are generally similar to those obtained by Hine (1953).

Other Routes of Exposure

In rats and mice, LD50 values for glycerol were between 4,420 and 10,100 mg/kg bw after intraperitoneal administration and 4250-6700 (rabbit LD50 = 53000) mg/kg bw after intravenous administration. Glycerol is much more toxic after subcutaneous administration (LD50 91-100 mg/kg bw). The key finding is that of haemolysis. Further details are available in the SIDS dossier.

Studies in Humans

A single human 'lowest-lethal-dose' (LDLo) value is reported to be 1428 mg/kg (Anon., 1969).

However, the reliability of this information is unknown, as the original literature reference was not available to the reviewer.

Anecdotal reports indicate that subcutaneous or intraperitoneal injection of glycerol in humans, results in albuminuria, haemoglobinuria, anaemia and renal damage.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

CASE REPORTS/ A three year old boy showed a unique intolerance to glycerol: 1-5 hrs after oral administration of glycerol in doses of 0.5-1.0 g/kg he had euphoria, mental confusion, drowsiness, nausea and vomiting, on one occasion the glycerol also provoked hypoglycemia; intravenously administered glycerol induced an immediate loss of consciousness with spontaneously recovery after 30 min., there were no changes in blood glucose values. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/CASE REPORTS/ Two cases of adverse effects after oral administration of glycerine in patients /were reported/. A 82-year old female (hypertensive, mentally senile) received 200 mL 50% glycerine orally for primary angle closure glaucoma. This woman developed headache, shaking of the arm, quivering of the eyes and nausea. A 68-year old female (diabetic) received 280 mL 50% glycerine orally within a period of 3 days. This glycerine was felt responsible for the ensuing severe diabetic acidosis. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

As taken from HSDB, 2012.

The oral median lethal dose (LD50) values for glycerol were reported to be 12,600–28,800 mg/kg bw (rat); 15,000–38,000 mg/kg bw (mouse); 7,750–11,500 mg/kg bw (guinea pig) and 17,600–27,000 mg/kg bw in the rabbit (BIBRA, 1993 [Documentation provided to EFSA n. 2]).

Diuresis was reported to occur in dogs 15 min after gavage of 2,500 g/kg bw (Johnson et al., 1933) and in rats after 'toxic dose levels' (no further details; Suzuki et al., 1977).

"The Panel considered that glycerol has a low acute toxicity."

"The Panel considered that the therapeutic oral use of glycerol at 1,000–1,500 mg/kg bw given as bolus in patients with glaucoma triggered an increase in plasma osmolality and dehydration and resulted in some patients in side effects such as headache, nausea and vomiting in some individuals. Given the dose- and time period-range reported in a study, the Panel calculated that the minimum dose of glycerol required to induce a therapeutic reduction in intracranial pressures was within the range of 125–333 mg/kg bw per hour. The Panel considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients."

As taken from EFSA, 2017.

Name	Route of Administration	Threshold	Information for the Package Leaflet
Glycerol	Oral	10 g per	May cause headache, stomach upset and

(E422)		dose	diarrhea
Glycerol (E422)	Rectal	1 g	May have a mild laxative effect

As taken from EMA, 2017.

Intraperitoneal LD50 rat – 8700 mg/kg (ChemSpider).

organism	testtype	route	dose	effect	reference
human	TDLo	oral	1428 mg/kg	BEHAVIORAL: HEADACHE; GASTROINTESTINAL: NAUSEA OR VOMITING	Toxicology of Drugs and Chemicals, Deichmann, W.B., New York, Academic Press, Inc., 1969, - (288), 1969
rat	LD50	oral	12600 mg/kg	BEHAVIORAL: GENERAL ANESTHETIC; BEHAVIORAL: MUSCLE WEAKNESS; LIVER: OTHER CHANGES	Federation Proceedings, Federation of American Societies for Experimental Biology., 4(142), 1945
rat	LC50	inhalation	570 mg/m3/1H		BIOFAX Industrial Bio-Test Laboratories, Inc., Data Sheets., 9-4/1970
rat	LD50	intraperitoneal	4420 mg/kg	BEHAVIORAL: TOXIC PSYCHOSIS; CARDIAC: OTHER CHANGES; KIDNEY, URETER, AND BLADDER: OTHER CHANGES	Research Communications in Chemical Pathology and Pharmacology., 56(125), 1987 [PMID:3589147]
rat	LD50	subcutaneous	100 mg/kg		Drugs in Japan, 6(215), 1982
rat	LD50	intravenous	5566 mg/kg		Arzneimittel-Forschung. Drug Research., 26(1581), 1976 [PMID:1036956]
rat	LDLo	intramuscular	10 mg/kg	KIDNEY, URETER, AND BLADDER: CHANGES IN TUBULES (INCLUDING ACUTE RENAL FAILURE, ACUTE TUBULAR NECROSIS); KIDNEY, URETER, AND BLADDER: OTHER CHANGES IN URINE COMPOSITION	Nephron., 20(47), 1978 [PMID:619300]

mouse	LD50	oral	4090 mg/kg		Farmatsevtichnii Zhurnal, (6)(56), 1977 [PMID:598477]
mouse	LD50	intravenous	4250 mg/kg		Journal of the American Pharmaceutical Association, Scientific Edition., 39(583), 1950
rabbit	LD50	oral	27 gm/kg		Delaware State Medical Journal., 31(276), 1959
rabbit	LD50	skin	10 gm/kg		BIOFAX Industrial Bio-Test Laboratories, Inc., Data Sheets., 9-4/1970
rabbit	LD50	intravenous	53 gm/kg		Drugs in Japan, 6(215), 1982
guinea pig	LD50	oral	7750 mg/kg		Journal of Industrial Hygiene and Toxicology., 23(259), 1941
mouse	LD50	intraperitoneal	8700 mg/kg	BEHAVIORAL: ALTERED SLEEP TIME (INCLUDING CHANGE IN RIGHTING REFLEX)	Arzneimittel-Forschung. Drug Research., 28(1579), 1978 [PMID:582559]
mouse	LD50	subcutaneous	91 mg/kg		Drugs in Japan, 6(215), 1982

As taken from PubChemRat 1 hour inhalation – LC50: 0.57 mg/L (PPDB, 2021).

5.2. Repeated dose toxicity

Studies in Animals

Inhalation

Two key studies were identified which had some limitations in the reporting and of the study protocols compared to current OECD guidelines (a reduced number of haematology and clinical chemistry investigations, and range of organs examined). However, these inhalation studies provide useful information as they used test and control groups of rats exposed to respirable aerosols of glycerol at measured concentrations and histopathological examination of a range of tissues including the respiratory tract was performed.

Sprague-Dawley rats (10/sex/treatment) were exposed nose-only to a respirable aerosol of glycerol during 14 days (5 days/week, 6 hours/day). The mean exposure concentrations achieved were 0, 1000, 1930 and 3910 mg/m³. The mass median aerodynamic diameter (MMAD) was reported to be < 1.5 micrometres. Two males at 1000 mg/m³ and 1 male and 1 female at 2000 mg/m³ died (which were incidental to treatment). Body weight gain was decreased in all treated animals. This effect may be attributed to stress due to nose-only exposure. Serum glucose was decreased in treated females, but since it did not appear in males and no relationship with concentration was established, the biological relevance of this effect is not considered to be of toxicological

significance. There was no effect on lung, liver, kidney, brain and heart weight nor any macroscopic findings reported. Histopathological examination of the respiratory tract, liver, kidneys and heart of controls and high dose animals revealed an increased incidence of minimal to mild squamous metaplasia of the epiglottis in all treated animals (1/10, 13/18, 16/19 and 13/14 at 0, 1000, 1930, and 3910 mg/m³, respectively). The frequency of animals with mild metaplasia was greatest at the highest exposure concentration. No systemic effects were seen at the highest dose tested (Anderson 1950, Renne 1992).

In a further study by the same authors using a similar protocol, nose-only exposure of rats (SD 15/sex/treatment) 6h/day, 5d/w for 13 weeks to a respirable aerosol (MMAD <2 micrometres) of glycerol at measured concentrations of 0, 33, 165 and 662 mg/m³ led to decreased triglyceride levels in males at 33 (34%) and 165 mg/m³ (22%). This effect appears to be of little toxicological significance as there was no dose-response relationship and was seen in males only. There were no treatment related effects on cageside observations, haematology, organs weights or gross pathology. Microscopic evaluation of the tissues showed “minimal” (10 animals) or “mild” (one animal) squamous metaplasia of the epiglottis in 11 animals in total at the highest concentration. Since the effect on triglycerides did not show a relationship with concentration, was seen in males only and in the absence of any systemic target organ toxicity, the biological relevance of this effect is not considered to be of toxicological significance. Based on an increased incidence of “minimal” to “mild” squamous metaplasia of the epiglottis, the NOAEC for local irritant effects to the upper respiratory tract is 165 mg/m³ and 662 mg/m³ for systemic effects (Anderson 1950, Renne 1992).

Oral

No OECD guideline studies are available although there are a large number of older studies available. In the best available dietary study, groups of 22 rats (Long-Evans)/sex/treatment received 5, 10 and 20% glycerol (natural or synthetic) in their diet (males 2000, 4000 and 8000 mg/kg bw; females 2500, 5000 and 10000 mg/kg bw) for 2 years. Routine clinical observations were made, and bodyweight and food consumption was determined weekly. Deviations from the OECD guideline included the absence of clinical chemistry investigation and a limited range of haematological and urinary analyses were performed. A limited range of organs was investigated at necropsy and the liver, spleen, adrenals, small intestine, gonads and urinary bladder were examined microscopically. Glycogen and fat content of the liver was determined in surviving rats from the 0 and 20 % dose groups. No individual data were reported. For high dosed animals treatment was discontinued after 1 year (reason not stated in report, presumably as an ‘interim’ assessment for carcinogenicity; 3.1.7). No data on mortality and clinical observations were reported. Food consumption was slightly increased in males treated with 5 and 10% natural glycerol. Incidental observations considered by the report-authors to be without relationship to treatment included: bronchiectasis, pneumonia, pulmonary abscesses, hydronephrosis and pyelonephritis. Although the results were not described in detail, based on this limited dietary study it can be concluded that no adverse effects were observed at up to 10,000 mg/kg bw (Hine 1953).

A number of other studies have been incorporated in Table 3.1.5. These studies are considered less reliable indicators of the systemic effects of glycerol following repeated administration, mainly because of limited toxicity assessments and/or deficient experimental design. The effects they do report are consistent with those observed in the key studies and as such they may contribute to the overall assessment of toxicity of glycerol.

Study type	Species	No. of animals	Doses(mg/kg)	Effects	NOAEL (mg/kg)	Reference
DIET						
28-day	Rat	n.i.	20% suppl. diet (= 8824 mg/kg)	No abnormal findings	-	Stoewsand 1966

			bw)			
50-wk	Dog	3	35% in diet (no reference value available to convert to mg/kg bw)	BWG↓	-	Johnson 1933
WATER						
3-mnth	Rat	n.i.	1-20% solution in drinking water (= 667-13340 mg/kg bw)	At 20%: Mortality 2/12 rats at top dose; initial growth and development ↓ (with recovery)	-	Loeser 1954, Bornmann 1955
6-mnth	Rat	5	females/ treatment 5% (natural or synthetic) in drinking water (= 3335 mg/kg bw)	Mortality 1/5 (synthetic) Small thymus and spleen, calcified kidney masses	-	Anderson 1950
GAVAGE						
6-mnth	Rat	n.i.	Oral 10 ml of 50% solution/ kg bw (= 6300 mg/kg bw)	No abnormal findings	-	Loeser 1954
50-day	Rat	n.i.	Oral 10 ml of 20% solution/kg bw (= 2520 mg/kg bw)	No abnormal findings	-	Loeser 1954
44-day	Rat	20	males/ treatment 1, 5, 10, 20% in water (gavage) (=115-2300 mg/kg bw)	Mortality 15% in all treatment groups and controls	-	Fisher 1949
44-day	Rat	20	males/ treatment	No treatment related effects	-	Fisher 1949

			~1260 mg/kg bw (gavage)			
21-day	Rat	8	males/ treatment ~1525 mg/kg bw (gavage)	Mortality at 1525 mg/kg 5/8, O2 consumption ↓	-	Fisher 1949
3-day	Rat	10	females/ treatment 950, 1900 and 3800 mg/kg bw (gavage)	GI-tract: hyperaemia, petechial haemorrhage or erosions (DR)	LOAEL (local) 950 mg/kg bw	Staples 1967
3-day	Dog	1-2/ treatment	950, 1900 and 3800 mg/kg bw (gavage)	GI-tract: hyperaemia, petechial haemorrhage or erosions (DR)	NOAEL (local) 950 mg/kg bw	Staples 1967
30-40 day	Guinea pig	10	Oral 5 ml of 50% solution (= 6300 mg/kg bw)	All animals died RBC ↓		Ostwald 1962
↑= increase BWG = body weight gain CHO = cholesterol CL = chloride n.i.= not indicated			↓= decrease RBC=red blood cells UV= urinary volume WC = water consumption DR = dose related			

Human experience

In cases where glycerol was given intravenously to control cerebral oedema, there were no identified toxic signs attributed to glycerol (Meyer 1971). Chronic ingestion of glycerol gave increased levels of triglycerides (MacDonald 1970).

Conclusion

A considerable number of studies have been performed. However, many of these studies are considered to be of indeterminable reliability due to deficiencies in reporting or methodology, primarily because they were performed before internationalised guidelines were available.

Based on the studies of better quality, it can be concluded that repeated oral exposure by gavage to glycerol does not induce adverse effects other than local irritation of the gastro-intestinal tract. The lowest effect value was 950 mg/kg bw and found in a 3 day study with rats (Staples 1967). The design of this study was considered not to be representative for a repeated dose study, because the duration of exposure was only 3 days and only irritant properties were investigated. The 2-year study of Hine (1953) was chosen to establish the overall NOAEL after prolonged treatment of rats with glycerol. It was concluded that the NOEL is 10,000 mg/kg bw (20% in diet), which is in

agreement with most of the findings reported in Table 3.1.3. At this dose level no systemic or local effects were observed in the parameters investigated. However, it is noted that gavage dosing with bolus administration of glycerol may enhance the local toxicity to the gastrointestinal tract compared with continuous administration via the diet, however, toxic effects are still only seen at relatively high dose levels and do not raise concern For inhalation exposure, irritant effects were observed at 662 mg/m³. No other target organ involvement was identified. The NOAEL for local effects on the respiratory tract following exposure by inhalation is 165 mg/m³.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	96 gm/kg/30D (intermittent)	Blood - changes in leukocyte (WBC) count Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - true cholinesterase	GISAAA Gigiena i Sanitariya. For English translation, see HYSAAV. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1936- Volume(issue)/page/year: 49(10),81,1984
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	560 gm/kg/8W (continuous)	Lungs, Thorax, or Respiration - structural or functional change in trachea or bronchi	EXLRDA Experimental Lung Research. (Hemisphere Publishing, c/o Taylor & Francis 1900 Frost Road, Suite 101, Bristol, PA 19007) V.1- 1980- Volume(issue)/page/year: 12,281,1987
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	2800 mg/kg/25W (continuous)	Skin and Appendages - tumors Tumorigenic - facilitates action of known carcinogen	CALEDQ Cancer Letters (Shannon, Ireland). (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1975- Volume(issue)/page/year: 155,61,2000

As taken from RTECS, 2019.

"The use of glycerol in the diets for animals is of interest because it is a residue of biodiesel production and rich in energy. Thus, this study aimed to evaluate metabolic and physiological parameters of rats receiving supplemental pure glycerol by gavage. We used 30 Wistar rats (initial weight 202.7 ± 29.98 g) receiving 0 (control/saline), 200, 400, 800 and 1600 mg glycerol/kg of body weight (bidistilled glycerine, 99.85% glycerol) beside food and water ad libitum for 28 days. We used a completely randomised design with five treatments and six replicates. At the end of the

experiment, the animals were killed, and the results showed that there was no change ($p > 0.05$) in the intake and excretion of water, the average daily weight gain, dry matter, ash and crude protein in the carcass or plasma triacylglycerols. There was a beneficial effect ($p < 0.05$) up to a dose of 800 mg/kg glycerol on feed intake, percentage of carcass fat, plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDLc) and low-/very low-density lipoprotein (LDLc + VLDLc). The levels of total cholesterol and glucose were increased with up to a dose of 800 mg/kg glycerol (but remained within the normal range); they were reduced with the dose of 1600 mg/kg. The total leucocyte count tended to be reduced, although it was within the reference values for rats. There were no renal or pancreatic lesions. In conclusion, glycerol presented as a safe supplement at the studied doses, even having some beneficial effects in a dose-dependent manner in rats." As taken from Lisenko KG et al. 2015. J. Anim. Physiol. Anim. Nutr. (Berl.) 99, 265-72. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24996054>

Rats

In a study by Staples et al. (1967), female rats (10 animals/group; strain not reported) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol by gavage (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day), control animals received 3 mL water by gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 0.75 mL/kg bw (2,800 mg/kg bw per day) and higher, the authors reported dose-dependent local irritant effects including slight to severe hyperaemia, petechial haemorrhages and erosions but no systemic toxicity. Administration of 3,800 mg glycerol/kg bw (thrice daily) as either a 20, 40, 60, 80 or 100% aqueous solution (dose volume varied between 3 (for 100%) and 15 mL (for 20%)) resulted in local irritant effects at all dilutions but with severity reduced by dilution such that 1 animal in 10 showed slight effects in the 20% group.

Thirty adult male Wistar rats (6 animals/group) were treated by gavage with 0 (control/saline), 200, 400, 800 and 1,600 mg glycerol/kg bw per day (bidistilled glycerine, 99.85% glycerol) for 28 days (Lisenko et al., 2015). Glycerol did not induce any statistically significant change in average

daily weight gain or water consumption or excretion levels. Glycerol supplementation did induce a significant decrease in the average daily feed intake levels. Supplementation with glycerol did not affect plasma triglyceride, glucose, total cholesterol, lipoprotein concentrations, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Plasma glucose and total cholesterol levels demonstrated a significant increase in values up to a dose of 800 mg/kg, followed by a decrease at 1,600 mg/kg bw per day. In terms of high-density lipoprotein levels, there was an increase up to a dose of 800 mg/kg bw per day, followed by a decrease at 1,600 mg/kg bw per day, but levels were still within the normal range for the species. For very low-density lipoprotein and low density lipoprotein levels, there was a decrease with increasing glycerol doses, which remained within the standards. The liver enzymes AST and ALT showed a decrease trend up to the dose of 800 mg/kg bw per day, with an increase at 1,600 mg/kg bw per day. Glycerol supplementation did not result in any change in relative organ weights. Leucocyte levels differed between the groups, although all were within the normal range. The differential count for lymphocytes and neutrophils did not change between the experimental groups. No lesions were observed in any tissue (pancreas, kidney, liver) from animals receiving different levels of glycerol.

In a limited study by Fischer et al. (1949), male rats (20 animals/group; strain not reported) were treated for 44 days with 0, 100, 500, 1,000 or 2,000 mg glycerol/kg bw per day by gavage. The authors reported no clinically adverse signs at any dose; no effects on mortality or body weight gain. For the haematological parameters examined, there were no effects reported on haemoglobin, erythrocyte, leucocyte and lymphocyte counts and differential blood counts. Histopathological examination was restricted to liver, kidney and urinary bladder, and no effects were observed.

Adult male Wistar rats were subjected to 6 weeks of aerobic training (after an acclimatisation to swimming, the animals swam for 60 min daily, at a frequency of 5 times per week, with relative overload of 5% of their body mass) (Andrade et al., 2014). In the last 4 weeks, the animals were treated with saline, glucose or glycerol (800 mg/kg bw per day) by gavage (it is not stated how frequently animals were dosed). Treatment with glycerol did not induce statistically significant differences in body mass, water consumption, urinary production or relative weight of liver or kidney compared to negative control. Glycerol supplementation did induce a significant reduction in food consumption compared to negative control. In terms of blood biochemical parameters, no difference was observed in glycaemic level or lipoprotein concentrations in plasma compared to negative control. However, a statistically significant increase in both plasma triacylglyceride and total cholesterol levels was observed after supplementation with glycerol. Statistically significant increases in adipocyte diameter and area, as well as villous: crypt ratios of the duodenum and ileum were observed following glycerol supplementation. No differences were observed in adipocyte density or the jejunum villous: crypt ratio in glycerol-supplemented animals compared to negative control.

In a study by Anderson et al. (1950), female rats (5 animals/group; strain not reported) were treated for 26 weeks with 0 or 5% glycerol by volume in the drinking water (either derived from natural sources or synthetically synthesised), equivalent to 0 and 4,500 mg glycerol/kg bw per day). Haematology was performed monthly and was composed of erythrocyte and leucocyte counts, haemoglobin and differential blood counts. At 26 weeks, necropsy and histopathology of heart, lungs, liver, spleen, stomach, intestines, kidneys, thymus, thyroid and adrenals was performed, and no effects were observed by the authors except mineralisation in renal tubules. Glycerol treatment had no effects on haematological parameters. The Panel considered mineralisation in renal tubules to be due to the high dose of glycerol used in the study.

In a 3-month study by L€oser et al. (1954), male and female rats (strain unspecified, 6 animals/group) were treated with glycerol as a 0, 1%, 2%, 5%, 10% or 20% solution in drinking water (equivalent to 0, 800, 1,600, 4,000, 8,100 and 16,200 mg/kg bw per day and 0, 900, 1,800, 4,500, 9,100 and 18,200 mg/kg bw per day for males and female, respectively). No effects were noted in the animals receiving 1%, 2%, 5% or 10% glycerol. In the group receiving 20% glycerol, 2 animals out of 12 animals died. The remaining 10 exhibited some retardation in weight gain and development. However, they recovered while under treatment and had no abnormalities at the end of the study. The authors reported that some organs were investigated (liver, spleen, kidney, endocrine organs) and that no effects of glycerol were found. Identical results for glycerol were reported in a shorter version in a publication, by Bornmann (1954). The Panel considered it probable that this paper reported the same study.

Dogs

In a study by Staples et al. (1967), mongrel dogs (1–2 animals/group) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day) by gavage, control animals received 3 mL water by gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 5,600 mg/kg bw per day and higher, the authors reported dose-dependent local irritating effects including hyperaemia, petechial haemorrhages and erosions.

Overall, the Panel noted that no studies were performed according to current test guidelines. The Panel also noted that in a subchronic toxicity study (in drinking water) in rats (L€oser et al., 1954) the effects reported were observed with doses in the range of the LD50 for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg bw per day, the lowest dose tested (Staples et al., 1967)), and dogs (100% glycerol at 5,600 mg/kg bw per day (Staples et al., 1967)) was likely due to the hygroscopic and osmotic effects of large doses of glycerol administered by gavage.

“Short-term or subchronic studies were not performed according to current test guidelines. In a subchronic toxicity study (in drinking water) in rats, the effects reported were observed with doses in the range of the oral median lethal dose (LD50) values for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg body weight (bw) per day, the lowest dose tested), and dogs (100% glycerol at 5,600 mg/kg bw per day) was likely due to the hygroscopic and osmotic effects of large bolus doses of glycerol administered by gavage.”

“From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in tumour incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.”

As taken from EFSA, 2017.

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use – Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - NOEL

POD Value – 3442.0

POD Owner - COSMOS TTC (NON-CANCER)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - dietary exposure for 1 GEN

NOEL/LEL Owner - MUNRO

Original NOEL - 3442.0 mg/kg bw/day

Original LEL - 6883.0 mg/kg bw/day

Critical Sites - UNSPECIFIED ORGANS

Critical Effects - UNSPECIFIED ORGANS - WEIGHT CHANGES

US FDA CFSAN

POD Method	POD Value	POD Owner
NOAEL	Not Calculated	US FDA CFSAN

Lowest-observed effect

Owner	Type	Value	Sites	Effects
US FDA CFSAN	LOAEL	1000.0 mg/kg bw/day	KIDNEY	KIDNEY
			-	-
			WEIGHT	WEIGHT
			CHANGES	CHANGES
			THYROID	THYROID
			-	-
			WEIGHT	WEIGHT
			CHANGES	CHANGES

No-observed effect

US FDA CFSAN: NOAEL: 5000.0 mg/kg bw/day

Adjustment factors

>Critical study: Target Organ Toxicity > Chronic Toxicity (Rat, Oral exposure) for 728 day

As taken from the COSMOS database available at <https://ng.cosmosdb.eu>

5.3. Reproduction toxicity

Studies in Animals

Effects on Fertility

In a two generation study not fully matching current OECD Guidelines, male and female rats (10/treatment) were dosed daily with glycerol (20% solution in water) during 8 weeks before mating. Females received glycerol throughout pregnancy or until weaning of the F1 generation (5 each). When the F1 generation was ~100 days of age, pups were killed except for 10/sex. These animals were used to produce the F2-generation. No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility, reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation. Although the data are limited, a NOAEL of 2000 mg/kg bw was identified from this study (Wegener 1953).

Intratesticular administration of glycerol decreased spermatogenesis, leading to complete loss of spermatogenic cells in rats, but did not affect sexual behaviour (Wiebe 1984). The intratesticular route of exposure is not considered appropriate to investigate effects on fertility. Furthermore, the author reports that no effects were observed following oral administration (data not shown).

Developmental Toxicity

Rats, mice and rabbits were treated daily with glycerol at dose levels up to 1310, 1280 and 1180 mg/kg bw (oral gavage), respectively, during part of the gestation period. The study protocol was in reasonable agreement with the requirements of the OECD 414 (1981). No maternal toxicity or teratogenic effects were seen at the highest dose levels tested (NTIS 1974). From these studies a NOAEL of 1180 mg/kg bw can be derived.

The results from a Frog Embryo Teratogenesis Assay-Xenopus (FETAX, see also 4.1.4) were ambiguous. Since there is no other evidence of developmental effects especially on mammals, the results of this screening assay for developmental toxicity are not considered to be relevant to mammals. The authors considered the response to be a false positive (Bantle 1999).

Human experience

A fertility study involving 64 males workers involved in glycerol manufacture reported no significant differences in sperm quality parameters (sperm counts and percent "normal" forms) (Venable 1980).

Conclusion

Based on the available data, it can be concluded that glycerol does not have any adverse effects on reproductive parameters. There was no evidence of teratogenicity. The NOAEL for developmental toxicity is 1180 mg/kg bw. The evidence of effects on spermatogenesis following intratesticular administration are not considered relevant as an exposure route. These data do not cause concern in relation to reproductive effects from anticipated routes of exposure.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

A seven-generation study of reproductive toxicity in rats given glycerol at a concentration of 0 or 30% (equivalent to 15 000 mg/kg bw per day) was evaluated On average, the pups of treated dams weighed 20% less than those of the control group. [WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of July 14, 2011: <http://www.inchem.org/pages/jecfa.html> **PEER REVIEWED**

As taken from HSDB, 2012.

Type of Test	Route of Exposure or	Species/Test System	Dose Data	Sex/Duration	Toxic Effects	Reference

	Administration					
TDLo - Lowest published toxic dose	Oral	Rodent - rat	100 mg/kg	male 1 day(s) pre-mating	Reproductive - Fertility - post-implantation mortality (e.g. dead and/or resorbed implants per total number of implants)	TGANAK Tsitologiya i Genetika. Cytology and Genetics. For English translation, see CYGEDX. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1967- Volume(issue)/page/year: 19,436,1985
TDLo - Lowest published toxic dose	Intratesticular	Rodent - rat	280 mg/kg	male 2 day(s) pre-mating	Reproductive - Paternal Effects - spermatogenesis (incl. genetic material, sperm morphology, motility, and count) Reproductive - Paternal Effects - testes, epididymis, sperm duct	CCPTAY Contraception. (Geron-X, Inc., POB 1108, Los Altos, CA 94022) V.1-1970- Volume(issue)/page/year: 29,291,1984
TDLo - Lowest published toxic dose	Intratesticular	Rodent - rat	1600 mg/kg	male 1 day(s) pre-mating	Reproductive - Fertility - male fertility index (e.g. # males impregnating females per # males exposed to fertile nonpregnant females)	CCPTAY Contraception. (Geron-X, Inc., POB 1108, Los Altos, CA 94022) V.1-1970- Volume(issue)/page/year: 29,291,1984
TDLo - Lowest published toxic dose	Intratesticular	Rodent - rat	862 mg/kg	male 1 day(s) pre-mating	Reproductive - Paternal Effects - spermatogenesis (incl. genetic material, sperm morphology, motility, and count)	LIFSAK Life Sciences. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1-8, 1962-69; V.14-1974- Volume(issue)/page/year: 34,1747,1984
TDLo - Lowest published toxic dose	Intratesticular	Primate - monkey	119 mg/kg	male 1 day(s) pre-mating	Reproductive - Paternal Effects - spermatogenesis (incl. genetic material, sperm morphology, motility, and count)	CCPTAY Contraception. (Geron-X, Inc., POB 1108, Los Altos, CA 94022) V.1-1970- Volume(issue)/page/year: 39,447,1989

					Reproductive - Paternal Effects - testes, epididymis, sperm duct	

As taken from RTECS, 2019.

“We evaluated the effect of glycerol on the perinuclear theca (PT) of boar sperm. Samples from six ejaculates obtained from three different boars were incubated in the detergent Brij 36-T. Spermatozoa were treated with a glycerol concentration of either 2 or 4%, and incubated for 10 or 30 min; two other samples were treated with protease inhibitors (PI; leupeptin or an inhibitor commercial cocktail), mixed with 4% glycerol, and incubated for 30 min. A third glycerol-free group was used as the control. The samples were processed for electron microscopy evaluation. The PT remained intact in 78% of the control samples while, after addition of glycerol for 30 min, the proportion of spermatozoa with disrupted or absent PT increased ($P < 0.05$). PT was preserved in PI samples, but PT changes increased ($P < 0.05$). Differences due to treatment with glycerol (2 or 4%) at 10 or 30 min were not observed. These results show, to our knowledge for the first time, the adverse effect of glycerol on the integrity of the PT.” As taken from Arenas Núñez MA et al. 2013. Zygote 21(2), 172-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22475502>

Reprotoxicity

In a study reported by Johnson et al. (1933), rats were kept on diets containing 61% starch and no glycerol (control), or 20% starch plus 41% glycerol (equivalent to 20,500 mg/kg bw per day), or no starch plus 61% glycerol (equivalent to 30,500 mg/kg bw per day), limited information, e.g. no data about number of animals per group. Reproduction was impaired by diets containing 41% glycerol and no pregnancies occurred in rats that were on diets containing 61% glycerol (undernourished conditions). However, this was an old study and the documentation of the results is not sufficient for evaluation of this endpoint.

In a study performed by Guerrant et al. (1947), groups of male and female rats (3 males and 6 females per group) received a synthetic diet containing 0 or 30% glycerol (equivalent to 15,000 mg/kg bw per day) and were carried through seven successive generations. Young rats from treated females weighed on average 20% less than controls. The Panel noted this study was limited: the number of animals per group was low and the diet changed during the generations.

Ten male and 10 female rats were orally gavaged daily either with glycerol (20% solution in water) at a dose level of 2,000 mg/kg bw per day or with distilled water (control) for 8 weeks before mating (Wegener, 1953). After mating, one subgroup of females ($n = 5$) received glycerol throughout pregnancy and up to parturition. The other subgroup ($n = 5$) was treated until weaning of the F1 generation. Body weights of F1 rats were measured every second day during the post-natal period from day 15–60. At post-natal day 100, F1 rats were sacrificed except for 10/sex which were used to produce the F2-generation. The authors reported that there were no effects of glycerol on fertility of parental (P) rats, F1 litter size or F1 weight gain (however, data were not presented). In total, 12 F1 rats died during post-natal day 1–50 in the treatment group and three rats in the control; this mortality was not further discussed and could not be judged by the Panel as the total number of pups/dam was not presented. The onset of oestrus cycle in F1 females was comparable in both groups and histopathology of F1 rats revealed no effects on testes, ovaries, thyroid glands, pituitary and adrenal glands (no other organs were examined). F2 litter size was comparable to control. No further parameters were investigated. The Panel noted that the study has limitations due to some methodological weaknesses (e.g. low number of pregnant P rats, no F1 pup weights, no data about external malformations or toxic effects in P generation) but there is no indication for adverse effects on reproductive performance of P rats at a dose of 2,000 mg/kg bw per day, the highest dose level tested. The Panel, therefore, considered that this study was limited as the number of animals per (sub) group was very low and the reporting was limited.

Developmental toxicity

Several developmental toxicity studies of glycerol were conducted in CD-1 mice, Wistar rats golden hamsters and Dutch-belted rabbits (FDRL, 1974a,b,c). Animals were administered different doses of glycerol (not specified) in water by gavage; the control groups were vehicle-treated (dose volume is not reported). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects. Groups of 25 adult female albino CD-1 outbred mice were dosed by gavage from gestation day (GD) 6 to 15 with 0, 12.8, 59.4, 276 or 1,280 mg glycerol/kg bw per day (dose volume 10 mL/kg bw per day) (FDRL 1974a). At necropsy on GD 17, the surviving dams appeared to be completely normal and the number of implantations, and live fetuses was comparable to the control group. Doses up to 1,280 mg glycerol/kg bw per day had no effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions and average implant sites and also fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 25–29 virgin adult female Wistar rats were dosed by gavage from GD 6 and to 15 with 0, 13.1, 60.8, 282 or 1,310 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2, or 5 mL/kg bw per day) (FDRL, 1974b). At necropsy on GD 20, doses up to 1,600 mg glycerol/kg bw per day appeared to be completely normal and had no effects on implantation or on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glyceroltreated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 15–20 virgin adult female Dutch-belted rabbits were treated by gavage once daily from GD 6 to 18 with doses of 0, 11.8, 54.8, 254.5 or 1,180 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2 or 5 mL/kg bw per day) (FDRL, 1974c). Only 10–14 rabbits became pregnant and were evaluated at termination. At necropsy on GD 29, the surviving dams appeared normal throughout the observation period and had normal fetuses. No effect was observed on the number of implantations. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glyceroltreated groups, did not differ from the number in vehicle-treated dams of the control group. The Panel noted that the identically performed tests with glycerol in mice, rats and rabbits by the FDRL (1974a,b,c) were reported in a limited way but cover the organogenesis period of the reproductive cycle. These prenatal developmental toxicity studies showed no dose-related developmental and maternal effects up to the highest dose tested 1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits.

“The reports of the two and multigeneration reproductive toxicity studies have limitations but no adverse effects were reported. Prenatal developmental toxicity studies were also limited but showed no dose-related developmental and maternal effects up to the highest dose tested (1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits, respectively).”

As taken from EFSA, 2017.

Glycerin (CAS RN 56-81-5):

MAK Pregnancy Risk Group C – There is no reason to fear damage to the embryo or foetus when MAK and BAT values are observed.

As taken from ACGIH, 2021.

5.4. Mutagenicity

Mutagenicity Studies:

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE

Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1538
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE

Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE

Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1538
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	E. COLI
Strain Indicator:	WP2 UVRA
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE
Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	E. COLI
Strain Indicator:	WP2 UVRA
Metabolic Activation:	RAT, LIVER, S-9, KC 500
Method:	PREINCUBATION
Dose:	5-5000 UG/PLATE (TEST MATERIAL SOLVENT: WATER)
Results:	NEGATIVE

Reference:	[SHIMIZU,H, SUZUKI,Y, TAKEMURA,N, GOTO,S AND MATSUSHITA,H; RESULTS OF MICROBIAL MUTATION TEST FOR FORTY-THREE INDUSTRIAL CHEMICALS; SANGYO IGAKU 27(6):400-419, 1985]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.1-10 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H, AOKI,N AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. IX; TOKYO-TORITSU EISEI KENKYUSHO KENKYU NENPO 45:191-199, 1994]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.1-10 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H, AOKI,N AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. IX; TOKYO-TORITSU EISEI KENKYUSHO KENKYU NENPO 45:191-199, 1994]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.1-10 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE

Reference:	[FUJITA,H, AOKI,N AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. IX; TOKYO-TORITSU EISEI KENKYUSHO KENKYU NENPO 45:191-199, 1994]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.1-10 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H, AOKI,N AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. IX; TOKYO-TORITSU EISEI KENKYUSHO KENKYU NENPO 45:191-199, 1994]
Test System:	CHO-WBL CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	NONE
Dose:	750; 1000 MM
Dose Regimen:	4 HR TREATMENT, 6 HR RECOVERY
Results:	NEGATIVE
Reference:	[GALLOWAY,M, DEASY,DM, BEAN,CL, KRAYNAK,AR, ARMSTRONG,MJ, AND BRADLEY,MO; EFFECTS OF HIGH OSMOTIC STRENGTH ON CHROMOSOME ABERRATIONS, SISTER-CHROMATID EXCHANGES AND DNA STRAND BREAKS, AND THE RELATION TO TOXICITY; MUTAT. RES. 189(1):15-25,1987]

As taken from CCRIS, 2008.

In vitro Studies

Glycerol did not induce mutations in bacteria in an Ames test, which used four Salmonella typhimurium strains both with and without metabolic activation (rat and hamster S-9). The test was performed in three different laboratories (Haworth 1983). No mutagenic effects were reported in an additional Ames test with 5 strains and rat S-9 as metabolic activation system (Doolittle 1988).

Glycerol was considered to be negative by the authors in a mammalian cell gene mutation test (HGPRT) since the increased number of mutations at the two highest dose levels was considered to be biologically irrelevant, because no concentration dependence was seen (Doolittle 1988). However, it is unknown why the concentrations tested were not maximised to those recommended in the OECD guideline and hence, the result is of uncertain biological relevance.

Chromosomal damage was investigated in the chromosomal aberration test using cultured mammalian cells (Chinese hamster ovary), which was reported as negative. In this test an isolated increase in number of aberrations was seen at 200 ug/mL (with metabolic activation). This finding was considered to be of no biological relevance, since there was no relationship with the concentration tested (Doolittle 1988). Glycerol did not induce sister chromatid exchanges in CHO cells (Doolittle 1988). In rat hepatocytes, the number of nuclear grains did not differ between glycerol treated and control cells (Doolittle 1988). Therefore, it can be concluded that no unscheduled DNA synthesis occurred.

The results of a bacterial recombination assay were positive, which may be attributed to the interference of glycerol with cellular surfaces or with osmotic effects (Nonakae 1989).

Test type	Result	Reference
Ames	negative	Stolzenberg 1979, Ishidate 1984, Clark 1979, Yamaguchi 1982
Chromosome aberration	negative	Ishidate 1984

In vivo Studies

Two in vivo assays are available for glycerol (Varilyak and Kozachuk, 1985). In a rat bone marrow chromosome aberration test glycerol did not induce a statistically significant increase in chromosomal aberrations compared to controls. However, the lack of sufficient reported details on clinical toxicity and absence of a positive control limit the significance which can be attached to this result.

In the same study a rat dominant lethal assay was conducted. Although a dose related increase was seen in post implantation loss, this increase was not statistically significant compared to controls. However, the limited details on methodology, small number of animals per dose group and absence of a positive control, mean no reliable conclusions can be drawn from the data.

Conclusion

There are no structural alerts (expert judgement) which raise concern for the inherent mutagenic potential of glycerol. In vitro, glycerol was negative (with and without metabolic activation) in Ames tests and did not induce chromosomal effects in mammalian cells. The responses seen in a limited gene mutation study in mammalian cells are of uncertain biological relevance as the doses were not maximised. Only two in vivo studies are available. A negative result was observed in a chromosome aberration test, and an increase (not statistically significant) in post implantation loss was seen in a rat dominant lethal assay. However, for both assays, the limited details reported and absence of a positive control, mean no reliable conclusions can be drawn from the in vivo data.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

“BACKGROUND: We investigated the effect of corn-derived biodiesel glycerol on microRNAs (miRNAs) and mRNAs, which play a central role in regulating cell survival, apoptosis and carcinogenesis. MATERIALS AND METHODS: Inbred Balb/c mice were treated with purified glycerol from biodiesel for 24 hours. After administration, we determined the expressions of miR-21, miR-27a, miR-34a, miR-93, miR-143, miR-146a, miR-148a, miR-155, miR-196a, miR-203, miR-205, miR-221 and nuclear factor kappa-light-chain enhancer of activated B-cells-1 (Nfkb1), mitogen-activated protein kinase-8 (Mapk8) and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras) genes in the liver of mice. RESULTS: We found a parallel altered expression of miRNAs and mRNAs in animals consuming biodiesel glycerol that compared to control mice; these alterations reached significant levels only in few cases. CONCLUSION: Biodiesel glycerol presents no higher risk for carcinogenicity or toxicity.” As taken from Szele E et al. 2013. In Vivo 27(1), 107-11. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23239858>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
DNA inhibition		Human Lymphocyte	200 mmol/L	PNASA6 Proceedings of the National Academy of Sciences of the United States of America. (National Academy of Sciences, Printing & Pub. Office, 2101 Constitution Ave., Washington, DC 20418) V.1- 1915- Volume(issue)/page/year: 79,1171,1982
Cytogenetic analysis	Oral	Rodent - rat	1 gm/kg	TGANAK Tsitologiya i Genetika. Cytology and Genetics. For English translation, see CYGEDX. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1967- Volume(issue)/page/year: 19,436,1985

As taken from RTECS, 2019.

The most reliable data for the bacterial reverse mutation assay in *Salmonella Typhimurium* strains TA100, TA1535, TA1537, TA98, TA1538 and *Escherichia coli* WP2uvrA were presented by Shimizu et al. (1985). The study is comparable to current standards. Glycerol concentrations up to 5 mg/plate were tested, the maximal dose level recommended in OECD Guideline 471. Negative results were obtained with and without addition of S9 mix (prepared from livers of male rats pre-treated with polychlorinated biphenyl). Vehicle and positive controls were performed and showed the expected results. No mutagenic activity was detected in other studies (Clark et al., 1979; Stolzenberg and Hine, 1979; Yamaguchi, 1982; Haworth et al., 1983; Ishidate et al., 1984; Doolittle et al., 1988; Higashimoto et al., 1991; Fujita et al., 1994).

Ishidate et al. (1984) published a chromosome aberration test in Chinese hamster lung fibroblast cells (see Table 12). The cells were exposed to three different doses for 24 and 48 h without addition of a metabolic activation system. The maximum dose tested was 1 mg/mL. This concentration represented the highest non-cytotoxic dose level tested in preliminary tests (50% cell-growth inhibition). One hundred metaphases per concentration were analysed for polyploid cells and structural chromosomal aberrations (no further details). Chromosome and chromatid gaps were included in the evaluation of clastogenic effects. No data were presented about concurrent positive controls but clastogenic effects were reported in parallel experiments with other test substances. The exposure to glycerol resulted in no increase in polyploidy or chromosome aberration. The authors considered glycerol to be non-clastogenic and non-mutagenic in this test system, but concentrations up to the cytotoxic threshold were not tested. However, no cytotoxic effects, nor clastogenic activity, was reported by Galloway et al. (1987) at higher concentrations (92 mg/mL), a dose level 18 times greater than the maximum dose level recommended in vitro by OECD Guideline no. 473. In this study, no concurrent positive control was tested for verification of the results. The concentrations used by Doolittle et al. (1988, see Table 12) also gave negative results but did not induce cytotoxic effects nor reach the recommended maximum concentration. Overall, however, the available studies are sufficient for evaluation of this endpoint and were considered by the Panel to be sufficiently robust to support the results reported, indicating no clastogenic activity of glycerol in in vitro studies.

Equivocal results were reported in a mammalian cell gene mutation assay in trials without addition of a metabolic activation system (Doolittle et al., 1988). An increase in the mutation frequency compared to negative (4.8-fold increase) and solvent control (12-fold increase) was found at a concentration of 0.8 mg/mL but no clear increase at the highest dose level of 1 mg/mL. The effect was not dose-dependent and therefore considered by the authors to be without biological relevance. The Panel noted that mutation frequencies in the concurrent untreated and solvent treated controls were unusually low. No independent second trial was performed. Negative results

were obtained with addition of a metabolic activation system. Other studies on this endpoint were not available.

No genotoxic activity was reported in limited studies on DNA damage in mammalian cells (Doolittle et al., 1988). There were only limited details reported for the rec assay in *B. subtilis* (Nonaka, 1982).

Barilyak and Kozachuk (1985) reported a bone marrow chromosome aberration assay and a dominant lethal assay, both performed in male rats. No other in vivo genotoxicity data were available for glycerol.

A single gavage dose of 1 g/kg bw glycerol (n = 10, controls n = 11) induced an increase in chromosomal aberrations (2.2 ± 0.6% vs 0% in control; without gaps) and polyploidy cells (3.2 ± 0.8% vs 0.5 ± 0.2% in control) (Barilyak and Kozachuk, 1985). The bone marrow was processed for cytogenetic analysis 50 h after treatment and at least 50 metaphases per animal were analysed. However, the Panel noted that incidence of chromosomal aberrations observed was low and fell within the range of values often observed in untreated or solvent control animals. Moreover, the Panel also noted methodological shortcomings (e.g. collection of bone marrow outside the recommended range of 12–36 h, no positive control; no aberrations in 1,000 control metaphases; no statistical analysis and one dose level only) and limited information (e.g. no data on toxic effects). On this basis, the Panel considered this study not reliable. In the same publication, a rat dominant lethal assay was documented. Male rats were exposed to 0, 10, 100 or 1,000 mg/kg bw glycerol 'at the stage of late spermatids' (2 weeks before fertilisation) and mated with untreated females (n = 11–12 per dose). A dose-dependent and significant increase in post-implantation loss was reported with a statistically significant increase at ≥ 100 mg/kg bw. The Panel noted that the reported positive results were based on insufficient number of female animals to assess dominant lethal mutations and the number of treated male animals was not indicated. On this basis, the Panel considered this study not reliable.

Overall, glycerol did not show any genotoxic activity in different in vitro assays, which include negative results in the bacterial reverse mutation assay (Ames test), in chromosome aberration assays and in studies on DNA damage in mammalian cells. Questionable results obtained in a hypoxanthineguanine phosphoribosyltransferase (HGPRT) gene mutation assay did not show a dose–response effect and were therefore judged of no biological significance. A lack of valid in vivo genotoxicity data is not of concern since clear negative findings were observed in in vitro assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

As taken from EFSA, 2017.

Glycerol was negative or equivocal in Ames tests in which *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were tested with up to 10 mg glycerol/plate in water or dimethyl sulphoxide, in the absence and presence of metabolic activation systems (NTP, a, b, c).

Test chemical	CAS No.	Physical state	Molecular weight	Mechanism	LLNA EC3 (%)	In vivo prediction	DPRA prediction	ADRA prediction
Glycerol	56-81-5	Liquid	92.09	Non-reactive	-	Non-sensitizer	Neg	Neg

As taken from OECD, 2019

5.5. Cytotoxicity

“Although nephrotoxic agents or nephrotoxins are known to induce acute renal cell injury, their cytotoxic action is not fully elucidated. It is thus crucial to explore such a cytotoxic mechanism and

the increasing volume of reports indicated a significant involvement of oxidative stress. To test this possibility, we investigated if a nephrotoxin would exert oxidative stress, leading to renal cell injury accompanied by certain biochemical alterations. We also examined if specific antioxidant might help prevent such oxidative cell injury. These studies may then help establish a prophylactic or preventive modality for renal cell injury induced by nephrotoxins. **METHODS:** As glycerol has been commonly used for studying acute renal failure in animals, whether it would induce cellular injury was tested in renal proximal tubular OK cells in vitro. Cells were exposed to the varying concentrations of glycerol and cell number/viability was determined in 24 hours. Severity of oxidative stress was assessed by lipid peroxidation assay. Possible effects of glycerol on biochemical parameters were also examined on glyoxalase I activity and heat shock protein 90 using spectrophotometric (enzymatic) assay and Western blot analysis. **RESULTS:** Glycerol (2.5%) was highly cytotoxic to OK cells, inducing 95% cell death in 24 hours. Lipid peroxidation assay indicated that nearly 3-fold greater oxidative stress was exerted by this glycerol. Concurrently, glyoxalase I activity was drastically lost by 75% and heat shock protein 90 was partially degraded following glycerol exposure. However, N-acetyl-cysteine, a potent glutathione-based antioxidant, was capable of almost completely preventing the glycerol-mediated adverse outcomes, such as cell death, glyoxalase I inactivation, and heat shock protein 90 degradation. **CONCLUSIONS:** Glycerol is cytotoxic, capable of inducing specific biochemical alterations such as inactivation of glyoxalase I and degradation of heat shock protein 90, which may reflect a breakdown of the cellular detoxification and defense systems, leading ultimately to OK cell death. Nevertheless, as N-acetylcysteine can provide full cytoprotection against such glycerol toxicity, it could be considered a prophylactic modality for nephrotoxin-induced oxidative renal cell injury and death ". As taken from Fishman AL et al. 2012. J. Clin. Med. Res. 4, 95-101. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22505981?dopt=AbstractPlus>.

The study/ compared the effect of glycerol and glucose on the function of human peritoneal mesothelial cells (HPMC) in vitro. The viability of HPMC was not affected by glycerol (up to 250 mM), whereas it was reduced by glucose in a time- and dose-dependent manner, as assessed by the /lactate dehydrogenase/ release. Although the incubation of HPMC with glycerol induced a dose-dependent decrease in HPMC proliferation, the effect was significantly less inhibitory than that produced by glucose. In HPMC treated with 90 mM of glycerol or glucose the incorporation of (3)H-thymidine had reached 79.0 +/- 19.3% and 55.3 +/- 4.0% of the control (p < 0.05 and p < 0.01), respectively. As measured by the [methyl-(14)C]-choline incorporation, the intracellular amount of newly synthesized phospholipids was reduced from (cpm/ug cellular protein) 147 +/- 58 in control HPMC to 59 +/- 15 in cells exposed to 90 mM of glucose (p < 0.01), but not affected by glycerol (163 +/- 65). On the other hand, both glycerol and glucose (90 mM) decreased the synthesis of proteins (as assessed by the (3)H-proline incorporation) and interfered with potassium ((86)Rb) transport mechanisms in HPMC. [Witowski J, Knapowski J; Int J Artif Organs 17 (5): 252-60 (1994)] **PEER REVIEWED**

As taken from HSDB, 2012.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Rabbit - ocular	0.035 gm/L/10M	In Vitro Toxicity Studies - cell viability (cell	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford,

				death), unspecified assay	NY 10523) V.1- 1987- Volume(issue)/page/year: 3,329,1989
IC50 Inhibitor Concentration 50 -	In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,688,2015
IC50 Inhibitor Concentration 50 -	In vitro	Human - other normal tissue	68391.33 mg/L/48H	In Vitro Toxicity Studies - cell viability (lysosomal damage): neutral red assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 25,1989,2011
IC20	In vitro	Human monocyte -	>10000 umol/L/24H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 199,51,2010
IC50 Inhibitor Concentration 50 -	In vitro	Mouse fibroblast -	289 mmol/L/48H	In Vitro Toxicity Studies - cell viability (lysosomal damage): neutral red assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 27,1357,2013
IC05	In vitro	Human leukemia cells -	>25000 mg/L/30M	In Vitro Toxicity Studies - cell counting	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 27,857,2013
IC50 Inhibitor Concentration 50 -	In vitro	Human - colon tumor	35.8 umol/L/24H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014

				etc.	
IC50 Inhibitor Concentration 50 -	In vitro	Human - colon tumor	21 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
IC50 Inhibitor Concentration 50 -	In vitro	Human - gastrointestinal tumor	34.5 umol/L/24H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
IC50 Inhibitor Concentration 50 -	In vitro	Human - gastrointestinal tumor	23 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
ICLo Inhibitor Concentration Low -	In vitro	Human - colon tumor	50 umol/L/24H	In Vitro Toxicity Studies - apoptosis in vitro	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
ICLo Inhibitor Concentration Low -	In vitro	Human - gastrointestinal tumor	50 umol/L/24H	In Vitro Toxicity Studies - apoptosis in vitro	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
IC50 Inhibitor Concentration 50 -	In vitro	Human - ocular	454605 mg/L/1H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS,	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 212,198,2012

				WSTs assays etc.	
IC30 Inhibitor Concentration 30	- In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,688,2015
IC30 Inhibitor Concentration 30	- In vitro	Human lymphocyte -	>200 mg/L/45H	In Vitro Toxicity Studies - cell viability (dye exclusion): trypan blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015
IC20	In vitro	Human - lung	>4 mmol/L	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 30,274,2015

As taken from RTECS, 2019.

“High concentrations of cryoprotective agents are required for cryopreservation techniques such as vitrification. Glycerol is a common cryoprotective agent used in cryopreservation protocols but this agent is toxic at high concentrations. This work is an attempt to mitigate the toxic effects of high concentrations of glycerol on intact chondrocytes in human knee articular cartilage from total knee arthroplasty patients by simultaneous exposure to glycerol and a variety of additive compounds. The resulting cell viability in the cartilage samples as measured by membrane integrity staining showed that, in at least one concentration or in combination, all of the tested additive compounds (tetramethylpyrazine, ascorbic acid, chondroitin sulphate, glucosamine sulphate) were able to reduce the deleterious effects of glycerol exposure when examination of membrane integrity took place on a delayed time frame. The use of additive compounds to reduce cryoprotectant toxicity in articular cartilage may help improve cell recovery after cryopreservation.” As taken from Hahn J et al. 2017. Cryobiology 75, 68-74. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28192075>

“The purpose of this study was to investigate the potential for cryopreservation of granulocytes using 30% glycerol. Recently reported permeability data was used to design two different methods for addition and removal of glycerol: a fast method that is predicted to keep cell volumes between 80% and 150% of the isotonic volume and a slow method that is predicted to keep cell volumes between 80% and 115% of the isotonic volume. The fast method resulted in cell recoveries of 31% ± 9% and 11% ± 3% before and after freezing, respectively, whereas the slow method resulted in even lower cell recoveries of 5% ± 2% and 4% ± 2%. The reduced cell recovery for the slow method is consistent with an increase in damage as a result of glycerol toxicity. Our results suggest

that cryopreservation of granulocytes in concentrated glycerol is not feasible.” As taken from Moss AC and Higgins AZ. 2016. Cryobiology 72(3), 290-3. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27182034>

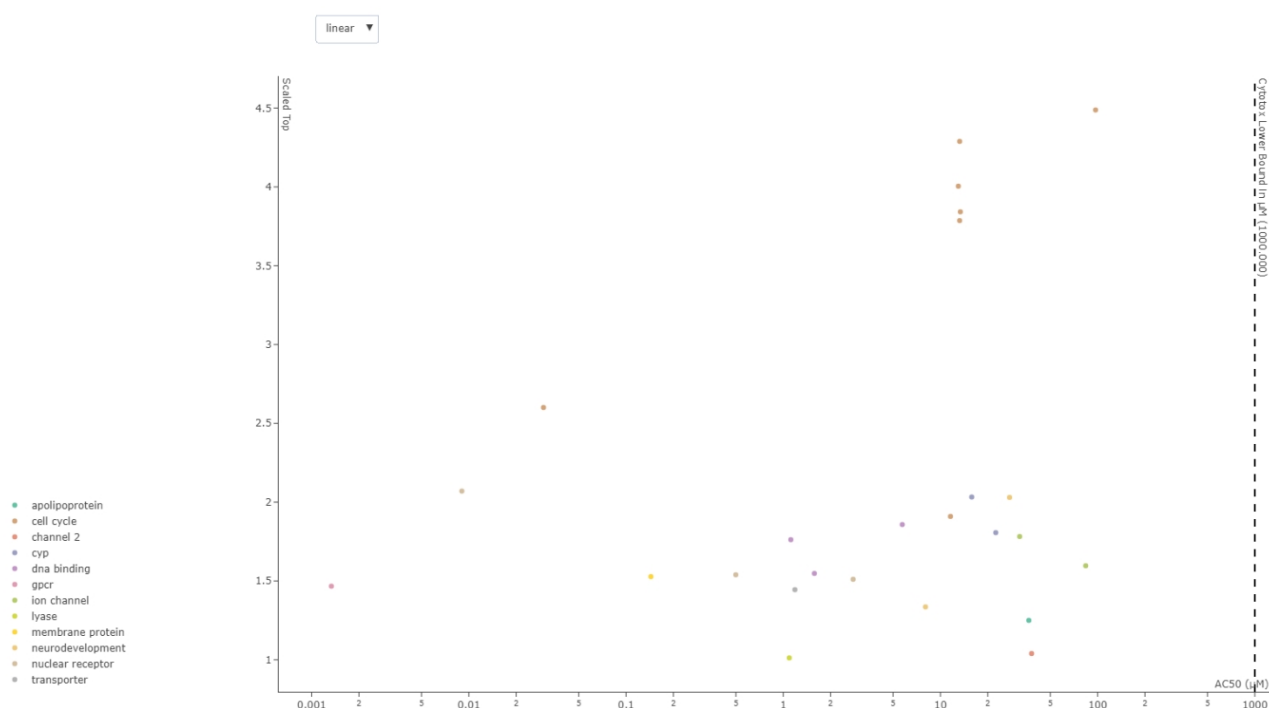
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated glycerol in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on glycerol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



“Background: The developing lung is uniquely susceptible and may be at increased risk of injury with exposure to e-cigarette constituents. We hypothesize that cellular toxicity and airway and vascular responses with exposure to flavored refill solutions may be altered in the immature lung. Methods: Fetal, neonatal, and adult ovine pulmonary artery smooth muscle cells (PASMC) were exposed to popular flavored nicotine-free e-cigarette refill solutions (menthol, strawberry, tobacco, and vanilla) and unflavored solvents: propylene glycol (PG) or vegetable glycerin (VG). Viability was assessed by lactate dehydrogenase assay. Brochodilation and vasoreactivity were determined on isolated ovine bronchial rings (BR) and pulmonary arteries (PA). Results: Neither PG or VG impacted viability of immature or adult cells; however, exposure to menthol and strawberry flavored solutions increased cell death. Neonatal cells were uniquely susceptible to menthol flavoring-

induced toxicity, and all four flavorings demonstrated lower lethal doses (LD50) in immature PASM. Exposure to flavored solutions induced bronchodilation of neonatal BR, while only menthol induced airway relaxation in adults. In contrast, PG/VG and flavored solutions did not impact vasoreactivity with the exception of menthol-induced relaxation of adult PAs. Conclusion: The immature lung is uniquely susceptible to cellular toxicity and altered airway responses with exposure to common flavored e-cigarette solutions.” As taken from Berkelhamer SK et al. 2019. *Int. J. Environ. Res. Public Health* 16(19), 3635. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31569724/>

“Background and objective: E-cigarettes are often marketed and thought of as emitting harmless vapour; however, verification of their safety for non-smokers is scarce. We have previously shown that E-cigarettes cause decreased phagocytosis of bacteria by macrophages via reductions in surface bacterial recognition receptors. This study assessed the effect of E-cigarette constituents, 3 E-liquid apple flavours, nicotine, vegetable glycerine and propylene glycol, on bronchial epithelial cell viability, apoptosis and cytokine secretion and macrophage phagocytosis of apoptotic airway cells and phagocytic recognition molecules. Methods: Cell necrosis and apoptosis were measured by Sytox Green stain and Annexin V. Efferocytosis was measured by internalization of pHrodo Green labelled apoptotic airway cells by macrophages. Expression of macrophage cell surface apoptotic cell receptors was measured by flow cytometry. Cytokine release by E-cigarette-exposed airway cells was measured by cytokine bead array. Results: E-cigarette vapour increased primary bronchial epithelial necrosis and apoptosis. E-cigarette vapour reduced efferocytosis (lowest flavour 12.1%) versus control (20.2%, $P = 0.032$). The efferocytosis receptor CD44 was reduced by one flavour (MFI 1863 vs 2332 control, $P = 0.016$) and all components reduced expression of CD36, including the glycol bases (MFI 1067-12 274 vs 1415 control). Reduced secretion of TNF- α , IL-6, IP-10, MIP-1 α and MIP-1 β was observed for all flavour variants. Conclusion: E-cigarettes can cause bronchial epithelial apoptosis and macrophage efferocytosis dysfunction via reduced expression of apoptotic cell recognition receptors. These data further show that E-cigarettes should not be considered harmless to non-smokers and their effects may go far beyond cytotoxicity to cells.” As taken from Ween MP et al. 2020. *Respirology*. 25(6), 620-628. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/31542893/>

“Information on the effects of propylene glycol (PG) and vegetable glycerin (VG) and on cytotoxicity and subsequent activation of the biological mediators is limited in periodontal diseases. This study analyzes the effect of unflavored PG/VG alone or in combination with nicotine on gingival epithelial cells. The cells were exposed to different PG/VG ($\pm$ nicotine) concentrations for 24 h and cytotoxicity was evaluated by calorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The expressions of interleukin (IL)-6, IL-8, and matrix metalloproteinases (MMPs)-9 were measured using an enzyme-linked immunosorbent assay and a western blotting. Stimulation with PG/VG mixtures reduced cell viability compared to nonexposed controls ($p < 0.05$). Adding PG/VG increased the levels of IL-6, IL-8, and MMP-9, and the amount of PG had more biological impact compared to the VG amount. The nicotine augmented this effect compared to its nicotine-free counterparts. In western blotting result, MMP-9 was clearly activated in almost all samples. These findings suggest that the main constituents PG/VG are cytotoxic and able to induce biological response in gingival cells in vitro. Despite being advertised as less harmful than conventional cigarettes, electronic cigarette liquid pose certain risks on periodontal cells. Awareness about the effects of electronic cigarettes on periodontal diseases must be increased.” As taken from Beklen A and Uckan D. 2021. *Hum. Exp. Toxicol.* 40(1), 25-34. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32729321/>

5.6. Carcinogenicity

“In a limited and non OECD Guideline 12-24 month dietary study in rats, evidence of malignant neoplasms were reported in 5/26, 1/22, 5/22, 0/22, 0/21, 5/22 and 0/22 animals in controls and at 5%, 10%, 20% (natural glycerol) and at 5%, 10%, 20% (synthetic glycerol). At the top dose, the

treatment period was one year. Benign neoplasms were encountered including pheochromacytomas and granulosa cell tumours in 0/26, 2/22, 1/22, 0/22, 4/21, 4/22 and 1/22 animals in controls and at 5%, 10%, 20% (natural glycerol) and at 5%, 10%, 20% (synthetic glycerol), respectively. The authors concluded that glycerol does not initiate tumour development in the rat (Hine 1953).

In male ddY mice administration of glycerol (5% in drinking water during 1-20 weeks) after a single s.c. injection with 4-nitroquinoline 1-oxide (4-NQO) was reported to enhance lung tumour development. Histopathologically most lung tumours were identified as adenomas (Nagahara 1987, Inayama 1986). The mechanism of tumour induction was independent from pulmonary cell kinetics (Nagahara 1987)".

Conclusion

No studies conducted to modern regulatory guidelines are available. The studies that are available are therefore of lower quality. No increase in tumour formation was observed in a limited dietary carcinogenicity study in the rat. Data from non-guideline studies designed to investigate tumour promotion activity in male mice suggest that oral administration of glycerol up to 20 weeks had a weak promotion effect on the incidence of lung tumour formation. In the same studies, treatment with glycerol alone (administered in the drinking water) did not result in an increase in the number of tumour bearing mice relative to controls. Overall, these data do not raise concern for carcinogenic potential.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

Glycerin, synthetic/natural (CAS 56-81-5):

Rat Target Sites		Mouse Target Sites		TD50 (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no positive (B)	no positive (B)	no test	no test	no positive	no test

(B) = experimental results were reported only for both sexes together.

As taken from CPDB, 2007.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	87.5 gm/kg/25W (intermittent)	Tumorigenic - equivocal tumorigenic agent by RTECS criteria Lungs, Thorax, or Respiration - tumors Tumorigenic - facilitates action of known carcinogen	CALEDQ Cancer Letters (Shannon, Ireland). (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1975- Volume(issue)/page/year: 229,181,2005

As taken from RTECS, 2019.

"BACKGROUND: We investigated the effect of corn-derived biodiesel glycerol on microRNAs (miRNAs) and mRNAs, which play a central role in regulating cell survival, apoptosis and

carcinogenesis. MATERIALS AND METHODS: Inbred Balb/c mice were treated with purified glycerol from biodiesel for 24 hours. After administration, we determined the expressions of miR-21, miR-27a, miR-34a, miR-93, miR-143, miR-146a, miR-148a, miR-155, miR-196a, miR-203, miR-205, miR-221 and nuclear factor kappa-light-chain enhancer of activated B-cells-1 (Nfkb1), mitogen-activated protein kinase-8 (Mapk8) and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras) genes in the liver of mice. RESULTS: We found a parallel altered expression of miRNAs and mRNAs in animals consuming biodiesel glycerol that compared to control mice; these alterations reached significant levels only in few cases. CONCLUSION: Biodiesel glycerol presents no higher risk for carcinogenicity or toxicity." As taken from Szele E et al. 2013. *In Vivo* 27(1), 107-11. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23239858>

"Aquaglyceroporin 9 (AQP9) is considered to be involved in numerous types of carcinogenic processes, particularly in liver carcinoma. AQP9 expression is significantly decreased in the human hepatocellular carcinoma when compared with the non-tumourigenic liver, which leads to increased resistance to apoptosis. In addition, AQP9 is permeable to glycerol and urea. The involvement of AQP9 in leukemia has not been fully delineated. It is proposed that abnormal proliferation of hematopoietic stem cells (HSCs) contributes to leukemia carcinogenesis. Therefore, the present study aimed to investigate the possible roles of AQP9 in HSCs and its effect on the intracellular glycerol content....The results indicated that the glycerol content in the HSCs was markedly greater than that in the nHSCs. AQP9 mRNA and protein expression was not detected in the HSCs and nHSCs, but was identified in the WBCs. Moreover, the HSC morphological characteristics included round or oval cells with round, slightly oval or irregularly shaped nuclei. Additionally, the nuclei occupied almost the entire cell, were located in the middle or were biased toward one side, and were stained light purple or red. Overall, our results indicated that intracellular glycerol is involved in HSC proliferation, despite the fact that glycerol is not mediated by AQP9. Hence, our findings may be useful in further understanding the mechanism of leukemia carcinogenesis, and these data may be valuable in developing future therapeutic strategies." As taken from Qin YS et al. 2014. *Oncol. Lett.* 8(1), 169-174. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24959239>

Witschi et al. (1989) investigated the effects on lung and liver tumour incidences in male and female CH3 mice (between 31 and 51 mice/group) after administration for 1 year of drinking water containing 0% or 5% glycerol (equivalent to 0 and 6,500 mg glycerol/kg bw per day for males and 0 and 8,300 mg/kg bw per day for female). Although the reporting of this study was limited, the Panel noted that lung and liver tumour incidences were not significantly elevated in male or female mice (no further parameters examined)..

As reported by JECFA (1976), male and female Sprague–Dawley rats (9 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% natural or synthetic glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 50 weeks (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976). At necropsy, growths in the pituitary and histopathological effects were observed in the kidney in females treated with either test compounds. The Panel noted limited methodological detail, effects only in females and data lacking any dose–response effects.

Hine et al. (1953) examined the effects of supplementing the diet with glycerol (natural and synthetic, no further details on test substances) for up to 2 years on Long-Evans male and female rats (between 10 and 14 animals per group) at 0, 2,500, 5,000 or 10,000 mg/kg bw per day. The Panel noted that the study has a number of limitations – all rats in the high-dose group were culled after 1 year, with no concurrent control group; no clinical chemistry; limited parameters in haematology and urinalysis and limited histopathology. Body weight and food consumption were measured weekly and body weight gain calculated at 4 week intervals. Clinical signs were recorded daily and careful inspections for health and activity were made once a week. Urinalysis and blood sampling for haematology were performed at 3, 6, 12, 18 and 24 month (n = 5 per dose, no data about sex). Urinalysis was limited to microscopical examination and determination of glucose and albumin concentrations. Haematology was limited to erythrocyte counts, leucocyte counts and

haemoglobin levels. High-dose rats were sacrificed after 1 year and liver glycogen and lipids determined (no control values were available). For the other groups, including the control group, the study was terminated after 105 weeks. In all groups, complete necropsy was performed and weights for the liver, lung, kidney, heart and spleen determined. Histopathology was limited to the liver, spleen, adrenals, kidney, small intestine, reproductive organs and urinary bladder. Lipid stains in the liver were prepared for all groups sacrificed after 105 weeks. The results were similar for the groups treated with natural or synthetic glycerol. No data were presented on food consumption, clinical signs or mortality. No significant effects were found on the body weight gain (statistical analysis restricted to mean values at week 13, 52 and 105); data on absolute body weight were not given. No consistent treatment-related effects were found in haematology, urinalysis, evaluation of organ weights and pathology. Liver glycogen and lipids did not differ between the two high-dose groups, a comparison with the control group was not possible. Similarly, data on organ weights and pathological effects in the high-dose group (10,000 mg/kg bw per day) were compared with a control group sacrificed at 105 weeks, limiting the validity of the results in the high-dose group. No no observed adverse effect level (NOAEL) was identified by the authors of the study. Although this study had minor limitations (e.g. lacking concurrent control in the 1-year study group), the Panel noted that no adverse effects were reported in animals receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in the tumours incidences up to doses of 5,000 mg/kg bw per day for 2 years, the highest dose tested.

JECFA reported a study in rat (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976) in which male and female Sprague–Dawley rats (24 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 2 years. Animals were treated with natural glycerol (99.8% glycerol, similar caloric density for test and control diets) or synthetic glycerol (99.76% glycerol and 1,2,3-butanetriol and 1,2,4-butanetriol, 0.09%, similar caloric density for test and control diets). For both natural and synthetic glycerol, at all dose levels, glycerol treatment resulted in an increase in body weight gain (no further details). At the 10% dose level, there was an increase in relative heart weights (no data regarding other dose levels). At necropsy, the 20% glycerol group was examined for mortality, body weight, efficiency of caloric utilisation, water consumption, urinalysis (volume, specific gravity, sediment, acetone, albumin, sugar and oxalate), haematology (white blood cells and differential count, red blood cells, haematocrit, and haemoglobin) and clinical chemistry (blood glucose, urea, nitrogen, plasma cholesterol, serum alkaline phosphatase and transaminases, and bromosulfophthalein retention). In addition, organ weights were determined and complete histopathology performed (no further details). The only reported change noted was an increase in relative kidney weight at 20% glycerol dose level with both natural and synthetic glycerol. The Panel considered that the increase in relative heart weights did not show a dose–response relationship and that the increase in relative kidney weight at 20% glycerol dose level in both studies as adaptive since no histopathological changes were reported.

Overall, the Panel considered glycerol to be of no concern with regard to carcinogenicity.

“From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in tumour incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.”

As taken from EFSA, 2017.

5.7. Irritation/immunotoxicity

Skin Irritation

There were no studies conducted to modern OECD guidelines. In a study conducted using contemporary protocols but prior to GLP standards it was demonstrated that dermal application of 0.5-mL glycerol to the rabbit's skin for 24 hours did not lead to signs of irritation 24 and 72 hours after application. Irritation scores according to Draize scale were 0-0.4, compared to a maximum score of 30 (Weil 1971).

Another test with similar methodology gave a similar outcome (Clark 1979) and there was no evidence of irritation in rabbits following repeated applications of 4 mL over 30% of the surface area 8 h/day for 90 days (Hine 1953).

Conclusion

No OECD guideline studies are available, however, the data available indicate that glycerol is not irritating when in contact with the skin.

Eye Irritation

In a study conducted to a contemporary protocol and prior to GLP standards, 0.1 mL undiluted glycerol was instilled in the eyes of 6 rabbit) caused no evidence of irritation after 1, 24 and 72 hours and after 7 days. The overall irritation score using the Draize system was 0-2 on a scale up to a maximum of 110 (Weil 1971). In another study of similar design, using 4 rabbits, irritation of unspecified severity observed at 1 h after instillation of glycerol was absent after 24 h (Hine 1953).

Another test with a similar design on a glycerol/water mixture (not further specified) gave a similar result and reactions, which were reversible within 24 h (Clark 1979). Due to the methodology and scoring systems used in these non-OECD guidelines it is not possible to directly compare the results to internationally agreed criteria for assessing eye irritation. However, it is apparent from these studies that glycerol has a very low potential to irritate the eyes. In an OECD guideline study reported in a secondary source, slight to moderate corneal irritation was observed in all rabbits after 1h, however the effects were found to regress after 24h and were fully reversed by 48h (Janssen and de Rooy). In a secondary source, there is an anecdotal report that in workers that glycerol caused a burning and stinging sensation with tear production but without injury (Grant 1974).

The data available indicate that glycerol is not irritating to the eyes.

Sensitization

Studies in Animals

No studies conducted to current OECD guidelines are available. A group of male 24 Guinea pigs receiving 10 0.1 mL injections of 0.1 % synthetic or natural glycerol in isotonic saline every alternate day over 20 days showed no indication of sensitisation following challenge with further 0.05 mL injections of 0.1 % glycerol after a 2 week exposure-free period (Hine 1953). However, it is unclear from the protocol and reporting whether or not the study was capable of detecting sensitisation since it was not apparent whether maximal dose-levels were used, and the use of positive controls or a measure of strain sensitivity were not reported.

Human experience

Data is available from a briefly reported study, in which skin patch tests were conducted on workers in a foam rubber factory. No sensitising effects of a glycerol/water mixture became apparent (El-Nagdy 1973). Considering the extensive, widespread dermal exposure to glycerol in preparations repeatedly applied to the skin, the absence of case reports of humans showing skin reactions is consistent with glycerol having a very low skin sensitisation potential.

Conclusion

Based on the available information, there is no human or animal data that indicates glycerol to be a skin sensitizer. Considering the extensive, widespread dermal exposure to glycerol in preparations repeatedly applied to the skin, the absence of case reports of humans showing skin reactions is consistent with glycerol having a very low skin sensitisation potential.

As taken from OECD SIDS (2002), Glycerol (56-81-5).

Glycerol is not generally considered an irritant of human skin, although some reactions have been observed. Similarly, a small proportion of individuals have been identified as glycerol sensitized (BIBRA, 1993).

"Glycerol has low irritancy and is considered to be a weak sensitizer based on human data (El-Nagdy et al. 1973; Preston and Finch, 2003; Fairhurst and Wilkinson, 2007; Tamagawa-Mineoka et al. 2007)".

As taken from EFSA, 2012.

Slightly irritating after 48 hours application of 0.05 mL on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

In a briefly reported study, in which skin patch tests were conducted on workers in a foam rubber factory. No sensitising effects of a glycerol/water mixture became apparent. [United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of July 14, 2009: <http://www.chem.unep.ch/irptc/sids/OECDsids/sidspub.html> **PEER REVIEWED**

When introduced into the anterior chamber of the rabbit eye, full strength glycerin causes an inflammatory reaction and edema of the cornea with wrinkling of the posterior surface and damage of endothelial cells. [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463] **PEER REVIEWED**

As taken from HSDB, 2012.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Rodent - rabbit	500 mg/24H	Mild	85JCAE "Prehled Prumyslove Toxikologie; Organické Latky," Marhold, J., Prague, Czechoslovakia, Avicenum, 1986 Volume(issue)/page/year: - ,207,1986
Standard Draize test	Administration into the eye	Rodent - rabbit	500 mg/24H	Mild	85JCAE "Prehled Prumyslove Toxikologie; Organické Latky," Marhold, J., Prague, Czechoslovakia, Avicenum, 1986 Volume(issue)/page/year: - ,207,1986

As taken from RTECS, 2019.

"PURPOSE: A previous, unblinded study demonstrated that an alcohol-free barrier film containing an acrylate terpolymer (ATP) was effective in reducing skin reactions compared with a 10% glycerine cream (sorbolene). The different appearances of these products precluded a blinded

comparison. To test the acrylate terpolymer principle in a double-blinded manner required the use of an alternative cream formulation, a moisturizing durable barrier cream (MDBC); the study was conducted by the Trans Tasman Radiation Oncology Group (TROG) as protocol 04.01. METHODS AND MATERIALS: A total of 333 patients were randomized; 1 patient was ineligible and 14 patients withdrew or had less than 7 weeks' observations, leaving 318 for analysis. The chest wall was divided into medial and lateral compartments, and patients were randomized to have MDBC applied daily to the medial or lateral compartment and sorbolene to the other compartment. Weekly observations, photographs, and symptom scores (pain and pruritus) were collected to week 12 or resolution of skin reactions if earlier. Skin dose was confirmed by centrally calibrated thermoluminescent dosimeters. RESULTS: Rates of medial and lateral compartment Common Toxicity Criteria (CTC), version 3, greater than or equal to grade 3 skin reactions were 23% and 41%, but rates by skin care product were identical at 32%. There was no significant difference between MDBC and sorbolene in the primary endpoint of peak skin reactions or secondary endpoints of area-under-the-curve skin reaction scores. CONCLUSIONS: The MDBC did not reduce the peak skin reaction compared to sorbolene. It is possible that this is related to the difference in the formulation of the cream compared with the film formulation. Skin dosimetry verification and double blinding are essential for radiation skin care comparative studies." As taken from Graham PH et al. 2013. *Int. J. Radiat. Oncol. Biol. Phys.* 86(1), 45-50. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23414763>

"Allergic contact dermatitis is a widespread health disorder and occupational skin disease. Hence, screening for contact-sensitizing chemicals is highly relevant to toxicology, dermatology, and occupational medicine. The use of animal tests for this purpose is constrained by ethical considerations, need for high-throughput screening, and legislation (e.g., for cosmetics in the European Union). T cell activation is the final and most specific key event of the "adverse outcome pathway" for skin sensitization and therefore a promising target for the development of in vitro sensitization assays. We present a novel in vitro sensitization assay with a lymphocyte endpoint as an add-on to the loose-fit coculture-based sensitization assay (LCSA): the LCSA-ly. While the LCSA measures dendritic cell activation, the LCSA-ly offers the option for an additional lymphocyte endpoint which can be measured concurrently. We incorporated lymphocytes in our previously established coculture of primary human keratinocytes and monocyte-derived dendritic cells and tested nine substances: five sensitizers [2,4-dinitrochlorobenzene (DNCB) 1.25-15 $\mu\text{mol/l}$, p-phenylenediamine (PPD) 15.6-125 $\mu\text{mol/l}$, 2-mercaptobenzothiazole (MBT) 50-1000 $\mu\text{mol/l}$, coumarin, and resorcinol (both: 250-1500 $\mu\text{mol/l}$)] and four non-sensitizers (monochlorobenzene, caprylic acid, glycerol, and salicylic acid (all: 125-1000 $\mu\text{mol/l}$)). DNCB and MBT increased a subset of IL-23 receptor α /IFN- γ receptor 1 (CD119)+ lymphocytes. DNCB, PPD, and MBT enhanced a subunit of the IL-4 receptor (CD124) and a memory marker (CD44) on lymphocytes. Remarkably, DNCB, PPD, and MBT raised IL-4 concentrations in coculture supernatants while IFN- γ levels decreased, which might point to Th2 activation in vitro. Coumarin, resorcinol, and non-sensitizers did not alter any of the tested surface markers or cytokines. IL-17 was not affected by any of the substances. Relative strength of sensitizers according to lymphocyte markers was DNCB > PPD > MBT, which corresponds to earlier results from the LCSA without lymphocyte endpoint, the murine local lymph node assay, and human data. This study is the first to prove the suitability of lymphocyte surface markers for sensitization testing and potency assessment." As taken from Frombach J et al. 2018. *Arch. Toxicol.* 92(4), 1495-1505. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29380012>

"The study objectives were to demonstrate that glycerol, when topically applied from a roll-on antiperspirant formulation, can be delivered directly to human skin ex vivo and the axillary stratum corneum (SC) in vivo, and to assess whether it improves the quality of the axillary skin barrier. Ex vivo human skin absorption of glycerol was measured following application of a roll-on antiperspirant formulation containing 4% $^{13}\text{C}_3$ -glycerol. Skin distribution of $^{13}\text{C}_3$ -glycerol over 24 h was assessed using gas chromatography-mass spectrometry. In vivo axillary SC penetration was measured by confocal Raman spectroscopy and multivariate curve-resolution software 1 h after

topical application of a roll-on antiperspirant formulation containing 8% deuterated glycerol (d5-glycerol). A clinical study was conducted to determine the efficacy of a roll-on antiperspirant formulation containing 4% glycerol in reducing shaving-induced visual irritation and in increasing axillary-skin hydration. Ex vivo skin absorption studies indicated that the formulation delivered ¹³C₃-glycerol into the SC at all timepoints over the 24-h period. In vivo Raman measurements (1 h after application) demonstrated that d5-glycerol was detectable to a depth of at least 10 µm in the axillary SC. Application of 4% glycerol from a roll-on antiperspirant formulation to the axilla was associated with significantly less visible irritation and greater skin hydration than observed with the control (glycerol-free) product. These studies demonstrate that glycerol, incorporated in a roll-on antiperspirant formulation, is delivered directly and rapidly to all depths of the axillary SC, and results in improvements in visible irritation and hydration in the axilla.” As taken from Evans RL et al. 2017. Arch. Dermatol. Res. 309(9), 739-748. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28889318>

“A thorough understanding of which of the effects assessed in the in vivo Draize eye test are responsible for driving UN GHS/EU CLP classification is critical for an adequate selection of chemicals to be used in the development and/or evaluation of alternative methods/strategies and for properly assessing their predictive capacity and limitations. For this reason, Cosmetics Europe has compiled a database of Draize data (Draize eye test Reference Database, DRD) from external lists that were created to support past validation activities. This database contains 681 independent in vivo studies on 634 individual chemicals representing a wide range of chemical classes. A description of all the ocular effects observed in vivo, i.e. degree of severity and persistence of corneal opacity (CO), iritis, and/or conjunctiva effects, was added for each individual study in the database, and the studies were categorised according to their UN GHS/EU CLP classification and the main effect driving the classification. An evaluation of the various in vivo drivers of classification compiled in the database was performed to establish which of these are most important from a regulatory point of view. These analyses established that the most important drivers for Cat 1 Classification are (1) CO mean ≥ 3 (days 1-3) (severity) and (2) CO persistence on day 21 in the absence of severity, and those for Cat 2 classification are (3) CO mean ≥ 1 and (4) conjunctival redness mean ≥ 2 . Moreover, it is shown that all classifiable effects (including persistence and CO = 4) should be present in ≥ 60 % of the animals to drive a classification. As a consequence, our analyses suggest the need for a critical revision of the UN GHS/EU CLP decision criteria for the Cat 1 classification of chemicals. Finally, a number of key criteria are identified that should be taken into consideration when selecting reference chemicals for the development, evaluation and/or validation of alternative methods and/or strategies for serious eye damage/eye irritation testing. Most important, the DRD is an invaluable tool for any future activity involving the selection of reference chemicals.” As taken from Barroso J et al. 2017. Arch. Toxicol. 91(2), 521-547. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/26997338>

In a validation study of the IL-8 Luc assay to detect skin sensitization effects of a number of compounds, coordinated by the Japanese Center for the Validation of Alternative Methods (JaCVAM), glycerol was described as non-sensitizing (OECD, 2017).

“PURPOSE OF REVIEW: Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma. RECENT FINDINGS: Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-

asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms. Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers.” As taken from Clapp PW & Jaspers I. 2017. *Curr. Allergy Asthma Rep.* 17(11), 79. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28983782>

“Background: An essential step in ensuring the toxicological safety of cosmetic or personal care products is the evaluation of the skin sensitizing potential of product ingredients. Objective: We used a standardized protocol from cosmetic trade industry and consumer safety groups to evaluate the sensitization potential of ingredients in 3 commercially available cleansing conditioners. Methods: A total of 33 ingredients were evaluated. Each ingredient underwent (1) dermatological evaluation, (2) in silico analysis for irritation and sensitization potential, and (3) a literature evaluation to determine risk of sensitization. Consumer exposure level was compared with the weight-of-evidence no-expected sensitization induction level for the constituent. If a no-expected sensitization induction level for a specific ingredient was not available, the dermal sensitization threshold approach was used. A margin of safety was calculated for each constituent. Results: The margins of safety for all evaluated ingredients in the cleansing conditioners were greater than 1. Conclusions: This analysis indicates that exposure to the individual ingredients present in these cleansing conditioners would not be expected to induce dermal sensitization in a consumer under the examined exposure scenario.” As taken from Monnot AD et al. 2019. *Dermatitis* 30(2), 116-128. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30829810/>

5.8. All other relevant types of toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to oral product (snus/nicotine pouch). Non-clinical in vitro studies were conducted to investigate the characteristics of extracts from tobacco free nicotine pouches alongside a reference snus product containing glycerol and/or 1R6F reference cigarette. Within the sensitivity and specificity of the bioassay(s) all smokeless products extracts were non-mutagenic, non-genotoxic, non-cytotoxic and non-irritant in contrast to results obtained for the 1R6F reference cigarette (Miller-Holt et al., 2022). Table below provides highest tested level(s) and specific endpoint(s).

Product type	Endpoint	Tested level (ppm)	Reference
Nicotine Pouch	In vitro genotoxicity	42000	Labstat International Inc. (2020)
	In vitro cytotoxicity	42000	Labstat International Inc. (2020)
	In vitro irritation (EpiGingival™ 3D)	42000	Charles River Laboratories (2022)

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing glycerol was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the

bioassay(s) the activity of the TPM was not increased by the addition of glycerol when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	52000	JTI KB Study Report(s)
In vitro cytotoxicity	52000	JTI KB Study Report(s)

“Marketed since 2004 as an alternative to nicotine delivery and advertised as a valid means to smoking cessation, the electronic (e)-cigarette has been the subject of much controversy but very little experimental study. This review provides a brief summary of the current knowledge of this product. Propylene glycol and glycerol, the main ingredients of the fluid that is vaporized, have proved to be harmless in the fog machines of the entertainment industry. However, in the case of the e-cigarette fluid, the composition is not properly labeled: additives like nicotine and flavors vary between and within brands and contamination with various chemicals has been detected. The short-term toxicity seems low, but the long-term toxicity is unknown. The usefulness of the e-cigarette in smoking cessation has still to be clinically established.” As taken from Bertholon JF et al. 2013. Respiration 86(5), 433-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24080743>

“Propylene glycol and glycerine have been shown to be harmless when used orally. However, when inhaled, as in the case of ECs [electronic cigarettes], their safety has not been clearly demonstrated.” As taken from Jimenez Ruiz CA et al. 2014. Archivos de Bronconeumología 50(8), 362. Full paper available at http://apps.elsevier.es/watermark/ctl_servlet? f=10&pidet_articulo=90337413&pidet_usuario=0&pcontactid=&pidet_revista=260&ty=165&accion=L&origen=bronco&web=www.archbronconeumol.org&lan=en&fichero=260v50n08a90337413pdf001.pdf

“....This is a review of published data on the human health effects of exposure to e-cigarettes and their components....Literature searches were conducted through September 2013 using multiple electronic databases....Forty-four articles are included in this analysis. E-cigarette aerosols may contain propylene glycol, glycerol, flavourings, other chemicals and, usually, nicotine. Aerosolised propylene glycol and glycerol produce mouth and throat irritation and dry cough....” As taken from Callahan-Lyon P et al. 2014. Tob. Control 23(Suppl. 2), ii36-40. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24732161>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - liver tumor	983 mmol/L/24H	In Vitro Toxicity Studies - cell protein synthesis	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 3,189,1989
IC50 - Inhibitor Concentration 50	In vitro	Human - other normal tissue	55.2 pph/60M	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 26,150,2012
IC20	In vitro	Human - monocyte	10 mmol/L/2H	In Vitro Toxicity Studies -	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000

				other assays	AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 199,51,2010
ICLo - Inhibitor Concentration Low	In vitro	Rat - brain(cerebrum)	267 mmol/L/44H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 27,1416,2013
ICLo - Inhibitor Concentration Low	In vitro	Human - leukemia cells	25000 mg/L/30M	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 27,857,2013
ICLo - Inhibitor Concentration Low	In vitro	Human - colon tumor	40 umol/L/24H	In Vitro Toxicity Studies - cell morphology: overgrowth of cell appendixes etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
ICLo - Inhibitor Concentration Low	In vitro	Human - gastrointestinal tumor	40 umol/L/24H	In Vitro Toxicity Studies - cell morphology: overgrowth of cell appendixes etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 74,311,2014
ICLo - Inhibitor Concentration Low	In vitro	Human - skin	0.01 pph/6H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 27,2213,2013
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,688,2015
IC30 - Inhibitor Concentration	In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies -	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House,

30				cell metabolic activity: Alamar Blue assay etc.	Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,688,2015
IC50 Inhibitor Concentration 50	- In vitro	Human lymphocyte	- >200 mg/L/45H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015

As taken from RTECS, 2019.

No irritation was apparently seen when undiluted glycerol was applied to the lining of the oral cavities of rats, rabbits and dogs (Informatics Inc, 1973, as referred to by BIBRA, 1993 [Documentation provided to EFSA n. 2]). As taken from EFSA, 2017.

“....chronically high levels of glycerol in the blood are associated with glycerol kinase deficiency (GKD). GKD causes the condition known as hyperglycerolemia, an accumulation of glycerol in the blood and urine. There are three clinically distinct forms of GKD: infantile, juvenile, and adult.”

“Patients with the adult form of GKD generally have no symptoms and are often detected fortuitously.”

As taken from the Human Metabolome Database, 2020.

Name	Route of Administration	Threshold	Information for the Package Leaflet
Glycerol (E422)	Oral	10 g per dose	May cause headache, stomach upset and diarrhea
Glycerol (E422)	Rectal	1 g	May have a mild laxative effect

As taken from EMA, 2017.

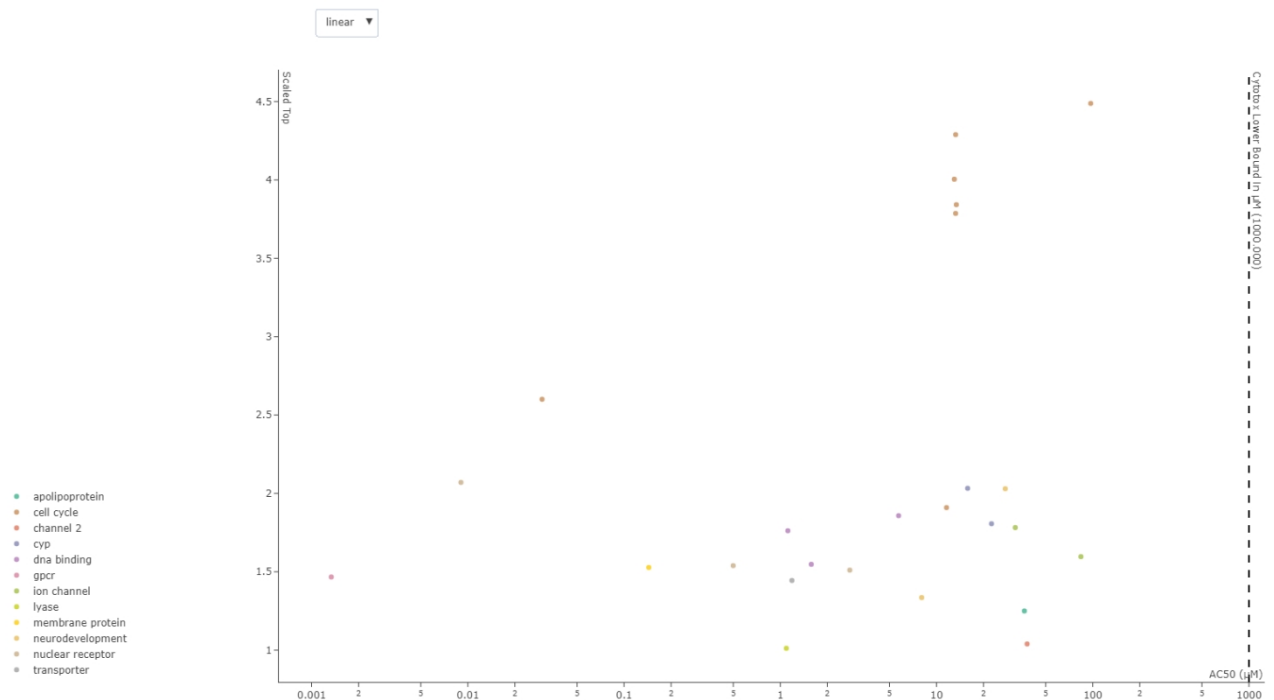
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated glycerol in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on glycerol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



“Background and aims: Monoamine oxidase inhibitors have been hypothesised to be important in tobacco dependence, reinforcing the brain's response to nicotine by delaying the degradation of neurotransmitters by monoamine oxidases. The development of electronic cigarettes has provided an alternative nicotine delivery system, which is widely viewed as less toxic than tobacco smoke. However, significant data gaps remain. This paper reports the results of measurements of monoamine oxidase inhibitory activity in a small sample of commercially available, flavoured e-liquids. Methods: Twelve e-liquids were tested for monoamine oxidase inhibitory activity, using the kynuramine assay and monoamine oxidase enzymes (human, recombinant). Control samples of carrier liquids, propylene glycol and glycerol, and nicotine were also tested. Results: Four e-liquids contained high levels of inhibitory activity, four more were moderately inhibitory. The remaining four e-liquids were mildly inhibitory, while the carrier liquids, and nicotine were inactive at relevant concentrations. The active compounds in the e-liquids were subsequently identified as vanillin and ethyl vanillin. Under some conditions of use, the sampled e-liquids with the highest concentrations of monoamine oxidase inhibitory activity have the potential to expose consumers to physiologically significant levels of MAO inhibitory activity. Conclusions: While only a small sample of e-liquids was tested, the findings suggest that some flavours have pharmacological actions, with potential to enhance the response to nicotine or to other drugs. The public health implications of these preliminary findings on addiction and smoking cessation warrant exploration and further research.” As taken from Truman P et al. 2019. *Neurotoxicology* 75, 123-128. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31536738/>

“In late 2019, hundreds of users of electronic products that aerosolize a liquid for inhalation were hospitalized with a variety of respiratory and gastrointestinal symptoms. While some investigations have attributed the disease to the presence of vitamin E acetate in liquids that also contained tetrahydrocannabinol, some evidence suggests that chronic inhalation of two common solvents used in electronic nicotine delivery systems (ENDS), propylene glycol (PG) and vegetable glycerin (VG), can interfere with the lipid components of pulmonary surfactant and cause or exacerbate pulmonary injury. The interaction between PG, VG, and lung surfactant is not yet understood. This study presents an examination of the molecular interactions of PG and VG with lung surfactant mimicked by 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). The interaction of DPPC and PG-VG is studied by attenuated total reflectance fourier transform infrared spectroscopy. The results showed that PG and VG altered the molecular alignment of the DPPC surfactant. The

orientation of the surfactant at the surface of the lung affects the surface tension at the air-water interface, thereby influencing breathing. These findings suggest that chronic aerosolization of the primary solvents in ENDS might alter the function of pulmonary surfactant.” As taken from Hayeck N et al. 2021. Chem. Res. Toxicol. 34(6), 1572-1577. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33945261/>

6. Functional effects on

6.1. Broncho/pulmonary system

A 73-year-old man was treated with oral glycerol solution for elevated intraocular pressure. Forty-five minutes later, he developed severe pulmonary edema. ... [Almog Y et al; Ann Ophthalmol 18 (1): 38-9 (1986)] **PEER REVIEWED**.

As taken from HSDB, 2012.

“PURPOSE OF REVIEW: Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma. RECENT FINDINGS: Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms. Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers.” As taken from Clapp PW & Jaspers I. 2017. Curr. Allergy Asthma Rep. 17(11), 79. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28983782>

Glycerol is listed as a respiratory toxicant on the FDA's list of chemicals identified as harmful or potentially harmful constituents in tobacco products and tobacco smoke (FDA, 2019).

6.2. Cardiovascular system

The route of administration is of influence on the toxicity of glycerol in humans. Toxic effects, apart from nausea and vomiting, do not occur after oral administration. Toxic effects reported after intraperitoneal and subcutaneous administration are albuminuria, hemoglobinuria, anemia and

renal damage. Glycerol has a dehydration effect on the central nervous system. Intraocular pressure begins to fall at plasma concentrations of 10 mmol per liter. Concentration and dilutant used are also of influence on toxicity. Use of saline as dilutant diminishes the toxic effects of glycerol. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

Severe dehydration, cardiac arrhythmias, and hyperosmolar nonketotic coma have been reported and may be fatal. [US Pharmacopeial Convention; US Pharmacopeia Dispensing Information (USP DI); Drug Information for the Health Care Professional 12th ed, V.I p.1456 (1992)] **PEER REVIEWED**

Side/adverse effects indicating need for medical attention: incidence less frequent - Confusion; incidence rare: irregular heartbeat. [US Pharmacopeial Convention; US Pharmacopeia Dispensing Information (USP DI); Drug Information for the Health Care Professional 12th ed, V.I p.1456 (1992)] **PEER REVIEWED**

As taken from HSDB, 2012.

"Glycerin elevates the blood plasma osmolality thereby extracting water from tissues into interstitial fluid and plasma."

As taken from PubChem.

"VG-only aerosols induced the most profound bradycardia, bradyarrhythmias (high-grade SVBs), and repolarization changes." As taken from Nat Commun. 2022. Available at: <https://pubmed.ncbi.nlm.nih.gov/36284091/>

6.3. Nervous system

Osmotic effect of glycerin may also produce tissue dehydration & decr in cerebrospinal fluid pressure. glycerin produces only very slight diuresis in healthy individuals receiving single dose of 1.5 g/kg or less. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773] **PEER REVIEWED**

Adverse effects following oral administration of glycerin include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea. [McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1774] **PEER REVIEWED**

A 46 year old male inadvertently consumed 500 mL of glycerol and presented with altered sensorium, focal neurologic signs and generalised seizures. He was managed conservatively and recovered fully within 48 hours. The case highlights the rare presentations of overdose and neurologic effects with glycerol, an otherwise safe drug used in neurology. [Singh R et al; Neurol India 49 (3): 320-1 (2001)] **PEER REVIEWED**

"CASE REPORTS/ /The authors/ report on a 72-year-old male who was referred to the Department of Neurology with progressive neurological symptoms that had developed 4 hr prior to admission. Temporally associated was the so-called glycerol test or Klockhoff test, which was performed for the diagnosis of suspected Meniere's disease. The test procedure starts with oral administration of glycerol, the maximal dose should not exceed 1.5 g/kg of body weight. Because of an apparently pathologically highly elevated serum concentration of triglycerides (3,465 mg/dL) measured 10 hr after glycerol administration, the suspicion of an overdose of glycerol rose. During the following day, the glycerol serum concentration was analyzed at three different times. Based on these measurements, /investigators/ determined pharmacokinetic parameters and estimated the initially ingested amount of glycerol of about 3.88-3.95 g/kg body weight. /The authors/ conclude that an accidental overdose of glycerol must have occurred during the glycerol test to the patient"

[Andresen H et al; Clin Toxicol (Phila) 47 (4): 312-6 (2009)] **PEER REVIEWED** PubMed Abstract.

As taken from HSDB, 2012.

"In a case report, a 46 years old male consumed about 450 - 500 grams of glycerol. The exposure caused neurologic symptoms and signs within 6 – 12 hours after consumption. Complete recovery was achieved in 48 hours. The major complications when serum osmolality of glycerol exceeds 310 – 320 mosm/Kg were hyperosmolality, hypernatraemia, neuronal injury, rebound oedema, metabolic injury, and renal failure (Singh et al., 2001. Ks: 2)". As taken from Bassan et al. 2012.

"Background: Cerebellar hypoplasia is a common problem in preterm infants and infants suffering from intraventricular hemorrhage (IVH). To evaluate the effects of IVH on cerebellar growth and development, we used a neonatal rabbit model of systemic glycerol to produce IVH. Methods: New Zealand White rabbit kits were surgically delivered 2 d preterm and treated with intraperitoneal glycerol (3.25-6.5 g/kg). Controls were born at term. IVH was documented by ultrasonography. Brain volumes determined by magnetic resonance imaging, cerebellar foliation, proliferation (Ki-67), and Purkinje cell density were assessed at 2 wk of life. Tissue glycerol and glutathione concentrations were measured. Results: Glycerol increased IVH, subarachnoid hemorrhages, and mortality in a dose-dependent manner. Total cerebellar volumes, cerebellar foliation, and cerebellar proliferation were decreased in a dose-dependent manner. Glycerol accumulated rapidly in blood, brain, and liver and was associated with increased glutathione concentration. All of these results were independent of IVH status. Conclusion: Cerebellar hypoplasia was induced after glycerol administration in a dose-dependent manner. Given the rapid tissue accumulation of glycerol, dose-dependent decrease in brain growth, and lack of IVH effect on measured outcomes, we question the validity of this model because glycerol toxicity cannot be ruled out. A better physiological model of IVH is needed." As taken from Traudt CM et al. 2013. *Pediatr. Res.* 75(3), 389-94. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24346111>

"Glossopharyngeal neuralgia is much less common in children and more difficult to relief its pain symptoms than the adults. We report an experience with peripheral glycerol injection for the control of pain in eight sick children with glossopharyngeal neuralgia. At the latest follow-up, 5 cases had a complete pain-free result after the treatment. There were two sick children who were recurred within three months, of which one child was respond to additional injections. It is concluded that the peripheral glycerol injection is safe and effective in the control of pain symptom among the children with glossopharyngeal neuralgia." As taken from Yue WL & Zhang Y. 2014. *Int. J. Pediatr. Otorhinolaryngol.* 78(3), 558-60. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24418185>

6.4. Other organ systems, dependent on the properties of the substance

The route of administration is of influence on the toxicity of glycerol in humans. Toxic effects, apart from nausea and vomiting, do not occur after oral administration. Toxic effects reported after intraperitoneal and subcutaneous administration are albuminuria, hemoglobinuria, anemia and renal damage. Glycerol has a dehydration effect on the central nervous system. Intraocular pressure begins to fall at plasma concentrations of 10 mmoles per liter. Concentration and dilutant used are also of influence on toxicity. Use of saline as dilutant diminishes the toxic effects of glycerol. [European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

Adverse effects following oral administration of glycerin include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea. [McEvoy, G.K. (ed.). *American Hospital Formulary Service - Drug Information* 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1774] **PEER REVIEWED**

Side/adverse effects indicating need for medical attention only if they continue or are bothersome: incidence more frequent - headache, nausea or vomiting; incidence less frequent, diarrhea, dizziness, dry mouth or increased thirst. [US Pharmacopeial Convention; US Pharmacopeia Dispensing Information (USP DI); Drug Information for the Health Care Professional 12th ed, V.I p.1456 (1992)] **PEER REVIEWED**

In human eyes, specular microscopy has shown that repeated application of 100% glycerin to the surface of the eye causes extensive changes in the appearance of the endothelium, but most of these changes disappear within 90 min after exposure is ended. [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463] **PEER REVIEWED**

As taken from HSDB, 2012.

"Acute kidney injury (AKI) is a common and potentially life-threatening complication after ischemia/reperfusion and exposure to nephrotoxic agents. In this study, we examined the efficacy and mechanism(s) of suramin in promoting recovery from glycerol-induced AKI, a model of rhabdomyolysis-induced AKI. After intramuscular glycerol injection (10 ml of 50% glycerol per kilogram) into male Sprague-Dawley rats, serum creatinine maximally increased at 24 to 72 h and then decreased at 120 h. Creatinine clearance (CrCl) decreased 75% at 24 to 72 h and increased at 120 h. Suramin (1 mg/kg i.v.) administered 24 h after glycerol accelerated recovery of renal function as demonstrated by increased CrCl, decreased renal kidney injury molecule-1, and improved histopathology 72 h after glycerol injection. Suramin treatment decreased interleukin-1 β (IL-1 β) mRNA, transforming growth factor- β (1) (TGF- β (1)), phospho-p65 of nuclear factor- κ B (NF- κ B), and cleaved caspase-3 at 48 h compared with glycerol alone. Suramin treatment also decreased glycerol-induced activation of intracellular adhesion molecule-1 (ICAM-1) and leukocyte infiltration at 72 h. Urinary/renal neutrophil gelatinase-associated lipocalin 2 (NGAL) levels, hemoxygenase-1 expression, and renal cell proliferation were increased by suramin compared with glycerol alone at 72 h. Mechanistically, suramin decreases early glycerol-induced proinflammatory (IL-1 β and NF- κ B) and growth inhibitory (TGF- β (1)) mediators, resulting in the prevention of late downstream inflammatory effects (ICAM-1 and leukocyte infiltration) and increasing compensatory nephrogenic repair. These results support the hypothesis that delayed administration of suramin is effective in abrogating apoptosis, attenuating inflammation, and enhancing nephrogenic repair after glycerol-induced AKI". As taken from Korrapati MC et al. 2012. J. Pharmacol. Expt. Therap. 341, 126-136. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22228809>.

"OBJECTIVE: To evaluate and compare the effects of oral isosorbide and glycerol on intraocular pressure (IOP), serum osmolality (SOSM), and blood glucose (BG) in normal dogs. METHODS: Ten normal dogs were administered an oral dose of either isosorbide (1.5 g/kg), glycerol (1.5g/kg) or control (water, 2 mL/kg) in a double blind protocol. Prior to dosing, baseline IOP, SOSM, and BG were measured in all dogs. IOP was subsequently evaluated every 30 min for 6h post-dosing. BG and SOSM were reassessed at times 1, 2, 4, and 6h post-dosing. After 1-week washout periods, every dog was subject to each of the three treatments. The dogs were held NPO for 4h after dosing. RESULTS: The maximal decrease in IOP was 17% by 1h and 13.5% by 30min after glycerol and isosorbide administration, respectively. However, the overall changes in IOP were not significant when compared to the controls. SOSM increased above baseline after dosing with glycerol but decreased after isosorbide, which difference was significant at 1, 2, and 4 h post-administration. BG significantly increased after administration of glycerol relative to the control but was not significantly affected by isosorbide. CONCLUSIONS: Neither glycerol nor isosorbide significantly affected IOP when compared to the control. However, glycerol induced significant elevations in both BG relative to the control and SOSM relative to isosorbide." As taken from Wasserman NT et al. 2013. Vet. Ophthalmol. 16(1), 20-4. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22512809?dopt=AbstractPlus>

"A replicated 3×3 Latin square study was conducted to evaluate the effects of replacing a portion of ground corn in the diet with dietary glycerol on rumen environment, blood metabolites, and nutrient digestibility. Six rumen-cannulated Holstein cows, averaging 56±18 DIM and 38.0±8.2 kg of milk/d, were used in the study. Experimental periods included 3 wk for treatment adjustment period followed by 1 wk for data collection. Diets were corn silage based and balanced to be isocaloric and isonitrogenous. Treatments were 0 g of glycerol/d (control, CON), 200 g of glycerol/d (G2), and 400 g of glycerol/d (G4). Dry matter intake (DMI) decreased as the amount of glycerol fed increased. Milk yield was higher for CON and G2 cows in comparison with G4 cows. Milk fat percentage and yield were reduced when glycerol was fed compared with CON cows but increased the milk protein percentage at the highest concentration of dietary glycerol. These changes resulted in decreased energy-corrected milk yield and efficiency (milk/DMI) in diets supplemented with G4 compared with CON. No differences were observed in ruminal pH and ammonia concentrations. Molar proportions of acetate, valerate, and acetate:propionate ratio decreased, whereas propionate increased as the amount glycerol fed increased. Molar proportions of butyrate were greatest when glycerol was included in the diet compared with CON. Nutrient intake and digestion were not different among treatments. Results of this trial suggest that feeding increasing amounts of glycerol may decrease DMI and alters ruminal fermentation, resulting in reduced yield of milk, fat, and energy-corrected milk." As taken from Boyd J et al. 2013. J. Dairy Sci. 96(1), 470-6. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23182356?dopt=AbstractPlus>

"Glycerin has been administered orally and/or intravenously to reduce intracranial pressure caused by various medical conditions. Glycerin has been used to reduce brain volume for neurosurgical procedures. It is also used as the active ingredient in laxative products (ie, glycerin suppositories)."

As taken from CIR, 2015.

"The purpose of this study was to investigate the protective effects of sulfated chitosan of C3 sulfation (TCTS) on the glycerol-induced acute renal failure. Compared with the normal group, rats from model group exhibited collecting duct and medullary ascending limb dilation and casts by glycerol treating. TCTS, which was injected to pretreat rats by glycerol, exerted a protective effect. The results showed that serum creatinine and blood urea nitrogen were markedly increased in glycerol-treated rats. It is proved that TCTS reduced their levels significantly. Ions level in plasma and urine were significantly changed in glycerol-treated rats, whereas TCTS almost recovered their levels back to normal. For female rats, administration of TCTS reduced their mortality. This study showed a noticeable renal morphologic and functional protection by TCTS in glycerol-induced acute renal failure." As taken from Xing R et al. 2014. Int. J. Biol. Macromol. 65, 383-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24508919>

"We evaluated training adaptation and physical performance parameters in rats orally supplemented with glycerol, glucose, or saline, and submitted to moderate aerobic exercise. Thirty male rats were trained for 6 weeks and administered the supplements during the last 4 weeks of the experiment. Animals were distributed in a completely randomized factorial 2 × 3 design (with or without exercise and 3 substrates). Data were subjected to analysis of variance (ANOVA) and means were compared using the Student-Newmann-Keuls test at 5%. Among the trained animals, none of the substances caused differences in the percentages of protein, fat, or water content in the carcass. Compared with the sedentary animals, the trained animals supplemented with saline and glucose showed a higher protein percentage in the carcass. The relative mass of the heart and adrenal glands was higher in the trained animals. Glycerol improved the protein content in non-trained animals and increased the relative adrenal mass in both groups. Glycerol reduced the variation in levels of lactate and aspartate aminotransferase (AST) during the last exercise session. There was no difference between groups regarding the relative mass of the thymus and gastrocnemius or with the diameter of muscle fibers or the neutrophil-lymphocyte ratio. Supplementation with glycerol was efficient at attenuating variation in AST and lactate levels during exercise." As taken from Andrade EF et al. 2015. Can. J. Physiol. Pharmacol. 93, 63-9. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25474597>

"The objective of this study was to evaluate the effect of three sources of glycerine (crude glycerine from soya bean oil - CGSO, mixed crude glycerine from frying oil and lard--MCG, and a semipurified glycerine from soya bean oil--SPGSO) in four concentrations in the diet (17.5, 35.0, 52.5 and 70.0 g of each type of glycerine per kg of feed) on the levels of plasmatic glycerol and the activity of the hepatic enzyme glycerol kinase in broilers of 22-35 days old (experiment I) and 33-42 days old (experiment II)....The source of glycerine influenced the glycerol kinase activity only in experiment II, where the use of CGSO in the diet increased the enzyme activity. For both experiments, the glycerol kinase activity increased with the inclusion of glycerine in the diet. In conclusion, based on the absence of saturation of the glycerol kinase activity for the three glycerine sources and for both rearing periods evaluated, the broilers can metabolize the glycerol (at the level of the phosphorylation of the glycerol to glycerol-3-phosphate in the liver) present in the glycerine when the diet is supplemented with up to 70 g of glycerine per kg of feed." As taken from Bernardino VM et al. 2014. J. Anim. Physiol. Anim. Nutr. (Berl.) 98(2), 328-37. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23692650>

"....Glycerol injection into muscle is known to induce myopathy, and glycerol exposure promotes detachment of transverse tubules from the sarcoplasmic reticulum...." As taken from Demonbreun AR et al. 2014. Am. J. Pathol. 184(1), 248-59. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24177035>

"The use of glycerol in the diets for animals is of interest because it is a residue of biodiesel production and rich in energy. Thus, this study aimed to evaluate metabolic and physiological parameters of rats receiving supplemental pure glycerol by gavage. We used 30 Wistar rats (initial weight 202.7 ± 29.98 g) receiving 0 (control/saline), 200, 400, 800 and 1600 mg glycerol/kg of body weight (bidistilled glycerine, 99.85% glycerol) beside food and water ad libitum for 28 days. We used a completely randomised design with five treatments and six replicates. At the end of the experiment, the animals were killed, and the results showed that there was no change ($p > 0.05$) in the intake and excretion of water, the average daily weight gain, dry matter, ash and crude protein in the carcass or plasma triacylglycerols. There was a beneficial effect ($p < 0.05$) up to a dose of 800 mg/kg glycerol on feed intake, percentage of carcass fat, plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDLc) and low-/very low-density lipoprotein (LDLc + VLDLc). The levels of total cholesterol and glucose were increased with up to a dose of 800 mg/kg glycerol (but remained within the normal range); they were reduced with the dose of 1600 mg/kg. The total leucocyte count tended to be reduced, although it was within the reference values for rats. There were no renal or pancreatic lesions. In conclusion, glycerol presented as a safe supplement at the studied doses, even having some beneficial effects in a dose-dependent manner in rats." As taken from Lisenko KG et al. 2015. J. Anim. Physiol. Anim. Nutr. (Berl.) 99, 265-72. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24996054>

"BACKGROUND: Rhabdomyolysis (RM)-induced acute renal failure (ARF) accounts for about 10-40% of all cases of ARF. AIM: The present study investigated the possible protective effect of two nitric oxides (NO)-releasing third generation β -blockers, carvedilol (Carv) and nebivolol (Nebi), against RM-mimicking glycerol (Gly)-induced ARF in rats. MATERIAL AND METHODS: After 24 h dehydration, rats received a single dose of 50% Gly (8 ml/kg, im). They were treated with vehicle, Carv (2.5 mg/kg/day, po) or Nebi (10 mg/kg, po) for 3 successive days starting from an hour prior to Gly injection. Evaluation of blood pressure and locomotor activity was performed during the experiment. 72 h following Gly administration, total protein in the urine, serum levels of creatinine, blood urea nitrogen, sodium and potassium as well as the renal contents of malondialdehyde, reduced glutathione and NO were assessed, together with a histopathological examination of renal tissues. RESULTS: Carv and Nebi attenuated Gly-induced renal dysfunction and histopathological alterations. They decreased the Gly-induced oxidative stress and increased renal NO concentration. Restoration of normal blood pressure and improvement of locomotor activity were also observed. CONCLUSION: The results clearly demonstrate protective effects of Carv and Nebi against renal damage involved in RM-induced ARF and suggest a role of their antioxidant and NO-

releasing properties.” As taken from Atwa A et al. 2016. Open Access Maced. J. Med. Sci. 4(3), 329-336. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27703551>

“OBJECTIVE: Contrast media (CM) are a major cause of nephropathy in high-risk patients. The aim of this study was to examine the effects of carnitine (CAR) in advanced nephrotoxicity due to CM administration in rats with glycerol-induced renal functional disorder. METHODS: 40 rats were divided randomly into five groups (n = 8): (1) healthy group; (2) glycerol only (GLY); (3) glycerol and CM (GLY + CM); (4) glycerol, CM and 200 mg kg⁻¹ carnitine (CAR200, Carnitene®; Sigma-tau/Santa Farma, Istanbul, Turkey); and (5) glycerol, CM and 400 mg kg⁻¹ carnitine (CAR400). Kidney injury was induced with a single-dose, intramuscular injection of 10 ml kg⁻¹ body weight (b.w.) of GLY. CAR was administered intraperitoneally. CM (8 ml kg⁻¹ b.w. iohexol, Omnipaque™; Opakim Medical Products, Istanbul, Turkey) was infused via the tail vein to the rats in Groups 3-5. RESULTS: L-carnitine administration significantly decreased serum creatinine and blood urea nitrogen levels. Superoxide dismutase and glutathione activity increased significantly in the treatment groups compared with the nephrotoxic groups. CAR400 significantly reduced malondialdehyde levels to healthy levels. In the treatment groups, tumour necrosis factor (TNF)-α, transforming growth factor 1β, interleukin 1β and caspase-3 gene expression decreased compared with the nephrotoxic groups. TNF-α and nuclear factor kappa-beta (NF-κB) protein expression increased after CM and CAR administration reduced both TNF-α and NF-κB expressions. Histopathologically, hyaline and haemorrhagic casts and necrosis in proximal tubules increased in the nephrotoxicity groups and decreased in the CAR groups. CONCLUSION: The results reveal that L-carnitine protects the oxidant/antioxidant balance and decreases proinflammatory cytokines and apoptosis in CM-induced nephrotoxicity in rats with underlying pathology. ADVANCES IN KNOWLEDGE: Depending on the underlying kidney pathologies, the incidence of CM-induced nephropathy (CIN) increases. Therefore, this is the best model to represent clinically observed CIN.” As taken from Kunak CS et al. 2016. Br. J. Radiol. 89(1058), 20140724. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26562095>

“Contrast media (CM) is known to have nephrotoxic adverse effects. Epigallocatechin-3-gallate (EGCG) is the most abundant and active catechin in green tea, and has strong antioxidant and anti-inflammatory properties. This study investigated whether EGCG can reduce contrast-induced nephrotoxicity (CIN), alone or with glycerol (GLY)-induced renal damage, and to understand its mechanisms of protection against toxicity, using models of GLY and CIN in rats. The rats were separated into eight groups (n = 6 in each), as follows: Healthy, GLY, CM, GLY + CM, CM + EGCG 50 mg/kg (po), GLY + CM + EGCG 50 mg/kg (po), CM + EGCG 100 mg/kg (po), and GLY + CM + EGCG 100 mg/kg (po). Both doses of EGCG protected against CM-induced renal dysfunction, as measured by serum creatinine and blood urea nitrogen (BUN). In addition, EGCG treatment markedly improved CIN-induced oxidative stress, and resulted in a significant down-regulatory effect on tumor necrosis factor (TNF)-α and nuclear factor (NF)-κB mRNA expression. Moreover, histopathological analysis showed that EGCG also attenuated CM-induced kidney damage. Considering the potential clinical use of CM and the numerous health benefits of EGCG, this study showed the protective role of multi-dose EGCG treatment on CIN and GLY-aggravated CIN through different mechanisms.” As taken from Palabiyik SS et al. 2017. Ren. Fail. 39(1), 314-322. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28100100>

“Glycerin is a trihydroxyalcohol with localized osmotic diuretic and laxative effects. Glycerin elevates the blood plasma osmolality thereby extracting water from tissues into interstitial fluid and plasma. This agent also prevents water reabsorption in the proximal tubule in the kidney leading to an increase in water and sodium excretion and a reduction in blood volume. Administered rectally, glycerin exerts a hyperosmotic laxative effect by attracting water into the rectum, thereby relieving constipation.”

As taken from PubChem.

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. *Burnt ingredient toxicity*

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing glycerol and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of glycerol. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	42,048	Carmines, 2002 & Rustemeier et al., 2002
	150,000	Carmines E.L. & Gaworski C.L.; 2005
	21,400	Baker et al., 2004a
	43,000	JTI KB Study Report(s)
	59740	Stabbert et al., 2019
In vitro genotoxicity	42,048	Carmines, 2002 & Röemer et al., 2002
	70,000	Baker et al., 2004c
	150,000	Carmines E.L. & Gaworski C.L.; 2005
	69,800	fGLH Study Report (2010)
	59740	Stabbert et al., 2019
In vitro cytotoxicity	42,048	Carmines, 2002 & Röemer et al., 2002
	150,000	Carmines E.L. & Gaworski C.L.; 2005
	70,000	Baker et al., 2004c
	69,800	fGLH Study Report (2010)
	59740	Stabbert et al., 2019
Inhalation study	11	Gaworski et al., 1998
	150,000	Carmines E.L. & Gaworski C.L.; 2005
	42,048	Carmines, 2002 & Vanscheeuwijck et al., 2002
Skin painting	70,000	Baker et al., 2004c
	24,000	Gaworski et al., 1999

Transfer studies:

In a pyrolysis study, 100% of glycerol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

Toxicological evaluation of an electrically heated cigarette. Part 4: Subchronic inhalation toxicology (Abstract). The biological activity of mainstream smoke from an electrically heated cigarette (EHC) with controlled combustion and from the University of Kentucky Reference Cigarette 1R4F was determined in Sprague Dawley rats exposed nose-only for 90 days, 6 h a day, 7 days per week. For an equivalent response comparison between the two cigarette types, two doses were chosen for the EHC where the anticipated results were in the dynamic range of the 1R4F dose-response curve (four concentrations) for most end points. The number of cigarettes smoked per m(3) of diluted smoke resulted in total particulate matter concentrations of 40 and 90

microg I (-1) for the EHC and 40-170 microg I (-1) for the 1R4F. Biomonitoring indicated achievement of target doses. Mainstream smoke yields were lower for the EHC, with the exception of formaldehyde. No smoke-related mortality, remarkable in-life observations or abnormal gross pathological findings were observed. Smoke- and dose- related clinical pathology and organ weight changes included: increases in segmented neutrophils, some liver parameters and lung and adrenal weight relative to body weight; and decreases in lymphocytes, glucose concentration and spleen weight. Smoke-related histopathological findings in the respiratory tract included epithelial cell hyperplasia, squamous metaplasia, atrophy and accumulation of pigmented alveolar macrophages; they were mostly dose-dependent, more pronounced in the upper than lower respiratory tract and completely or partially reversed by 6 weeks post-inhalation. Qualitatively, the biological effects seen for the EHC and the 1R4F were comparable and similar to those observed in other mainstream smoke inhalation studies. Quantitatively, the biological activity of the EHC mainstream smoke was, on average, 65% lower than that of the 1R4F mainstream smoke on an equal cigarette basis and equivalent activity on an equal TPM basis (Terpstra PM et al., 2003)

“Glycerin is applied to cigarette tobacco at levels in the range of about 1-5% to improve moisture holding characteristics of tobacco and act as a surface active agent for flavor application. Neat material pyrolysis studies, smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, in vivo micronucleus, and sub-chronic rodent inhalation) with mainstream smoke, or mainstream smoke preparations from cigarettes containing various target levels (5%, 10%, and 15%) of the glycerin were performed to provide data for an assessment of the use of glycerin as a cigarette tobacco ingredient. The actual levels of glycerin in the respective test cigarettes were determined to be 3.2%, 6.2% and 8.4% after cigarette production. At simulated tobacco burning temperatures up to 900 degrees C, neat glycerin did not pyrolyze extensively suggesting that glycerin would transfer intact to mainstream smoke (smoke was not analyzed for glycerin in this study). On a tar basis, nicotine in smoke was significantly decreased at 10% and 15% glycerin while water was increased at all addition levels. Addition of 10% or 15% glycerin also resulted in a statistically significant increase in acrolein (9%) and a decrease in acetaldehyde, propionaldehyde, aromatic amines, nitrogen oxides, tobacco specific nitrosamines, and phenols. Addition of 5% glycerin produced the same decrease in smoke constituents as the 10% and 15% groups but there was no concomitant increase in acrolein. Biological tests indicated no relevant differences in the genotoxic or cytotoxic potential of either mainstream smoke (or smoke preparations) from cigarettes with added glycerin compared to control cigarettes. Cigarette smoke atmosphere dilution, coupled with the lower nicotine delivery in the test cigarettes containing glycerin resulted in a lower nicotine delivery to the glycerin cigarette smoke exposed rats of the 90-day inhalation study. Smoke atmosphere acrolein was also reduced in a concentration-related manner. Incorporation of glycerin at target levels up to 15% did not produce any adverse effects in rats exposed for 90-days. The major observation in the study was a reduced biological activity of the smoke as indicated by a reduction in the severity and/or incidence of focal macrophage accumulation in the lungs and goblet cell hyperplasia/hypertrophy in the nose (level 1), and goblet cell staining depletion in the nose (level 1). The results of these studies with glycerin applied to cigarette tobacco suggest that adding glycerin to cigarette tobacco at typical use levels does not adversely alter the smoke chemistry or biological effects normally associated with exposure to mainstream cigarette smoke”. As taken from Carmines EL & Gaworski CL. 2005. *Fd Chem. Toxicol.* 43, 1521-1539. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/15967561?dopt=AbstractPlus>.

Glycerin ...and propylene glycol ...are commonly used as humectant ingredients in manufactured cigarettes to control and maintain the moisture content of the cut tobacco filler. The potential of these added humectants to affect the toxicity of cigarette smoke was investigated in a subchronic nose-only smoke inhalation study in rats. Filtered test cigarettes were prepared from an American-style tobacco blend containing either glycerin added at 5.1% wt/wt tobacco, propylene glycol at 2.2% wt/wt tobacco, or combinations of these humectants totaling 2.3%, 3.9%, and 7.2% wt/wt tobacco. Other groups of animals were exposed similarly to the smoke of reference cigarettes without added humectants, or to filtered air (sham control). Smoke exposures were conducted for 1

hr/day, 5 days/wk for 13 wk, at target smoke particulate concentrations of 350 mg/cu m. All smoke-exposed groups had equivalent increases in blood carboxyhemoglobin, serum nicotine, and serum cotinine relative to the air controls. Smoke-associated reductions in body weights and occasional increases in heart and lung weights were generally similar among the various exposure conditions at necropsy. Increases in serum alkaline phosphatase and decreases in serum glucose and cholesterol were observed among smoke-exposed females relative to air controls. However, no significant differences in these parameters were evident between the humectant-containing and reference cigarette smoke exposure groups. Assessments of respiration conducted after 3, 6, 9, and 12 wk of smoke exposure indicated an initial smoke-related but not humectant-related decrease in respiratory rate, tidal volume, and minute volume during the first 20 min of each smoke exposure. Respiratory-tract histopathology was consistent across sexes and smoke groups, comprising (1) diffuse and focal alveolar pigmented macrophages and chronic interstitial inflammation in the lung, (2) laryngeal epithelial hyperplasia, squamous metaplasia, and scab formation, and (3) epithelial hyperplasia in the anterior nose. Smoke-related histopathology resolved substantially during a 6-wk postexposure recovery period. Addition of the tested humectants to cigarettes, singly or in combination, had no meaningful effect on the site, occurrence, or severity of respiratory-tract changes or on the measured indices of pulmonary function. It was concluded that the addition of glycerin and propylene glycol to cigarettes does not significantly affect the biological activity of inhaled cigarette smoke in this rat model. [Heck JD; Inhal Toxicol 14 (11): 1135-1152 (2002)] **PEER REVIEWED**

As taken from HSDB, 2012.

"Tobacco smoke contains more than 5600 constituents, of which approximately 150 are toxicants. This paper describes the activities in the Neutral Red uptake (NRU) assay, the Salmonella mutagenicity test (SAL), the mouse lymphoma mammalian cell mutation assay (MLA) and the in vitro micronucleus test (IVMNT) of Particulate Matter (PM) obtained from experimental cigarettes (ECs), designed to produce reduced levels of toxicants. The designs included tobacco substitute sheet (TSS) containing glycerol, which dilutes toxicants in smoke, or the incorporation of blend-treated (BT) tobacco to reduce the levels of nitrogenous toxicant precursors and some polyphenols. All samples were cytotoxic in the NRU, however TSS reduced PM cytotoxicity in this assay. All PMs were mutagenic in the SAL, MLA and IVMNT. Reductions in bacterial mutagenicity were observed in the SAL, for cigarettes with BT tobacco, compared with their respective controls. The quantitative changes in bacterial mutagenicity could be explained by analytical chemistry data on smoke generated from the ECs used in the study. These observations, and the absence of consistent qualitative differences in the activities of the experimental, control and reference cigarettes, suggest that reduced toxicity cigarettes, as measured by the tests described in this paper, may be developed without introducing any additional cytotoxic or genotoxic hazards, but the impact of this on human health risks remains unknown." As taken from Combes R et al. 2013. Toxicol. In Vitro 27(5), 1533-41. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23542039>

"BACKGROUND: ELECTRONIC CIGARETTES (ECS) HAVE BEEN MARKETED AS AN ALTERNATIVE-TO-SMOKING HABIT. Besides chemical studies of the content of EC liquids or vapour, little research has been conducted on their in vitro effects. Smoking is an important risk factor for cardiovascular disease and cigarette smoke (CS) has well-established cytotoxic effects on myocardial cells. The purpose of this study was to evaluate the cytotoxic potential of the vapour of 20 EC liquid samples and a "base" liquid sample (50% glycerol and 50% propylene glycol, with no nicotine or flavourings) on cultured myocardial cells. Included were 4 samples produced by using cured tobacco leaves in order to extract the tobacco flavour. METHODS: Cytotoxicity was tested according to the ISO 10993-5 standard. By activating an EC device at 3.7 volts (6.2 watts-all samples, including the "base" liquid) and at 4.5 volts (9.2 watts-four randomly selected samples), 200 mg of liquid evaporated and was extracted in 20 mL of culture medium. Cigarette smoke (CS) extract from three tobacco cigarettes was produced according to ISO 3308 method (2 s puffs of 35 mL volume, one puff every 60 s). The extracts, undiluted (100%) and in four dilutions (50%, 25%, 12.5%, and 6.25%), were applied to myocardial cells (H9c2); percent-viability was measured after

24 h incubation. According to ISO 10993-5, viability of <70% was considered cytotoxic. RESULTS: CS extract was cytotoxic at extract concentrations >6.25% (viability: 76.9 ± 2.0% at 6.25%, 38.2 ± 0.5% at 12.5%, 3.1 ± 0.2% at 25%, 5.2 ± 0.8% at 50%, and 3.9 ± 0.2% at 100% extract concentration). Three EC extracts (produced by tobacco leaves) were cytotoxic at 100% and 50% extract concentrations (viability range: 2.2%-39.1% and 7.4%-66.9% respectively) and one ("Cinnamon-Cookies" flavour) was cytotoxic at 100% concentration only (viability: 64.8 ± 2.5%). Inhibitory concentration 50 was >3 times lower in CS extract compared to the worst-performing EC vapour extract. For EC extracts produced by high-voltage and energy, viability was reduced but no sample was cytotoxic according to ISO 10993-5 definition. Vapour produced by the "base" liquid was not cytotoxic at any extract concentration. Cell survival was not associated with nicotine concentration of EC liquids. CONCLUSIONS: This study indicates that some EC samples have cytotoxic properties on cultured cardiomyoblasts, associated with the production process and materials used in flavourings. However, all EC vapour extracts were significantly less cytotoxic compared to CS extract." As taken from Farsalinos KE et al. 2013. Int. J. Environ. Res. Public Health 10(10), 5146-62. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24135821>

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing glycerol was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of glycerol when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	52000	JTI KB Study Report(s)
In vitro cytotoxicity	52000	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Glycerol was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	611,523	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	611,523	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	611,523	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	487,050	Logic (2019a)
Inhalation study	487,050	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Glycerol was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those

observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.98 mg/310 mg tobacco in tobacco capsule and 447 ppm in liquid	Logic (2019b)
In vitro genotoxicity	0.98 mg/310 mg tobacco in tobacco capsule and 447 ppm in liquid	Logic (2019b)
In vitro cytotoxicity	0.98 mg/310 mg tobacco in tobacco capsule and 447 ppm in liquid	Logic (2019b)
In vivo genotoxicity	0.98 mg/310 mg tobacco in tobacco capsule and 447 ppm in liquid	Logic (2019b)
Inhalation study	0.98 mg/310 mg tobacco in tobacco capsule and 447 ppm in liquid	Logic (2019b)

Aerosol from heated tobacco stick(s) containing Glycerol was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	51.5	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	51.5	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	51.5	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

A prototype electronic cigarette device and three formulations were evaluated in a 90-day rat inhalation study followed by a 42-day recovery period. Animals were randomly assigned to groups for exposure to low-, mid- and high-dose levels of aerosols composed of vehicle (glycerin and propylene glycol mixture); vehicle and 2.0% nicotine; or vehicle, 2.0% nicotine and flavor mixture. Daily targeted aerosol total particulate matter (TPM) doses of 3.2, 9.6 and 32.0 mg/kg/day were achieved by exposure to 1 mg/L aerosol for 16, 48 and 160 min, respectively. Pre-study evaluations included indirect ophthalmoscopy, virology and bacteriological screening. Body weights, clinical observations and food consumption were monitored weekly. Plasma nicotine and cotinine and carboxyhemoglobin levels were measured at days 28 and 90. After days 28, 56 and 90, lung function measurements were obtained. Biological endpoints after 90-day exposure and 42-day recovery period included clinical pathology, urinalysis, bronchoalveolar fluid (BALF) analysis, necropsy and histopathology. Treatment-related effects following 90 days of exposure included changes in body weight, food consumption and respiratory rate. Dose-related decreases in thymus and spleen weights, and increased BALF lactate dehydrogenase, total protein, alveolar macrophages, neutrophils and lung weights were observed. Histopathology evaluations revealed sporadic increases in nasal section 1-4 epithelial hyperplasia and vacuolization. Following the recovery period, effects in the nose and BALF were persistent while other effects were resolved. The no observed effect level based upon body weight decreases is considered to be the mid-dose level for each formulation, equivalent to a daily TPM exposure dose of approximately 9.6 mg/kg/day. As taken from Werley et al. 2016. Inhal Toxicol. 28(1):22-38. PubMed, 2016 available at

<http://www.ncbi.nlm.nih.gov/pubmed/26787428>

“Propylene glycol and glycerine, both organic compounds, are used in varying proportions to make the e-liquids for EC. The RCP report (3) summarised that in relation to both PG and glycerine, studies with animals have been generally reassuring in relation to health consequences.

Glasser (73) reported that PG and vegetable glycerine in EC liquids were not cytotoxic for any human and animal cell types.

Heating PG or glycerine can release aldehydes such as formaldehyde, acrolein and acetaldehyde, which are also produced during smoking.

A study analysing the exhaled breath of EC users reported that of chemicals inhaled, only 6% of nicotine, 8% of PG and 16% of glycerine was exhaled (79)."

As taken from Public Health England, 2018.

"PURPOSE OF REVIEW: Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma. RECENT FINDINGS: Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms. Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers." As taken from Clapp PW & Jaspers I. 2017. *Curr. Allergy Asthma Rep.* 17(11), 79. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/28983782>

"The electronic cigarette solvents propylene glycol and glycerol are known to produce toxic byproducts such as formaldehyde, acetaldehyde and acrolein. However, the aerosol toxin yield depends upon a variety of chemical and physical variables. The formaldehyde hemiacetals derived from these solvents were reported as major electronic cigarette aerosol components by us in 2015. In the study described herein, the formaldehyde hemiacetals were found at higher levels than those of free formaldehyde via an orthogonal sample collection protocol. In addition, the common aldehyde collection methods for electronic cigarettes, such as impingers and sorbent tubes containing DNPH, significantly underestimate the levels of formaldehyde. The reason for this is that formaldehyde hemiacetals follow other reaction pathways, such as the formation of a less reactive full cyclic acetal catalyzed by the acidity of the DNPH solution and the silica. We found that formaldehyde hemiacetals are a considerable fraction of the total formaldehyde produced in electronic cigarette that cannot be determined accurately by DNPH derivatization methods. Although the health effects of the hemiacetals are not yet known, they warrant further investigation." As taken from Salamanca JC et al. 2017. *Sci. Rep.* 7(1), 11044. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/28887552>

“BACKGROUND: In December 2016, the Surgeon General published a report that concluded e-cigarette use among youth and young adults is becoming a major public health concern in the United States of America. METHODS: Re-analysis of key data sources on nicotine toxicity and prevalence of youth use of e-cigarettes cited in the Surgeon General report as the basis for its conclusions. RESULTS: Multiple years of nationally representative surveys indicate the majority of e-cigarette use among US youth is either infrequent or experimental, and negligible among never-smoking youth. The majority of the very small proportion of US youth who use e-cigarettes on a regular basis, consume nicotine-free products. The sharpest declines in US youth smoking rates have occurred as e-cigarettes have become increasingly available. Most of the evidence presented in the Surgeon General's discussion of nicotine harm is not applicable to e-cigarette use, because it relies almost exclusively on exposure to nicotine in the cigarette smoke and not to nicotine present in e-cigarette aerosol emissions. Moreover, the referenced literature describes effects in adults, not youth, and in animal models that have little relevance to real-world e-cigarette use by youth. The Surgeon General's report is an excellent reference document for the adverse outcomes due to nicotine in combination with several other toxicants present in tobacco smoke, but fails to address the risks of nicotine decoupled from tobacco smoke constituents. The report exaggerates the toxicity of propylene glycol (PG) and vegetable glycerin (VG) by focusing on experimental conditions that do not reflect use in the real-world and provides little discussion of emerging evidence that e-cigarettes may significantly reduce harm to smokers who have completely switched. CONCLUSIONS: The U.S. Surgeon General's claim that e-cigarette use among U.S. youth and young adults is an emerging public health concern does not appear to be supported by the best available evidence on the health risks of nicotine use and population survey data on prevalence of frequent e-cigarette use. Nonetheless, patterns of e-cigarettes use in youth must be constantly monitored for early detection of significant changes. The next US Surgeon General should consider the possibility that future generations of young Americans will be less likely to start smoking tobacco because of, not in spite of, the availability of e-cigarettes.” As taken from Polosa R et al. 2017. *Harm Reduc. J.* 14(1), 61. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/28874159>

“Knowledge of the long-term toxicological and immunological effects of e-cigarette (e-cig) aerosols remains elusive due to the relatively short existence of vaping. Therefore, we performed a systematic search of articles published in public databases and analysed the research evidence in order to provide critical information regarding e-cig safety. Electronic nicotine delivery systems (or e-cigs) are an alternative to traditional cigarettes for the delivery of nicotine and are typically filled with glycerol or propylene glycol-based solutions known as e-liquids. Though present in lower quantities, e-cig aerosols are known to contain many of the harmful chemicals found in tobacco smoke. However, due to the paucity of experimental data and contradictory evidence, it is difficult to draw conclusive outcomes regarding toxicological, immunological and clinical impacts of e-cig aerosols. Excessive vaping has been reported to induce inflammatory responses including mitogen-activated protein kinase, Janus tyrosine kinase/signal transducer and activator of transcription and nuclear factor- κ B signalling, similar to that induced by tobacco smoke. Based on recent evidence, prolonged exposure to some constituents of e-cig aerosols might result in respiratory complications such as asthma, chronic obstructive pulmonary disease and inflammation. Future studies are warranted that focus on establishing correlations between e-cig types, generations and e-liquid flavours and immunological and toxicological profiles to broaden our understanding about the effects of vaping.” As taken from Kaur G et al. 2018b. *Eur. Respir. Rev.* 27(147), 170119. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/29491036>

“Electronic cigarette usage is increasing worldwide, yet there is a paucity of information on the respiratory health effects of electronic cigarette aerosol exposure. This study aimed to assess whether exposure to electronic cigarette (e-cigarette) aerosol would alter lung function and

pulmonary inflammation in mice and to compare the severity of any alterations with mice exposed to mainstream tobacco smoke. Female BALB/c mice were exposed for 8 wk to tobacco smoke, medical air (control), or one of four different types of e-cigarette aerosol. E-cigarette aerosols varied depending on nicotine content (0 or 12 mg/ml) and the main excipient (propylene glycol or glycerin). Twenty-four hours after the final exposure, we measured pulmonary inflammation, lung volume, lung mechanics, and responsiveness to methacholine. Mice exposed to tobacco cigarette smoke had increased pulmonary inflammation and responsiveness to methacholine compared with air controls. Mice exposed to e-cigarette aerosol did not have increased inflammation but did display decrements in parenchymal lung function at both functional residual capacity and high transrespiratory pressures. Mice exposed to glycerin-based e-cigarette aerosols were also hyperresponsive to methacholine regardless of the presence or absence of nicotine. This study shows, for the first time, that exposure to e-cigarette aerosol during adolescence and early adulthood is not harmless to the lungs and can result in significant impairments in lung function.” As taken from Larcombe AN et al. 2017. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 313(1), L67-L79. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/28360111>

“BACKGROUND: Electronic cigarette (e-cigarette) use has increased substantially in recent years. While e-cigarettes have been proposed as a potentially effective smoking cessation tool, dual use in smokers is common and e-cigarettes are widely used by non-smokers, including youth and young-adult non-smokers. Nicotine, the primary addictive component in cigarettes, is present at varying levels in many e-liquids. E-cigarettes may lead to initiation of nicotine use in adult and youth non-smokers, re-initiation of nicotine dependence in ex-smokers or increased severity of nicotine dependence in dual-users of cigarettes and e-cigarettes. As such, there are important clinical and policy implications to understanding factors impacting nicotine exposure from e-cigarettes. However, the broad and rapidly changing range of e-liquid constituents and e-cigarette hardware which could impact nicotine exposure presents a challenge. Recent changes in regulatory oversight of e-cigarettes underscore the importance of synthesizing current knowledge on common factors which may impact nicotine exposure. METHODS: This review focuses on factors which may impact nicotine exposure by changing e-cigarette use behavior, puff topography, altering the nicotine yield (amount of nicotine exiting the e-cigarette mouth piece including nicotine exhaled as vapor) or more directly by altering nicotine absorption and bioavailability. RESULTS: Topics reviewed include e-liquid components or characteristics including flavor additives (e.g., menthol), base e-liquid ingredients (propylene glycol, vegetable glycerin), components commonly used to dissolve flavorants (e.g., ethanol), and resulting properties of the e-liquid (e.g., pH), e-cigarette device characteristics (e.g., wattage, temperature, model) and user behavior (e.g., puff topography) which may impact nicotine exposure. CONCLUSION: E-liquid characteristics and components, e-cigarette hardware and settings, and user behavior can all contribute substantially to nicotine exposure from e-cigarettes.” As taken from DeVito EE and Krishnan-Sarin S. 2018. *Curr. Neuropharmacol.* 16(4), 438-459. PubMed, 2019 available at

<https://www.ncbi.nlm.nih.gov/pubmed/29046158>

“In the United States, the recent surge of electronic cigarette (e-cig) use has raised questions concerning the safety of these devices. This study seeks to assess the pro-inflammatory and cellular stress effects of the vaped humectants propylene glycol (PG) and glycerol (GLY) on airway epithelial cells (16HBE cells and differentiated human bronchial epithelial cells) with a newly developed aerosol exposure system. This system allows for chemical characterization of e-cig generated aerosol particles as well as in vitro exposures of 16HBE cells at an air-liquid interface to vaped PG and GLY aerosol. Our data demonstrate that the process of vaping results in the formation of PG- and GLY-derived oligomers in the aerosol particles. Our in vitro data demonstrate an increase in pro-inflammatory cytokines IL-6 and IL-8 levels in response to vaped PG and GLY exposures. Vaped GLY also causes an increase in cellular stress signals HMOX1, NQO1, and carbonylated proteins when the e-cig device is operated at high wattages. Additionally, we find that

the exposure of vaped PG causes elevated IL-6 expression, while the exposure of vaped GLY increases HMOX1 expression in human bronchial epithelial cells when the device is operated at high wattages. These findings suggest that vaporizing PG and GLY results in the formation of novel compounds and the exposure of vaped PG and GLY are detrimental to airway cells. Since PG and/or GLY is universally contained in all e-cig liquids, we conclude that these components alone can cause harm to the airway epithelium.” As taken from Escobar YH et al. 2020a. Chem. Res. Toxicol. 33(7), 1677-1688. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32223225/>

“Cigarette smoking is associated with chronic obstructive pulmonary disease and chronic bronchitis. Acquired ion transport abnormalities, including cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction, caused by cigarette smoking have been proposed as potential mechanisms for mucus obstruction in chronic bronchitis. Although e-cigarette use is popular and perceived to be safe, whether it harms the airways via mechanisms altering ion transport remains unclear. In the present study, we sought to determine if e-cigarette vapor, like cigarette smoke, has the potential to induce acquired CFTR dysfunction, and to what degree. Electrophysiological methods demonstrated reduced chloride transport caused by vaporized e-cigarette liquid or vegetable glycerin at various exposures (30 min, 57.2% and 14.4% respectively, vs. control; $P < 0.0001$), but not by unvaporized liquid (60 min, 17.6% vs. untreated), indicating that thermal degradation of these products is required to induce the observed defects. We also observed reduced ATP-dependent responses (-10.8 ± 3.0 vs. -18.8 ± 5.1 $\mu\text{A}/\text{cm}^2$ control) and epithelial sodium channel activity (95.8% reduction) in primary human bronchial epithelial cells after 5 minutes, suggesting that exposures dramatically inhibit epithelial ion transport beyond CFTR, even without diminished transepithelial resistance or cytotoxicity. Vaporizing e-cigarette liquid produced reactive aldehydes, including acrolein (shown to induce acquired CFTR dysfunction), as quantified by mass spectrometry, demonstrating that respiratory toxicants in cigarette smoke can also be found in e-cigarette vapor (30 min air, 224.5 ± 15.99 ; unvaporized liquid, 284.8 ± 35.03 ; vapor, $54,468 \pm 3,908$ ng/ml; $P < 0.0001$). E-cigarettes can induce ion channel dysfunction in airway epithelial cells, partly through acrolein production. These findings indicate a heretofore unknown toxicity of e-cigarette use known to be associated with chronic bronchitis onset and progression, as well as with chronic obstructive pulmonary disease severity.” As taken from Lin VY et al. 2019. Am. J. Respir. Cell. Mol. Biol. 61(2), 162-173. PubMed, 2020 available at

<https://pubmed.ncbi.nlm.nih.gov/30576219/>

The use of electronic cigarettes (E-cig) is popular because of the perception that they are less addictive and safer compared to the traditional cigarettes. Nevertheless, there are still harmful effects associated with chemicals emitted from E-cig. Identifying the sources of the emitted compounds can be challenging because of the differences in the design of E-cig devices and the variability in the composition of E-cig liquids used in these devices. In this study, the emission profiles from impurity-free E-liquids containing only propylene glycol and glycerol in various percentage ratios along with two commercially available E-liquids were evaluated using gas chromatography-mass spectrometry (GC-MS). This study approach allows the elucidation of the transformation pathways of the major emitted compounds without the confounding effects of existing impurities or flavor ingredients added to E-liquids. Analysis of the vapor phases of E-cig emissions detected toxicants such as acetaldehyde, acrolein, benzaldehyde, as well as benzene, toluene, ethylbenzene, and xylene (BTEX) compounds. The amount of glycerol in the E-liquids has a major effect on the concentration of these hazardous compounds emitted because the concentration of these chemicals increased with increasing glycerol percentage in the E-liquid. Acetaldehyde and acrolein increased by 175-fold and 28-fold, respectively, when the glycerol composition was increased from 0 to 80%. Benzaldehyde, naphthalene, diphenyl ether, and glycerol along with menthol and nicotine that were present in the commercial E-liquids were also detected in the aerosol condensates. The cascade impactor data on the distribution of the nicotine and menthol in different size fractions from >2.5 to <2.5 μm allow the estimates of the extent of toxicant deposition in different parts of the pulmonary system including the oropharynx region, the trachea as well as inside the alveoli and bronchioles. In summary, users of E-cig are exposed to

harmful chemicals even if the E-liquids contain only propylene glycol and glycerol without flavorings, nicotine, or impurities. Furthermore, this study shows that E-liquids containing higher percentages of glycerol will produce higher levels of toxicants compared to E-liquids with similar percentages of propylene glycol. This finding has important implications to E-cigarette vendors and manufacturers, consumers, and regulatory agencies. Ooi BG et al. (2019). Influence of the E-Cigarette Emission Profile by the Ratio of Glycerol to Propylene Glycol in E-Liquid Composition. ACS Omega 4(8), 13338-13348. DOI: 10.1021/acsomega.9b01504. PubMed, 2020 available at

<https://pubmed.ncbi.nlm.nih.gov/31460462/>

A screening method allowing the quantification of 24 aerosol constituents using gas chromatography-mass spectrometry has been developed to assess the aerosol chemistry of heat-not-burn tobacco products. The aim of this method was to quantify phenol, o-cresol, m-cresol, p-cresol, catechol, resorcinol, hydroquinone, 1,3-butadiene, isoprene, benzene, acrylonitrile, toluene, pyridine, styrene, 1,2-propylene glycol, menthol, 2-furanmethanol, acrylamide, naphthalene, nicotine, acetamide, quinoline, triacetin, and glycerine in the aerosol emitted by heated tobacco products. The aerosol was generated by an electrically heated tobacco system (PMI's Heated Tobacco System (THS 2.4)) with one single aerosol collection method, using the Health Canada smoking regimen and analyzed with two analytical methods. The method was validated according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Association of Official Analytical Chemists guidelines. A regression model based on a linear relationship between concentration and response ratio with a 1/x weighting factor was selected for phenol, o-cresol, m-cresol, p-cresol, 1,3-butadiene, isoprene, benzene, acrylonitrile, toluene, pyridine, styrene, 2-furanmethanol, acrylamide, naphthalene and acetamide. A quadratic regression model with a 1/x weighting factor was chosen for catechol, resorcinol, hydroquinone, 1,2-propylene glycol, menthol, nicotine, quinoline, triacetin and glycerine. Coefficients of variation for repeatability were determined between 7.9% and 17.8% and for intermediate precision between 8.1% and 19.9%. The matrix effect of the heated tobacco aerosol extract was assessed by performing a recovery study, where the aerosol extracts were spiked at different concentrations for the compounds to be analyzed. In addition, the mainstream smoke from 3R4F reference cigarettes was analyzed, and results were compared with previously published studies. The method was successfully validated, providing data consistent with published data and it was shown to be selective, precise and accurate." As taken from Hofer I et al. 2019. Beiträge zur Tabakforschung 28(7), 317-328. Available at

https://content.sciendo.com/view/journals/cttr/28/7/article-p317.xml?rskey=sSWjME&result=1&tab_body=pdf-69320

"Introduction: IQOS is an emerging heated tobacco product marketed by Philip Morris International (PMI). Because the tobacco in IQOS is electrically heated and not combusted, PMI claims that it generates significantly lower toxicant levels than combustible cigarettes. To date, a few independent studies have addressed IQOS toxicant emissions, and none have reported reactive oxygen species (ROS), and the form of the nicotine emitted by the device. Methods: In this study, IQOS aerosol was generated using a custom-made puffing machine. Two puffing regimens were used: Health Canada Intense and ISO. ROS, carbonyl compounds (CCs), and total nicotine and its partitioning between free-base and protonated forms were quantified in the IQOS aerosol by fluorescence, high-performance liquid chromatography, and gas chromatography, respectively. The same toxicants were also quantified in combustible cigarette aerosols for comparison. In addition, propylene glycol and vegetable glycerin were also measured in the IQOS tobacco and aerosol. Results: IQOS and combustible cigarettes were found to emit similar quantities of total and free-base nicotine. IQOS total ROS (6.26 ± 2.72 nmol H₂O₂/session) and CC emissions (472 ± 19 µg/session) were significant, but 85% and 77% lower than levels emitted by combustible cigarettes. Conclusions: IQOS emits harmful constituents that are linked to cancer, pulmonary disease, and addiction in cigarette smokers. For a given nicotine intake, inhalation exposure to ROS and CCs from IQOS is likely to be significantly less than that for combustible cigarettes. Implications: IQOS is

PMI's new heated tobacco product. PMI claims that because IQOS heats and does not burn tobacco it generates low toxicant yields. We found that one IQOS stick can emit similar free-base and total nicotine yields as a combustible cigarette. A pack-a-day equivalent user of IQOS may experience significant inhalation exposure of ROS and CCs compared to background air. However, substituting IQOS for combustible cigarettes will likely result in far lower ROS and carbonyl inhalation exposure for a given daily nicotine intake." As taken from Salman R et al. 2019. *Nicotine Tob. Res.* 21(9), 1285-1288. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30476301/>

"An electronic cigarette (e-cigarette) is a product used to smoke aerosol by heating a solution of "e-liquid" that consists of propylene glycol (PG) and glycerol (GLY) containing nicotine and flavors. In this study, thermal decomposition products generated from three brands of e-cigarettes were determined at various electric power levels. When using neat PG or GLY instead of e-liquid, propylene oxide was detected only in the gas phase from PG and not detected from GLY. In contrast, glycidol was detected only from GLY and not from PG. Almost all of the glyoxal and acrolein was detected from GLY, but formaldehyde and methyl glyoxal were detected from both PG and GLY. Using commercially available e-liquids, the same results were obtained. Nearly all chemical compounds generated from e-cigarettes have a carbon number of 3 or less except for nicotine and flavors. We measured chemical compounds generated from e-cigarettes at various electric power levels (1-85 W). At an electric power of 10 W, the generation of chemical compounds was very low; however, when the electric power exceeded 40 W, it increased exponentially. As thermal decomposition products of e-liquid, acetaldehyde, acrolein, and propylene oxide mainly occur as gaseous matter, while glyoxal, methylglyoxal, and glycidol mainly occur as particulate matter. Formaldehyde exists in both gaseous and particulate matter forms. Thermal decomposition products can be divided into three groups: thermal decomposition products originating from PG and GLY, those originating from other sources, and those directly generated. Concentrations of these thermal decomposition products were mostly higher than those in traditional cigarettes. In particular, thermal decomposition products generated from one of the studied e-cigarettes were very high; e.g., formaldehyde reached 4400 µg/15 puffs at 50 W. E-cigarette users must know that hazardous substances are generated even within the recommended electric power limits." As taken from Uchiyama S et al. 2020. *Chem. Res. Toxicol.* 33(2), 576-583. PubMed, 2020 available at

<https://pubmed.ncbi.nlm.nih.gov/31950825/>

"Glycidol is a thermal byproduct of glycerol, a common component in e-liquids. In other words, glycidol can form and appear in the aerosol when a glycerol-containing solvent such as an e-liquid is heated and aerosol is produced (Refs. 1-2)."

As taken from FDA, 2019

"In the United States, millions of adults use electronic cigarettes (e-cigs), and a majority of these users are former or current cigarette smokers. It is unclear, whether prior smoking status affects biological responses induced by e-cigs. In this study, differentiated human nasal epithelial cells (hNECs) from nonsmokers and smokers at air-liquid interface were acutely exposed to the e-cig generated aerosols of humectants, propylene glycol (PG), and glycerol (GLY). Mucin levels were examined in the apical washes, and cytokine levels were assessed in the basolateral supernatants 24 h postexposure. The aerosol from the GLY exposure increased mucin 5, subtype AC (MUC5AC) levels in the apical wash of hNECs from nonsmokers, but not smokers. However, the aerosol from GLY induced pro-inflammatory responses in hNECs from smokers. We also exposed hNECs from nonsmokers and smokers to e-cig generated aerosol from PG:GLY with freebase nicotine or nicotine salt. The PG:GLY with freebase nicotine exposure increased MUC5AC and mucin 5, subtype B (MUC5B) levels in hNECs from nonsmokers, but the nicotine salt exposure did not. The PG:GLY with nicotine salt exposure increased pro-inflammatory cytokines in hNECs from smokers, which was not seen with the freebase nicotine exposure. Taken together, these data indicate that the e-cig generated aerosols from the humectants, mostly GLY, and the type of nicotine used cause differential effects in airway epithelial cells from nonsmokers and smokers. As e-cig use is

increasing, it is important to understand that the biological effects of e-cig use are likely dependent on prior cigarette smoke exposure.” As taken from Escobar YH et al. 2020b. *Am. J. Physiol. Lung Cell Mol. Physiol.* 320(6), L1064-L1073. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33825493/>

“To provide insights into the cause of e-cigarette (e-cig) associated lung injury, we examined the effects of propylene glycol (PG) and glycerol (G), two common solvent carriers used to deliver nicotine/ flavor, on markers of oxidative stress and inflammation in female B6C3F1 mice which had been used successfully in tobacco smoke (TS)-induced lung carcinogenesis. Mice exposed to air and TS were used as negative and positive controls, respectively. Using LC-MS/MS, we showed that PG/G alone, in the absence of nicotine, significantly increased the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG or its tautomer 8-oxodG), a biomarker of DNA oxidative damage, in lung and plasma of mice; moreover, addition of nicotine (12 and 24 mg/mL) in e-cig liquid appears to suppress the levels of 8-oxodG. Exposure to e-cig aerosols or TS induced nonsignificant increases of plasma C-reactive protein (CRP), a biomarker of inflammation; nonetheless, the levels of fibronectin (FN), a biomarker of tissue injury, were significantly increased by e-cig aerosols or TS. Although preliminary, our data showed that exposure to e-cig aerosols induced a higher score of lung injury than did control air or TS exposure. Our results indicate that the B6C3F1 mouse model may be suitable for an in-depth examination of the impact of e-cig on lung injury associated with oxidative stress and inflammation and this study adds to the growing evidence that the use of e-cig can lead to lung damage.” As taken from Sun YW et al. 2021. *Chem. Res. Toxicol.* 34(4), 1161-1168. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33761748/>

“Vaping has become increasingly popular over the past decade. This pragmatic review presents the published biological effects of electronic cigarette vapour inhalation with a focus on the pulmonary effects. Special attention has been devoted to providing the documented effects specific to each major ingredient, namely propylene glycol/glycerol, nicotine and flavouring agents. For each ingredient, findings are divided according to the methodology used, being in vitro studies, animal studies and clinical studies. Finally, we provide thoughts and insights on the current state of understanding of the pulmonary effects of vaping, as well as novel research avenues and methodologies.” As taken from Lechasseur A and Morissette MC. 2020. *Eur. Respir. Rev.* 29(157), 200268. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33060167/>

“The formation of carbonyls and epoxides in e-cigarette (EC) aerosol is possible due to heating of the liquid constituents. However, high background levels of these compounds have inhibited a clear assessment of exposure during use of ECs. An EC containing an e-liquid replaced with 10% of ¹³C-labeled propylene glycol and glycerol was used in a controlled use clinical study with 20 EC users. In addition, five smokers smoked cigarettes spiked with the described e-liquid. Seven carbonyls (formaldehyde, acetaldehyde, acrolein, acetone, crotonaldehyde, methacrolein, propionaldehyde) were measured in the aerosol and the mainstream smoke. Corresponding biomarkers of exposure were determined in the user's urine samples. ¹³C-labeled formaldehyde, acetaldehyde and acrolein were found in EC aerosol, while all seven labeled carbonyls were detected in smoke. The labeled biomarkers of exposure to formaldehyde (¹³C-thiazolidine carboxylic acid and ¹³C-N-(1,3-thiazolidine-4-carbonyl)glycine), acrolein (¹³C-3-hydroxypropylmercapturic acid) and glycidol (¹³C-3-dihydroxypropylmercapturic acid) were present in the urine of vapers indicating an EC use-specific exposure to these toxicants. However, other sources than vaping contribute to a much higher extent by several orders of magnitude to the overall exposure of these toxicants. Comparing data for the native (unlabeled) and the labeled (exposure-specific) biomarkers revealed vaping as a minor source of user's exposure to these toxicants while other carbonyls and epoxides were not detectable in the EC aerosol.” As taken from Landmesser A et al. (2021). *Assessment of the potential vaping-related exposure to carbonyls and epoxides using stable isotope-labeled precursors in the e-liquid.* *Arch. Toxicol.* Epub ahead of print. DOI: 10.1007/s00204-021-03097-x. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34159432/>

E-cigarette aerosol is a complex mixture of gases and particles with a composition that is dependent on the e-liquid formulation, puffing regimen, and device operational parameters. This work investigated mainstream aerosols from a third generation device, as a function of coil temperature (315-510 °F, or 157-266 °C), puff duration (2-4 s), and the ratio of propylene glycol (PG) to vegetable glycerin (VG) in e-liquid (100:0-0:100). Targeted and untargeted analyses using liquid chromatography high-resolution mass spectrometry, gas chromatography, in situ chemical ionization mass spectrometry, and gravimetry were used for chemical characterizations. PG and VG were found to be the major constituents (>99%) in both phases of the aerosol. Most e-cigarette components were observed to be volatile or semivolatile under the conditions tested. PG was found almost entirely in the gas phase, while VG had a sizable particle component. Nicotine was only observed in the particle phase. The production of aerosol mass and carbonyl degradation products dramatically increased with higher coil temperature and puff duration, but decreased with increasing VG fraction in the e-liquid. An exception is acrolein, which increased with increasing VG. The formation of carbonyls was dominated by the heat-induced dehydration mechanism in the temperature range studied, yet radical reactions also played an important role. The findings from this study identified open questions regarding both pathways. The vaping process consumed PG significantly faster than VG under all tested conditions, suggesting that e-liquids become more enriched in VG and the exposure to acrolein significantly increases as vaping continues. It can be estimated that a 30:70 initial ratio of PG:VG in the e-liquid becomes almost entirely VG when 60-70% of e-liquid remains during the vaping process at 375 °F (191 °C). This work underscores the need for further research on the puffing lifecycle of e-cigarettes.” As taken from Li Y et al. (2021). Impact of e-Liquid Composition, Coil Temperature, and Puff Topography on the Aerosol Chemistry of Electronic Cigarettes. Chem. Res. Toxicol. 34(6), 1640-1654. DOI: 10.1021/acs.chemrestox.1c00070. PubMed, 2021available at <https://pubmed.ncbi.nlm.nih.gov/33949191/>

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that glycerol is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Experimental Biodegradation result (%)	63
Reference (biodegradation result)	Chemicals Inspection and Testing Institute.; Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan; Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1; 1992
Predicted Ultimate degradation half-life (days)	8.67
MITI probability of biodegradation	0.9744

TOPKAT probability of biodegradation	1
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.5709

ROBUST STUDY SUMMARY - Persistence

Item	Yes	No
<u>Reference</u> : Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992. Chemicals Inspection and Testing Institute (Ed.). Published by: Japan Chemical Industry Ecology-Toxicology & Information Center.		
<u>Test Substance</u> (CAS # and name): The list of the substances is attached (see file on N drive)		
Chemical composition of the substance (including purity, by-products)		X
<u>Method</u>		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Not applicable	
<u>Test design / conditions</u>		
Study type (photodegradation, hydrolysis, biodegradation, other – <u>specify, do not assess</u>): Biodegradation		
Test type (aerobic or anaerobic - <u>specify, do not assess</u>):		
Test medium (air, water, soil, sediment - <u>specify, do not assess</u>):		
Information on stability of the substance in the media of concern is reported?	X	
Controls (positive or negative) Both	X	
Number of replicates (including controls)		X
Temperature (specify) 25±1 °C	X	
Duration of the experiment (specify) 14 or 28 days	X	
For photodegradation only		
Light source (specify):	Not applicable	
Light spectrum and relative intensity based on sunlight intensity:	Not applicable	
For hydrolysis only		
Measured concentrations reported?	Not applicable	

Basic water properties (pH, hardness, etc.)	Not applicable	
For biodegradation only		
Ready or inherent biodegradation (specify): Both types (see file on N drive)	X	
Innoculum (concentration and source)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (<u>do not assess this item</u>): % biodegradation by BOD or TOC (see file on N drive)		
Information on breakdown products (<u>do not assess this item</u>)		X
<u>Overall score</u> : 8/10 = 80%		
EC Reliability code: 1		
Reliability category (high, satisfactory, low): high		
Comments:		

Data accessed May 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

Environmental

Fate/Exposure

Summary:

Glycerin's production and use in industrial, cosmetic, food, pharmaceutical and domestic products may result in its release to the environment through various waste streams. Glycerin was reported present in Cork Oak, *Quercus suber* L. If released to air, a vapor pressure of 1.68×10^{-4} mm Hg at 25 deg C indicates glycerin will exist in both the vapor and particulate phases in the atmosphere. Vapor-phase glycerin will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 7 hours. Particulate-phase glycerin will be removed from the atmosphere by wet or dry deposition. Glycerin does not contain chromophores that absorb at wavelengths >290 nm, and therefore is not expected to be susceptible to direct photolysis by sunlight. If released to soil, glycerin is expected to have very high mobility based upon an estimated Koc of 1. Volatilization from moist soil surfaces is not expected to be an important fate process based upon a Henry's Law constant of 1.73×10^{-8} atm-cu m/mole. Glycerin may not volatilize from dry soil surfaces based upon its vapor pressure. A 63% of theoretical BOD using activated sludge in the Japanese MITI test suggests that biodegradation is an important environmental fate process in soil and water. If released into water, glycerin is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions. Occupational exposure to glycerin may occur through inhalation and dermal contact with this compound at workplaces where glycerin is produced or used. Use and limited monitoring data indicate that the general population may be exposed to glycerin via ingestion of food, some pharmaceuticals and drinking water, and dermal contact with consumer products containing glycerin. (SRC)

PEER REVIEWED

Artificial**Pollution****Sources:**

Glycerin's production and use in industrial, cosmetic, food, pharmaceutical and domestic products(1) may result in its release to the environment through various waste streams(SRC). It's many uses include use as a solvent, humectant, plasticizer, emollient, sweetener, in the manufacture of nitroglycerol (dynamite), cosmetics, liquid soaps, liqueurs, confectioneries, blacking, printing and copying inks, lubricants, elastic glues, lead oxide cements; to keep fabrics pliable; to preserve printing on cotton; for printing rollers, hectographs; to keep frost from windshields; as antifreeze in automobiles, gas meters and hydraulic jacks, in shock absorber fluids; in fermentation nutrients in the production of antibiotics and as a pharmaceutical aid(1). [(1) O'Neil MJ, ed; The Merck Index. 14 th ed., Whitehouse Station, NJ: Merck and Co., Inc., p. 775 (2006)] **PEER REVIEWED**

Environmental**Fate:**

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 1(SRC), determined from a structure estimation method(2), indicates that glycerin is expected to have very high mobility in soil(SRC). Volatilization of glycerin from moist soil surfaces is not expected to be an important fate process(SRC) given a Henry's Law constant of 1.73×10^{-8} atm-cu m/mole(3). Glycerin is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 1.68×10^{-4} mm Hg at 25 deg C(4). A 63% of theoretical BOD using activated sludge in the Japanese MITI test(5) suggests that biodegradation is an important environmental fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Meylan WM et al; Environ Sci Technol 26: 1560-67 (1992) (3) Hine J, Mookerjee PK; J Org Chem 40: 292-8 (1975) (4) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989) (5) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jul 12, 2011: <http://www.safe.nite.go.jp/english/db.html> **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 1(SRC), determined from a structure estimation method(2), indicates that glycerin is not expected to adsorb to suspended solids and sediment(SRC). Volatilization from water surfaces is not expected(3) based upon a Henry's Law constant of 1.73×10^{-8} atm-cu m/mole(4). According to a classification scheme(5), an estimated BCF of 3(SRC), from its log Kow of -1.76(6) and a regression-derived equation(7), suggests the potential for bioconcentration in aquatic organisms is low(SRC). A 63% of theoretical BOD using activated sludge in the Japanese MITI test(8) suggests that biodegradation is an important environmental fate process in water(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Meylan WM et al; Environ Sci Technol 26: 1560-67 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (4) Hine J, Mookerjee PK; J Org Chem 40: 292-8 (1975) (5) Franke C et al; Chemosphere 29: 1501-14 (1994) (6) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 7 (1995) (7) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Jan, 2010. Available from, as of Jul 12, 2011: <http://www.epa.gov/oppt/exposure/pubs/episuite.html> (8) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jul 12, 2011: <http://www.safe.nite.go.jp/english/db.html> **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), glycerin, which has a vapor pressure of 1.68×10^{-4} mm Hg at 25 deg C(2), will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase glycerin is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 7 hours(SRC), calculated from its rate constant of 1.9×10^{-11} cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(3). Particulate-phase glycerin may be removed from the air by wet or dry deposition(SRC). Glycerin does not contain chromophores that absorb at wavelengths >290

nm(4), and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

Environmental

Biodegradation:

AEROBIC: Glycerin, present at 100 mg/L, reached 63% of its theoretical BOD in 2 weeks using an activated sludge inoculum at 30 mg/L in the Japanese MITI test(1). Biodegradation rate constants of 0.258/day and 0.200/day in respirometric test systems employing activated sludge have also been reported, corresponding to 68% and 78% degradation, respectively(2). [(1) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jul 12, 2011: <http://www.safe.nite.go.jp/english/db.html> (2) Reuschenbach P et al; Water Res 37: 1571-1582 (2003)] **PEER REVIEWED**

AEROBIC: When incubated with a filtered effluent from a sanitary waste treatment plant, glycerin displayed a 5 day BOD of 82%(1). Inoculation of glycerin with activated sewage sludge resulted in 43.5-52.9% 5 day BOD(2). Glycerin underwent 94-97% removal after 24 hrs when incubated with activated sludge from a waste water treatment plant(3-4). A 98.7% COD was observed in 120 hrs after inoculation with an adapted activated sludge seed(5). Incubation with an activated sludge seed gave a 5 day BOD of 68%(6). In screening studies, 5 day BODs for glycerin of 31%(7), 52% using activated sludge(8), 78.3% using domestic sludge(9), and 24.4% using seawater(10) were observed. Glycerin is listed as a substance easily degraded in a sewage treatment plant(11). [(1) Bridie AL et al; Water Res 13: 627-30 (1979) (2) Belly RT, Goodhue CT; Proc Int Biodegrad Symp 3: 1103-7 (1976) (3) Matsui S et al; Prog Water Technol 7: 645-59 (1975) (4) Matsui S et al; Wat Sci Tech 20: 201-10 (1988) (5) Pitter P; Water Res 10: 231-5 (1976) (6) Placak OR, Ruchhoft CC; Sewage Works J 19: 423-40 (1947) (7) Dore M et al; Trib Cebedeau 28: 3-11 (1975) (8) Urano K, Kato Z; J Hazard Mater 13: 147-59 (1986) (9) Wagner R; Vom Wasser 47: 241-65 (1976) (10) Takemoto S et al; Suishitsu Odaku Kenkyu 4: 80-90 (1981) (11) Thom NS, Agg AR; Proc Royal Soc Lond B189: 347-57 (1975)] **PEER REVIEWED**

ANAEROBIC: Glycerin underwent 90% degradation after an 8 day lag period when incubated with enriched methane cultures under anaerobic conditions(1). [(1) Chou WL et al; Biotech Bioeng Symp 8: 391-414 (1979)] **PEER REVIEWED**

PURE CULTURE: Pure cultures of Aerobacter and lactobacillus were found to degrade glycerin under anaerobic conditions(1,2). [(1) Chou WL et al; Biotech Bioeng Symp 8: 391-414 (1979) (2) Kazanskaya TB, Anyukhina YG; Mikrobiologiya 49: 240-3 (1980)] **PEER REVIEWED**

Environmental

Abiotic

Degradation:

The rate constant for the vapor-phase reaction of glycerin with photochemically-produced hydroxyl radicals has been estimated as 1.9×10^{-11} cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 7 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals per cu cm(1). Glycerin is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under environmental conditions(2). Glycerin does not contain chromophores that absorb at wavelengths >290 nm(2), and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5, 8-12 (1990)] **PEER REVIEWED**

Environmental

Bioconcentration:

An estimated BCF of 3 was calculated in fish for glycerin(SRC), using a log Kow of -1.76(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) Hansch C et al; Exploring

QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 7 (1995) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Jan, 2010. Available from, as of Jul 12, 2011: <http://www.epa.gov/oppt/exposure/pubs/episuite.html> (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Soil

Adsorption/Mobility:

Using a structure estimation method based on molecular connectivity indices(1), the Koc of glycerin can be estimated to be 1(SRC). According to a classification scheme(2), this estimated Koc value suggests that glycerin is expected to have very high mobility in soil. [(1) Meylan WM et al; Environ Sci Technol 26: 1560-67 (1992) (2) Swann RL et al; Res Rev 85: 17-28 (1983)] **PEER REVIEWED**

Volatilization

from

Water/Soil:

The Henry's Law constant for glycerin is 1.73×10^{-8} atm-cu m/mole(1). This Henry's Law constant indicates that glycerin is expected to be essentially nonvolatile from water and moist soil surfaces(2). Glycerin is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 1.68×10^{-4} mm Hg(3). [(1) Hine J, Mookerjee PK; J Org Chem 40: 292-8 (1975) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (3) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989)] **PEER REVIEWED**

Effluent

Concentrations:

Glycerin was found but not quantified in the primary waste treatment effluent of the Oak Ridge treatment facility in 1974(1). It was identified in the influent to the industrial wastewater treatment plant of the Kashima petrochemical complex, Japan(2). [(1) USEPA; Preliminary Assessment of Suspected Carcinogens in Drinking Water Interim Report to Congress (1975) (2) Matsui S et al; Wat Sci Tech 20: 201-10 (1988)] **PEER REVIEWED**

Sediment/Soil

Concentrations:

SOIL: Glycerin was detected in fine soil and sand particles during winter in the metropolitan area of Riyadh, Saudi Arabia at percent relative concentrations of (percent relative concentration): 1.11 (outside of city, close to traffic; mostly sand), 1.48 outside city, palm tree farm; mostly soil), 7.95 (Ministry of Health locale in city; mixture of sand and soil), 0.61 (City center; mixture of soil and sand). It was not detected in the Azizia market place in the city nor in a sand dune 50 miles north of the city. Samples were collected in November 2002(1). [(1) Rushdi Al et al; Arch Environ Contam Toxicol 49: 457-470 (2005)] **PEER REVIEWED**

Atmospheric

Concentrations:

URBAN/SUBURBAN: Glycerin was present in daytime and nighttime samples at 1.4 to 9.6 (6 of 21 samples positive) and 19.9 ng/cu m (1 of 10 samples positive), respectively; samples were collected during southeastern aerosol and visibility study conducted at the Great Smoky Mountain National Park, TN from July 15 to August 25, 1995(1). Glycerin was detected in urban organic aerosols (PM_{2.5}) from Nanjing, a "mega-city" in China. Samples were collected in July 2004 and January 2005. Mean daytime and nighttime summer concentrations were 56.5 and 44.9 ng/cu m, respectively; range of 12.1 to 220 and 26.0 to 76.3 ng/cu m, respectively. Mean daytime and nighttime winter concentrations were 25.0 and 26.1; range of 12.7 to 36.1 and 8.42 to 48.4 ng/cu m, respectively(2). [(1) Yu LE et al; Environ Sci Technol 39: 707-715 (2005) (2) Wang G, Kawamura K; Environ Sci Technol 39: 7430-7438 (2005)] **PEER REVIEWED**

Plant

Concentrations:

Glycerin was reported present in Cork Oak, *Quercus suber* L., in the cork at 65,000 ppm(1). [(1) Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Glycerin. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Jul 12, 2011: <http://www.ars-grin.gov/duke/> **PEER REVIEWED**

As taken from HSDB, 2012.

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 75.2

Units : %14-CO2 RECOVERED

Test Method : RADIORESPIROMETRY

Oxygen Condition : AEROBIC

Analysis Method : 14-CO2

Incub Time (days): 5

Microbial Pop : 25-40 (% SLUDGE)

Inoculum : ACTIVATED SLUDGE

Temp (deg C) : 25

pH : 7.3

Reference : BELLY,RT & GOODHUE,CT (1976)

Study Biodeg Eval: BFA

Rate : 36; 63

Units : %BODT

Test Method : BOD5

Incub Time (days): 5; 5

Inoculum : SEWAGE

Temp (deg C) : 20

Remarks : SECOND SAMPLE USED ADAPTED SEED

Reference : BRIDIE,AL ET AL. (1979)

Rate : 68

Test Method : WARBURG

Analysis Method : MANO

Chem Conc (ppm) : 50-250

Microbial Pop : 10 (%)

Reference : HEUKELEKIAN,H & RAND,MC (1955)

Rate : 64

Test Method : BOD

Analysis Method : BOD

Chem Conc (ppm) : 150-500

Rate : 55.75

Rate : 28.19

Units : %BODT UTILIZATION IN 22-24 HRS

Incub Time (days): 1

Chem Conc (ppm) : 720

Microbial Pop : 2316 (MG/L)

Reference : PLACAK,OR & RUCHHOFT,CC (1947)

Rate : 52.57

Reference : STAFFORD,W & NORTHUP,HJ (1955)

Rate : 75.3

Half Life (days) : .89

Test Method : SEWAGE DIE-AWAY

Incub Time (days): 8-10

Chem Conc (ppm) : 6000

Reference : WAGNER,R (1976)

Study Biodeg Eval: NE

Oxygen Condition : ANAEROBIC-WATER

Remarks : AMENABLE TO ULTIMATE ANAEROBIC BIODEGRADATION

Reference : SPEECE,RE (1983)

As taken from BIODEG.

Soil Column Transport:

SCOL GLYCEROL CAS# 56-81-5

Soil Col Transp: PERMEABILITY INVERSLY CORRELATED TO LOGP AND NOT SOLVENT DENSITY OR VISCOSITY, INCR AS BULK DENSITY OF SOIL DECR, BREAK THROUGH RELATED TO SOLVENT WITH LOW DIELECRIC CONSTANT

Soil Type : RANGER SHALE

% Org. Content : .28

Temperature (C): 22

Test Cond. : 1000-2000 G COMPACTED CLAY, OPTIMUM MOISTURE CONTENT, REPORTED PERMEABILITY RATES AT EQUILIBRIUM, 35 DAYS

Soil Movement : 0.9E-9 CM/SEC

Abbrev. Ref. : GREEN,WJ ET AL. (1981)

Oxidation and other reactions:

OXID GLYCEROL CAS# 56-81-5

Reactant : OH RADICAL- PH 7

Rate : .95E 09 L/MOL-SEC

Analyt. Meth. : CHEM

Test Cond. : IN AQ SOLN, RT, LITERATURE VALUE FOR OH RADICAL CONC IN WATER IS 1E-17 MOL/L

Abbrev. Ref. : ANBAR,M & NETA,P (1967)

Reactant : OH

Rate : .19E 10 L/MOL-SEC

Test Cond. : IN WATER, OH FORMED BY PULSED RADIOLYSIS, NEUTRAL PH, TEMP
NS

Remarks : RATE REL TO FERROCYANIDE ($K = 9.3E9$ L/MOL-SEC), RELIABLE
VALUE, H ABSTRACTION REACTION

Abbrev. Ref. : DORFMAN,LM & ADAMS,GE (1973)

Degradation in natural systems

NSYD GLYCEROL CAS# 56-81-5

Summary : DEGR IN ANAEROBIC REACTOR

System : WATER

Rate : 66% UTILIZATION

Analyt. Meth. : COD OR TOC REDUCTION

Test Cond. : ANAEROBIC COMPLETELY MIXED SYSTEM, 20 DAY HYDRAULIC
RETENTION TIME, INOCULUM MAINTAINED ON ACETATE, 500 MG/L,
RESULTS DAY 70-90

Abbrev. Ref. : CHOU,WL ET AL. (1979)

Microbial degradation

MICD GLYCEROL CAS# 56-81-5

Summary : RAPID DEGR

Species Name :

1 : PSEUDOMONAS AERUGINOSA QMB 1468

2 : ASPERGILLUS VERSICOLOR OM 432

Rate :

1 : 100% DEGR IN 7 DAYS

2 : DEGR

Analyt. Meth. : GROWTH

Test Cond. : 2% TEST CMPD, SOLE C SOURCE, MINERAL SALT MEDIUM, SHAKEN, 30
DEGC, 7 DAY INCUBATION

Abbrev. Ref. : STAHL,WH & PESSEN,H (1953)

1 : ALCALIGENES SP., MC11

2 : ALCALIGENES SP., TE8

3 : ALCALIGENES SP., PE18

4 : CORYNEBACTERIUM SP., OEH8

1 : GREW WELL

2 : GREW WELL

3 : GREW WELL

4 : GREW WELL

Test Cond. : MICROBES ISOLATED FROM SOIL EXCEPT PE18 ISOLATED FROM ACTIV

SLUDGE, 1% SUBSTRATE AS SOLE C SOURCE, MINERAL SALTS, SHAKER
FLASK, 30 DEGC, 6 DAY

Abbrev. Ref. : HARADA,T & NAGASHIMA,Y (1975)

Species Name : ALCALIGENES FAECALIS

Rate : DEGR

Analyt. Meth. : WARBURG TECHNIQUE

Test Cond. : 20 DEGC, 115-195 HR INCUBATION, MINERAL SALTS MEDIUM

Abbrev. Ref. : MARION,CV & MALANEY,GW (1963)

Summary : DEGR

1 : MYCOBACTERIUM SMEGMATIS 422

2 : MYCOBACTERIUM RHODOCHROUS 382

1 : DEGR

Analyt. Meth. : COLORIM

Test Cond. : MICROBES GROWN ON GLYCEROL, TEST CMPD .2% (V/V), MINERAL
SALTS, SHAKEN, PH 7.0, 1 HR INCUBATION

Abbrev. Ref. : LUKINS,HB & FOSTER,JW (1963)

1 : ARTHROBACTER- 2 STRAINS

2 : BACILLUS- 3 STRAINS

3 : ENTEROBACTERIACEAE- 4 STRAINS

4 : PSEUDOMONAS- 5 STRAINS

1 : GROWTH

2 : GROWTH (NA41, NA50), NO GROWTH (NA44)

3 : GROWTH

4 : GROWTH

Test Cond. : MINERAL SALT MEDIUM, TEST CMPD SOLE C AND ENERGY SOURCE,
INCUBATED ON METHYLAMINE AS SOLE N SOURCE, 25 DEGC, 3-6 DAY
INCUBATION

Abbrev. Ref. : BICKNELL,B & OWENS,JD (1980)

Species Name : ARTHROBACTER GLOBIFORMIS- 10 STRAINS

Rate : GOOD TO EXCELLENT DEGR

Test Cond. : MICROBES ISOLATED FROM SOIL, INCUBATED 4-5 DAYS, 25 DEGC,
MINERAL SALTS, SOLE C SOURCE

Abbrev. Ref. : JENSEN,HL (1964)

As taken from CHEMFATE.

EPISuite provides the following data:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	6.35E-009 atm-m3/mole (6.43E-004 Pa-m3/mole)

Group Method:	1.51E-015 atm-m3/mole (1.53E-010 Pa-m3/mole)
Exper Database	1.73E-08 atm-m3/mole (1.75E-003 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 9.671E-012 atm-m3/mole (9.799E-007 Pa-m3/mole) VP: 7.98E-005 mm Hg (source: MPBPVP) WS: 1E+006 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	-1.76 (exp database)
Log Kaw used:	-6.150 (exp database)
Log Koa (KOAWIN v1.10 estimate):	4.390
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	1.1799
Biowin2 (Non-Linear Model) :	0.9937
Biowin3 (Ultimate Survey Model):	3.4756 (days-weeks)
Biowin4 (Primary Survey Model) :	4.1032 (days)
Biowin5 (MITI Linear Model) :	0.9697
Biowin6 (MITI Non-Linear Model):	0.9744
Biowin7 (Anaerobic Linear Model):	1.1205
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	0.0224 Pa (0.000168 mm Hg)
Log Koa (Koawin est):	4.390
Kp (particle/gas partition coef. (m3/ug)):	0.000134
Mackay model:	6.03E-009
Octanol/air (Koa) model:	

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.00481
Mackay model:	0.0106

Octanol/air (Koa) model:	4.55E-009

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	18.7343 E-12 cm ³ /molecule-sec
Half-Life =	0.571 Days (12-hr day; 1.5E6 OH/cm ³)
Half-Life =	6.851 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.00771 (Junge-Pankow, Mackay avg) 4.82E-007 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	1 L/kg (MCI method)
Log Koc:	0.000 (MCI method)
Koc :	0.1345 L/kg (Kow method)
Log Koc:	-0.871 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 1.73E-008 atm-m³/mole (Henry experimental database)

Half-Life from Model River:	3.248E+004 hours (1353 days)
Half-Life from Model Lake:	3.544E+005 hours (1.477E+004 days)

Removal In Wastewater Treatment:

Total removal:	1.85 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.75 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.484	13.7	1000

Water	35.3	208	1000
Soil	64.2	416	1000
Sediment	0.0624	1.87e+003	0

Persistence Time: 346 hr

“Glycerol, a non-biodegradable by-product during biodiesel production is a major concern to the emerging biodiesel industry. Many microbes in natural environments have the ability to utilize glycerol as a sole carbon and energy source. The focus of this study was to screen for microorganisms from soil, capable of glycerol utilization and its conversion to value added products such as ethanol and 1,3-propanediol (1,3-PDO). Twelve bacterial isolates were screened for glycerol utilization ability in shake flask fermentations using M9 media supplemented with analytical grade glycerol (30 g/L) at various pH values (6, 7 and 8) and temperatures (30°C, 35°C and 40°C). Among these, six bacterial isolates (SM1, SM3, SM4, SM5, SM7 and SM8) with high glycerol degradation efficiency (>80%) were selected for further analysis. Highest level of 1,3-PDO production (15 g/L) was observed with isolate SM7 at pH 7 and 30°C, while superior ethanol production (14 g/L) was achieved by isolate SM9 at pH 8 and 35°C, at a glycerol concentration of 30 g/L. The selected strains were further evaluated for their bioconversion efficiency at elevated glycerol concentrations (50-110 g/L). Maximum 1,3-PDO production (46 g/L and 35 g/L) was achieved at a glycerol concentration of 70 g/L by isolates SM4 and SM7 respectively, with high glycerol degradation efficiency (>90). Three isolates (SM4, SM5 and SM7) also showed greater glycerol tolerance (up to 110 g/L). The isolates SM4 and SM7 were identified as *Klebsiella pneumoniae* and SM5 as *Enterobacter aerogenes* by 16S rDNA analysis. These novel isolates with greater glycerol tolerance could be used for the biodegradation of glycerol waste generated from the biodiesel industry into value-added commercial products.” As taken from Raghunandan K et al. 2014. J. Environ. Sci. Health A Tox. Hazard, Subst. Environ. Eng. 49(1), 85-92. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24117087>

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that glycerol is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	5000
Experimental result iT (mg/l)	5000
Test species iT (Latin)	<i>Carassius auratus</i>
Test species iT (Common)	Goldfish
Final EndPoint iT	LC50
Exposure duration iT (hours)	24
Reference iT	Water Res. 13(7):623-626 (OECDG Data File)
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	184,000
Toxicity to fish (LC50 in mg/l) as predicted by PNN	1,395.65311
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	19,400

Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	77,712.039
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	1,593.083
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.84E+005

ROBUST STUDY SUMMARY - Inherent Toxicity

Reference: Bridié, A., C. Wolff and M. Winter, 1979. "The acute toxicity of some petrochemicals to goldfish", Water Research, vol 13, pp. 623-626.		
<u>Test Substance</u> (CAS # and name): 56815 Glycerol		
Item	Yes	No
<u>Test Substance</u>		
*Chemical composition of the substance (including purity, by-products)	X	
Persistence/stability of test substance in aquatic solution		
<u>Method</u>		
References	X	
*OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Na	
*GLP (Good Laboratory Practice)	Na	
<u>Test organisms</u> (specify common and Latin names): Carrasius auratus (Goldfish)		
Latin names reported?	X	
Life cycle age / stage of test organism		X
Sex		X
Length and weight of test organisms	X	
Number of test organisms per replicate	X	
Food type / feeding periods (acclimation/during test)		X
<u>Test design / conditions</u>		
Test type – acute or chronic (specify, but <u>do not assess this item</u>): 24-hr acute TLM		
Experiment type (laboratory or field) specified?	X	
System type (static, semi-static, flow through)?	X	
Negative or positive controls (specify)?		X

Number of replicates (including controls) and concentrations	X	
Exposure pathways (food, water, both)	X	
Exposure duration	X	
*Measured concentrations reported?		X
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	X	
Photoperiod and light intensity		X
Stock and test solution preparation		X
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances)	Na	
Analytical monitoring intervals	X	
Statistical methods used	X	
<u>Results</u>		
Toxicity values (LC ₅₀ , EC ₅₀ , or IC ₅₀ - specify, <u>do not assess this item</u>): 24-hr TLm >5000 mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, <u>do not assess this item</u>):		
*Was toxicity value below the chemical's water solubility?	X	
Other adverse effects (carcinogenicity, mutagenicity, etc. <u>Do not assess this item</u>):		
<u>Score</u> : major items - ...3/4; overall score: 15/23 = 65%		
EC Reliability code: 2		
EC Reliability category (high, satisfactory, low): Satisfactory		
Comments		
This paper was marked down due to the lack of detail concerning some aspects of the bioassay procedure. The actual toxicity was not established; the highest tested concentration had no effect. GLP is not applicable.		

Data accessed May 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

Type:	Static
Species:	Leuciscus idus melanotus (Fish, fresh water)
Unit:	mg/l
LC0:	> 10000
LC50:	> 10000
LC100:	> 10000
Year:	1978

Method:	TEST ORGANISMS
Result:	- Nominal concentrations (mg/L): 10000 - Mortality: none - Other effects: no data - Dose related effects: no data
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Conclusion:	Most appropriate study available.
Reliability:	(4) not assignable The report was essentially confined to what is included in the current summary. There was no information on control mortality, physical-chemical parameters, actual tested concentrations, feeding rate, number and size of fish tested and photoperiod during the test and the duration of the test.
Flag:	Critical study for SIDS endpoint 19.12.2001 (38)
Type:	Static
Species:	Carassius auratus (Fish, fresh water)
Exposure period:	24 hour(s)
Unit:	mg/l
LC50:	> 5000
Analytical monitoring:	Yes
Method:	other: Standard Methods for Examination of Water and Wastewater. Am. Publ. Health Assoc. Inc., New York, Method No. 231
Year:	1979
Method:	TEST ORGANISMS - Species: Carassius auratus - Size;weight;loading: 62+/-7 mm; 3.3+/-1.0 g; 1.3 g/L DILUTION WATER - Source: local tap-water - Chemistry: Alkalinity 30 mg Na+/L; 65 mg/L (Cl)-, 4 mg/L (NO3)-, 35 mg/L (SO4)2-, 0.15 mg/L (PO4)3-, 25 mg/L (HCO3)-, 25 mg/L SiO2, 0.05 mg/L Fe, 100 mg/L (Ca)2+, 8 mg/L (Mg)2+; pH 7.8 TEST SYSTEM - Test type: static - Concentrations: 5000 mg/L

	- Exposure vessel type: 33 L glass vessels containing 25 L of test solution. - Number of fish: 10 per replicate, 1 replicate/treatment - Test temperature: 20±1 °C - Dissolved oxygen: >4 mg/L DURATION OF THE TEST: 24 hours TEST PARAMETER: mortality OBSERVATION TIMES: 24 hours ANALYSES: - Method: TOC analysis or extraction followed by GC analysis - Sampling times: 0 and 24 hours
Result:	- Nominal concentrations (mg/L): 5000 - Measured concentrations (mg/L): not reported - Mortality: <50%
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.
Conclusion:	Although the study has an exposure time of 24-hours, it is marked as the critical study, because the underlying publication provides relative much information on the test design.
Reliability:	(4) not assignable 1. The information in the report (Secondary literature) was essentially confined to what is included in the current summary. Actually the report consists of an overview of the fish toxicity for a number of petrochemicals. No actual mortality rates at the tested concentration was reported. 2. The loading is with 1.3 g/L slightly higher than recommended by OECD 203 (1 g/L).
Flag:	Critical study for SIDS endpoint 23.01.2002 (39)
Type:	other: embryogenesis
Species:	Cyprinus carpio (Fish, fresh water)
Type:	Static
Species:	Leuciscus idus melanotus (Fish, fresh water)
Unit:	mg/l
LC0:	> 10000
LC50:	> 10000
LC100:	> 10000
Year:	1978
Method:	TEST ORGANISMS

	- Species: <i>Leuciscus idus melanotus</i> - Supplier: not specified - Age/size/weight/loading: not specified - Feeding (pretreatment): not specified - Feeding during test: not specified STOCK AND TEST SOLUTION AND THEIR PREPARATION - no information included in the report DILUTION WATER - no information included in the report TEST SYSTEM - only information available about the test system was that this test was based on the static fish toxicity test with the Goldorfe after Mann (1975/1976) DURATION OF THE TEST: not specified TEST PARAMETER: mortality OBSERVATION TIMES: not specified STATISTICAL METHOD: not specified
Result:	RESULTS: - Nominal concentrations (mg/L): 10000 - Mortality: none - Other effects: no data - Dose related effects: no data
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Conclusion:	Most appropriate study available
Reliability:	(4) not assignable The report was essentially confined to what is included in the current summary. There was no information on control mortality, physical-chemical parameters, actual tested concentrations, feeding rate, number and size of fish tested and photoperiod during the test and the duration of the test.
Flag:	Critical study for SIDS endpoint. 19.12.2001 (38)
Type:	Static
Year:	1997
Test substance:	other TS
Method:	This test was set up to study the toxicity of different cryoprotective agents on

	carp embryos at different developmental stages. This is important for the design of cryopreservation protocols for carp embryos. Embryos were selected at three developmental stages (morula, halfepiboly and heartbeat) and subsequently equilibrated for 5 minutes and 1 hour at 24 C in 1 M solution of glycerol in water (=92 g/L). A control group was included in which embryos were equilibrated to water of 24 C. At the end of the equilibration period, embryos were transferred into water and incubated at 24 C. Survival was defined as percentage of treated embryos that hatched. Statistical method: Chi-square method.	
Result:	Hatching rates of carp embryos (%) for embryos exposed respectively at morula stage, half-epiboly stage and heartbeat stage: - Control: 95, 94, 91 - Glycerol (5 min): 80*, 94, 86* - Glycerol (1 h): 14*, 14*, 78* *Statistically different from control treatment	
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability:	(4) not assignable 1. The information in the report was confined to the above mentioned. 2. The study was designed to test the toxicity of glycerol, when used as a cryoprotective substance. The results of this test are not relevant for ecotoxicological purposes, because the tested concentration is extremely high. 08.01.2002 (40)	
Type:	static	
Species:	Leuciscus idus (Fish, fresh water)	
Exposure period:	48 hour(s)	
Unit:	Mg/l	
LC0:	> 250	
Method:	other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15	
Year:	1968	
GLP:	No	
Remark:	250 mg/l was highest concentration tested. Test method conforms with OECD Guideline 203. 10 fish per tested concentration were used.	
Test substance:	CAS 56-81-5 (Glycerine), purity 99.5%.02.01.2002 (41)	
Type:	static	
Species:	Oncorhynchus mykiss (Fish, fresh water)	

Exposure period:	96 hour(s)
Unit:	Mg/l
LC100:	= 51000 - 57000
Year:	1980
Remark:	Literature could not be retrieved.
Source:	Unichema Chemie B.V. Gouda
Test condition:	- Rainbow trout of 0.9 g; - 12 °C, hardness 40-50 mg/l CaCO₃, alkalinity 30-35 mg/l CaCO₃, pH 7.2-7.5; - Reconstituted dilution water; - Unmeasured concentration. 25.01.2002 (42)

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type:	static
Species:	Daphnia magna (Crustacea)
Exposure period:	24 hour(s)
Unit:	Mg/l
EC50:	> 10000
EC100:	> 10000
Analytical monitoring:	no data
Year:	1982
GLP:	no data
Method:	TEST ORGANISMS - Species: Daphnia magna STRAUS IRCHA - Source/supplier: not indicated - Breeding method: 20-30 Daphnia in 2L beaker glasses with at least 1.6 L water (286 mg CaCO₃/L, pH 7.6-7.7), fed daily, 9 h light, 20 C; young daphnids were removed daily - Age: ≤24 h - Feeding (pretreatment): daily - Feeding during test: not specified

	STOCK AND TEST SOLUTION AND THEIR PREPARATION Vehicle, solvent: none DILUTION WATER - Source: synthetic test medium in accordance with ISO 6341 (medium recommended by OECD 202) - Chemistry (sum of Ca and Mg: 2.5 mmol/L; Na/K ratio: 10/1; pH: 8.0+/- 0.2) TEST SYSTEM - Test type: static - Concentrations: not specified - Exposure vessel type: 50 mL glass beakers containing 20 mL test medium - Number of individuals: 10 per replicate, 2 replicates/treatment - Photoperiod (intensity of irradiation): not specified PHYSICAL MEASUREMENTS - Measuring times: 0 and 24 hours - Test temperature: 20 C - Dissolved oxygen: measured but not reported - pH: 8.0+/-0.2 (0 hour), measured but not reported (24 hours) - Adjustment of pH: no DURATION OF THE TEST: 24 hours TEST PARAMETER: immobility OBSERVATION TIMES: 24 hours REFERENCE SUBSTANCE: Potassium dichromate STATISTICAL METHOD: Chi-Square test
Result:	RESULTS: - Nominal concentrations (mg/L): 10 000 - Immobility: <50% - Other effects: no data - Dose related effects: no data RESULTS: TEST WITH REFERENCE SUBSTANCE - Results: EC50 1.3 mg/L
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Conclusion:	Most elaborate study available.
Reliability:	(4) not assignable The report was essentially confined to what is included in the current summary. There was no information or limited information on control mortality, physico-chemical parameters, actual tested concentrations and feeding rate.

Flag:	Critical study for SIDS endpoint. 19.12.2001 (43)
Type:	static
Species:	Daphnia magna (Crustacea)
Exposure period:	24 hour(s)
Unit:	Mg/l
EC50:	> 10000
Analytical monitoring:	No data
Method:	other: not indicated
Year:	1977
GLP:	No data
Test substance:	other TS
Remark:	Daphnia magna collected from small pond, were exposed to several test substance concentrations for 24 h at 20 - 22 degr. C (pH before addition of test substance: 7.6 - 7.7, no adjustment of pH after addition of test substance). For the experiment 10 animals per replicate and 3 replicates were used.
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.
Reliability:	(4) not assignable The information in the report was confined to the above. 19.12.2001 (44)
Species:	Daphnia magna (Crustacea)
Exposure period:	24 hour(s)
Unit:	mg/l
EC0:	> 500
Analytical monitoring:	No
Method:	other: Daphnien-Kurzzeittest, DIN 38412 Teil 11, Bestimmung
	der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse
GLP:	No
Remark:	500 mg/l was highest concentration tested. Test method conforms with OECD Guideline

	202 A. Literature could not be retrieved.	
Source:	Unichema Chemie B.V. Gouda. 15.11.2001 (45)	
Species:	other: Daphnia	
Exposure period:	48 hour(s)	
Unit:	mg/l	
LC50:	= 153000	
Method:	other: calculated	
Year:	1999	
Remark:	Calculated with EPIWIN, part ECOSAR v0.99f.	
Reliability:	(4) not assignable	

TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species:	Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint:	other: inhibition of cell growth after 8 days
Exposure period:	8 day(s)
Unit:	mg/l
EC3:	2900
Year:	1978
Method:	TEST ORGANISMS - Species: Microcystis aeruginosa - Source/supplier: not specified - Method of cultivation: stemculture and preculture were maintained for 10 days in closed 100 mL Erlenmeyer containing 20 mL medium at 27 C and relative humidity of 50% before a new culture was set up. - Initial cell concentration: equivalent to a turbidity value corresponding to TE/F/578 nm = 20 DILUTION WATER - Source: dist. water GROWTH/TEST MEDIUM CHEMISTRY - Test medium contains 24.8 mg/L NaNO₃, 2.0 mg/L K₂HPO₄, 75 mg/L MgSO₄·7H₂O, 36 mg/L CaCl₂·2H₂O, 40 mg/L Na₂SiO₃, 58 mg/L Na₂CO₃, 3 mg/L C₆H₈O₇·H₂O, 3 mg/L C₆H₅FeO₇·5H₂O, 10 mg/L

	C10H14N2Na2O8.2H2O, 114 ug/L H3BO3, 72 ug/L MnCl2.4H2O, 8.8 ug/L ZnSO4.7H2O, 3.2 ug/L CuSO4.5H2O, 0.96 ug/L Na2MoO4.2H2O, 1.6 ug/L CoCl2.6H2O - Chemistry (Hardness: 0.55 mmol/L Ca+Mg; P: 0.34 mg/L; N: 4.1 mg/L; chelators 0.03 mmol/L) - pH 7.0 TEST SYSTEM - Test type: static, daily shaken - Concentrations: 10 solutions with unspecified concentrations (stock solution is 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 times diluted) - Exposure vessel type: culture tubes containing 10 mL test solution - Number of replicates: 3 (test substance), 1 (control) - Photoperiod (intensity of irradiation): continuous PHYSICAL MEASUREMENTS - no data DURATION OF TEST: 8 days TEST PARAMETER: inhibition of turbidity after 8 days (extinction at 578 nm); more than 3% difference in extinction is considered as an effect OBSERVATION TIMES: after 8 days STATISTICAL METHOD: not specified
Result:	EC3 2900 mg/L
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Conclusion:	Most sensitive toxicity threshold value available.
Reliability:	(4) not assignable 1. The test was not in accordance with OECD 201: test medium was different and extinction was only measured after 8 days. No quantitative relationship changes between extinction and growth inhibition was provided. Therefore it was not possible to deduce a 72-hours EC50 value for the test substance. It is clear from this report, that glycerine is not very toxic for algae in the aquatic environment. It is not possible to confirm, however, whether algae were in the exponential growth phase for the duration of the test. 2. The turbidity of the test solution is expressed in "TE/F/578 nm". This refers to a measurement of the extinction of the test solution at 578 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027). 3. All publications refer to the same study. 4. Review articles containing multiple substances. Bringmann & Kuehn (GWF Wasser/abwasser, 117, 410-413, 1976) contained a comparison of the toxicity of <i>Pseudomonas putida</i> with <i>Microcystis aeruginosa</i> and in Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) the toxicity of <i>Microcystis aeruginosa</i> was compared to the toxicity of <i>Scenedesmus quadricauda</i>.

Flag:	25.01.2002 (46) (47) (48) (49)
Species:	Scenedesmus quadricauda (Algae)
Endpoint:	other: inhibition in cell growth after 8 days
Exposure period:	8 day(s)
Unit:	mg/l
EC3:	> 10000
Year:	1978
Method:	TEST ORGANISMS - Species: Scenedesmus quadricauda - Source/supplier: not specified - Method of cultivation: stemculture and preculture were maintained for 10 days in closed 100 mL erlenmeyer containing 20 mL medium at 27 C and relative humidity of 50% before a new culture was set up - Initial cell concentration: corresponding to an extinction value corresponding to a turbidity value of TE/F/578 nm = 20 DILUTION WATER - Source: dist. water GROWTH/TEST MEDIUM CHEMISTRY - Chemistry (Hardness: 0.55 mmol/L Ca+Mg; P: 0.34 mg/L; N: 4.1 mg/L; chelators 0-0.03 mmol/L) - pH 7.0 TEST SYSTEM - Test type: static, daily shaken - Concentrations: not specified - Exposure vessel type: culture tubes containing 10 mL of test solution - Number of replicates: 3 - Photoperiod (intensity of irradiation): continuous PHYSICAL MEASUREMENTS - no data DURATION OF TEST: 7-8 days TEST PARAMETER: inhibition of turbidity after 8 days (extinction at 578 nm); 3% reduction of extinction is considered as an inhibitory effect OBSERVATION TIMES: after 8 days

	STATISTICAL METHOD: not specified - Species: <i>Scenedesmus quadricauda</i> - Source/supplier: not specified - Method of cultivation: stemculture and preculture were maintained for 10 days in closed 100 mL erlenmeyer containing 20 mL medium at 27 °C and relative humidity of 50% before a new culture was set up - Initial cell concentration: corresponding to an extinction value corresponding to a turbidity value of TE/F/578 nm = 20 DILUTION WATER - Source: dist. water GROWTH/TEST MEDIUM CHEMISTRY - Chemistry (Hardness: 0.55 mmol/L Ca+Mg; P: 0.34 mg/L; N: 4.1 mg/L; chelators 0-0.03 mmol/L) - pH 7.0 TEST SYSTEM - Test type: static, daily shaken - Concentrations: not specified - Exposure vessel type: culture tubes containing 10 mL of test solution - Number of replicates: 3 - Photoperiod (intensity of irradiation): continuous PHYSICAL MEASUREMENTS - no data DURATION OF TEST: 7-8 days TEST PARAMETER: inhibition of turbidity after 8 days (extinction at 578 nm); 3% reduction of extinction is considered as an inhibitory effect OBSERVATION TIMES: after 8 days STATISTICAL METHOD: not specified
Result:	EC3 >10000 mg/L All publications refer to the same study.
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Reliability:	(4) not assignable 1. The test was not in accordance with OECD 201: the test medium was different and extinction was only measured after 8 days. No quantitative relationship changes between extinction and growth inhibition were provided. Therefore it was not possible to deduce a 72-hours EC50 value for the test substance. It is clear from this report, that glycerine is not very toxic for algae in the aquatic environment.

2. The turbidity of the test solution is expressed in "TE/F/578 nm". This refers to a measurement of the extinction of the test solution at 578 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027). 3. All publications probably refer to the same study. 4. In Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) a comparison was made for the toxicity of several substances for <i>Microcystis aeruginosa</i> and <i>Scenedesmus quadricauda</i>. 19.12.2001 (46) (50) (51) (48)	
Species:	other algae: Green Algae
Exposure period:	96 hour(s)
Unit:	mg/l
EC50:	= 77712
Method:	other: calculated
Year:	1999
Remark:	Calculated with EPIWIN, part ECOSAR v0.99f.
Reliability:	(4) not assignable 19.12.2001 (10)
Species:	other aquatic plant
Endpoint:	growth rate
Exposure period:	28 day(s)
Year:	1970
Method:	TEST ORGANISMS - Species: several species with their suppliers are included below. CHLOROPHYCEAE: 1. <i>Dunaliella tertiolecta</i> Butcher (Wood Hole clone "Dun", Dr J. Strickland) 2. <i>Tetraselmis maculata</i> Butcher (Nanaimo strain "TMD", Dr T. Parsons) 3. <i>Nannochloris oculata</i> Droop (Millport strain no. 66, Dr M. Droop) 4. <i>Brachiomonas submarina</i> (Bohlin) Droop var. <i>pulsifera</i> 7/2a (Millport strain no. 44, Dr M. Droop) CHRYSOPHYCEAE 5. <i>Monochrysis lutheri</i> Droop (Millport strain no. 60, Dr J. Strickland) 6. <i>Isochrysis galbana</i> Parke (Woods Hole clone "Iso", Dr J. Strickland) 7. <i>Coccolithus huxleyi</i> (Lohm) Kamptner (Woods Hole clone "BT-6", Dr T. Parsons) 8. <i>Prymnesium parvum</i> Carter (Reich's Israeli strain, Dr M. Rahat)

	BACILLARIOPHYCEAE

TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type:	aquatic
Species:	Pseudomonas putida (Bacteria)
Exposure period:	16 hour(s)
Unit:	mg/l
EC0:	10000
Method:	DIN 38412, part8
Year:	1994
GLP:	Yes- Species: Pseudomonas putida MIGULA Stamm Berlin 33/2 (DSM 50026 - Source/supplier: TTB-Mikrobiologie, Henkel KGaA TEST DESCRIPTION A stock solution of glycerine of 99.6 g/L (pH 5.3) was prepared. Test solutions (100 mL) were prepared by adding together the required volume of stock solution, nutrient medium (according to DIN 38412), water and inoculum of Pseudomonas putida. Test concentrations were 100, 300, 1000, 3000 and 10000 mg/l. One replicate from each treatment was shaken (100 rpm) for 16 h at 21-22°C. For the highest test concentration 3 replicates were included and also 3 replicates of a control treatment were included. At the end of the test, the extinction (436 nm) was measured.
Result:	Based on the turbidity of the solution the growth of the bacteria can be estimated. For all test concentrations (100-10000 mg/L) no effects on the growth of bacteria were found. Compared to the control a positive effect of 0.5-3.4% was seen on the growth.
Test substance:	CAS 56-81-5 (glycerine), purity 99.5%.
Reliability:	(2) valid with restrictions 1. The EC0 is set on the highest tested concentration of 10000 mg/L. 2. The guideline DIN 38412, part 8 contains the following validity criterium: the turbidity in the control treatment should be increased with a factor of 100 during the test. Because there is no information on the initial turbidity, it is not clear whether this validity criterium was met. If however the test was performed in accordance with the guideline mentioned in the report, the initial turbidity was "TE/F=5". From the report it was clear that at the end of the test the turbidity in the control was "TE/F=592", which suggests that the validity criterium was met. 3. The turbidity of the test solution is expressed in "TE/F/436 nm". This refers to a measurement of the extinction of the test solution at 436 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027). 4. The report was essentially confined to what is included in the current summary. According to the reviewer there is enough information to give this study a reliability of 2. 19.12.2001 (54)

Type:	aquatic
Species:	Chilomonas paramecium (Protozoa)
Exposure period:	48 hour(s)
Unit:	mg/l
EC5:	> 10000
Year:	1980
Test substance:	other TS
Method:	TEST ORGANISMS - Species: Chilomonas paramecium Ehrenberg - Laboratory culture: yes - Initial cell concentration: 3.0E3 cells/ml STOCK AND TEST SOLUTION AND THEIR PREPARATION - Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a total test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water. DILUTION WATER - bidistilled water GROWTH/TEST MEDIUM CHEMISTRY - Medium (pre- and stem culture): 3190 mg/L glycine, 115 mg/L Ca(NO₃)₂·4H₂O, 28 mg/L Mg(NO₃)₂·7H₂O, 16 mg/L KNO₃, 10 mg/L K₂HPO₄, 0.8 mg/L biotin (as D-Biotin), 8.0 mg/L nicotinic acid, 4.0 mg/L thiamin.HCl, 4.0 mg/L p-aminobenzoic acid, 2.0 mg/L panthotenic acid (as D-Panthotenic acid, Na-salt), 20 mg/L pyridoxamine (as pyridoxaminedihydrochlorid), 8.0 mg/L cyanocobalamin (vitamine B12); sterilised and pasteurised. - Test medium: mixture of solution 1 (sterilised) an solution 2 (pasteurised) containing 7940 mg/L glycine, 288 mg/L Ca(NO₃)₂·4H₂O, 69 mg/L Mg(NO₃)₂·7H₂O, 40 mg/L KNO₃, 26 mg/L K₂HPO₄, 15 ug/L biotin (as DBiotin), 149 ug/L nicotinic acid, 74 ug/L thiamin.HCl, 74 ug/L paminobenzoic acid, 37 ug/L panthotenic acid (as D-Panthotenic acid, Nasalt), 372 ug/L pyridoxamine (as pyridoxaminedihydrochlorid), 149 ug/L cyanocobalamin (vitamine B12) TEST SYSTEM - Test cultures are prepared from pre-cultures (7 d, 20 C). Pre-cultures are prepared from stem cultures (3-4 d, 20 C). - Test solution: 8 mL of test substance solution, 8 mL of test medium and 4 mL of pre-culture (15,000 cells/mL). - Temperature: 20 C DURATION OF TEST: 48 hours TEST PARAMETER: cell growth using a Coulter counter

	OBSERVATION TIMES: 48 hours
Result:	At 10,000 mg/L the effect of glycerine was less than 5%.
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.
Conclusion:	>10,000 mg/L
Reliability:	(4) not assignable The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (171 substances included). 19.12.2001 (55) (56)
Type:	aquatic
Species:	Clostridium sp. (Bacteria)
Year:	1992
Method:	Clostridium pasteurianum is known as a classical acid producer and usually ferments carbohydrates to butyrate, acetate, carbon dioxide and molecular hydrogen. Recent reports indicate the ability of Clostridium pasteurianum to produce acetone, butanol and ethanol in media with high glucose concentrations. Therefore this test was set up to study the ability of Clostridium pasteurianum to produce solvents from renewable biomass. In the test glucose and glycerol were included, but in this summary only the part related to glycerol is included. GROWTH MEDIUM - Components: 1.74 g/L K₂HPO₄, 0.66 g/L NH₄Cl, 0.251 g/L MgSO₄·7H₂O, 0.596 g/L KCl, 69 mg/L Fe-Na-EDTA, 6 g/L NaHCO₃, 4 mg p-aminobenzoic acid, 0.24 mg/L biotin, 0.5 g/L yeast extract, 1 mg/L resazurin, 0.5 g/L cysteine-HCl and 20-200 g/L glycerol - pH 7.0 - gas phase: 80% v/v N₂ and 20% v/v CO₂ TEST DESIGN - EXPERIMENT 1: The effect on different concentrations of glycerol in the growth medium is determined using concentrations of 20, 40, 60, 80, 100, 120, 170 and 200 g glycerol/L. - EXPERIMENT 2: The effect of iron limitation on glycerol fermentation is tested using an adapted growth medium (no yeast extract or Fe-Na-EDTA, supplemental FeSO₄·H₂O added (0, 3 or 20 µM) containing 40 g/L glycerol. ENDPOINT - Growth: measured spectrophotometrically by determination of the optical density at 578 nm. - Products (ethanol, acetate, butanol, butyrate and propanediol) are

	- measured by a gas chromatographic method
Result:	EXPERIMENT 1: - Fermentation resulted in the production of mainly ethanol, propanediol and butanol. Acetate and butyrate were only produced in trace amounts. -The tolerance of <i>C. pasteurianum</i> to glycerol was rather high. -Good growth was obtained at glycerol concentrations of up to 170 g/L. The optimum growth was found at glycerol concentrations of 100 g/L. EXPERIMENT 2: -Less iron in the medium resulted in lower amounts of reaction products with a changed pattern (ethanol/butanol decreased, lactate increased)
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Conclusion:	Good growth at glycerol concentrations up to 170 g/L.
Reliability:	(4) not assignable 1. Secondary literature. 2. The test was not set up to determine the toxicity of glycerol, but can be used for this purpose. The sensitivity of the tested organism in comparison with other micro-organisms is not known. 19.12.2001 (57)
Type:	aquatic
Species:	Entosiphon sulcatum (Protozoa)
Exposure period:	72 hour(s)
Unit:	mg/l
EC5:	3200
Year:	1980
Method:	TEST ORGANIS- Species: Entosiphon sulcatum - Laboratory culture: yes - Initial cell concentration: 1.5E3 cells/ml STOCK AND TEST SOLUTION AND THEIR PREPARATION - Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a total test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water.

	DILUTION WATER - bidistilled water GROWTH/TEST MEDIUM CHEMISTRY - Medium (stem-, pre- and test culture): 290 mg/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 70 mg/L $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 40 mg/L KNO_3; pH 6.9. TEST SYSTEM - Test cultures are prepared from precultures (72 h, 25 C). Pre-cultures are prepared from stem cultures (72-96 h, 25 C). - Test solution: 8 mL of test substance solution, 8 mL of test medium, 2 mL bacteria suspension and 2 mL of preculture (15,000 cells/mL). - Temperature: 25 C DURATION OF TEST: 72 hours TEST PARAMETER: cell growth using a Coulter counter OBSERVATION TIME: 72 hours MS
Result:	EC5 = 3200 mg/L
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.
Reliability:	(4) not assignable
	The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (171 substances included). 19.12.2001 (50) (55) (58)
Type:	aquatic
Species:	<i>Pseudomonas putida</i> (Bacteria)
Exposure period:	16 hour(s)
Unit:	mg/l
EC3:	> 10000
Analytical monitoring:	no data
Method:	other: cell multiplication inhibition test
Year:	1980
GLP:	no data
Test substance:	other TS: glycerol. No indication about purity.
Method:	TEST ORGANISMS

	Species: <i>Pseudomonas putida</i> Source/supplier: not specified Method of cultivation: stemculture and preculture were renewed weekly (incubation at 25 C for 24 h) Initial cell concentration: equivalent to an extinction value corresponding to a turbidity value of TE/F/436 nm = 10 DILUTION WATER Source: bidistilled water ROWTH/TEST MEDIUM CHEMISTRY Chemistry: 1 g/L glucose, 0.21 g/L NaNO₃, 0.12 g/L K₂HPO₄, 60 mg/L H₂PO₄, 10 mg/L FeSO₄.7H₂O, 0.2 g/L MgSO₄.7H₂O, 0.083 mg/L I₂(SO₄)₃.18H₂O, 0.042 mg/L KI, 0.042 mg/L KBr, 0.083 mg/L TiO₂, .042 mg/L SnCl₂.2H₂O, 0.042 mg/L LiCl, 0.58 mg/L MnCl₂.4H₂O, 0.92 g/L H₃BO₃, 0.083 mg/L ZnSO₄.7H₂O, 0.083 mg/L CuSO₄.5H₂O, 0.089 g/L NiSO₄.6H₂O, 0.083 mg/L Co(NO₃)₂.6H₂O ECD SIDS GLYCEROL TEST SYSTEM - Test type: static - Concentrations: not specified, dilutions of stock in 1 to 16384 (2EXP0-2EXP14) parts of stock; controls containing saline instead of bacteriasuspension - Temperature: 25 C DURATION OF TEST: 16 h TEST PARAMETER: inhibition of turbity after 16 hours (extinction at 436 nm); 3% reduction of extinction is considered as an inhibitory effect OBSERVATION TIMES: after 16 hours STATISTICAL METHOD: not specified Result: EC₃ >10000 mg/L
Test substance:	CAS 56-81-5 (glycerine), purity not indicated
Reliability:	(4) not assignable 1. The test is not in accordance with the current accepted guidelines, but gives an indication of the toxicity of glycerine to <i>Pseudomonas putida</i>. 2. The turbidity of the test solution is expressed in "TE/F/436 nm". This refers to a measurement of the extinction of the test solution at 436 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027). 3. Secondary literature with information essentially confined to what is included in the current summary. 25.01.2002 (59) (50) (51)
Type:	aquatic
Species:	<i>Pseudomonas putida</i> (Bacteria)
Exposure period:	75 minute(s)
Unit:	mg/l

Year:	1998
GLP:	no data
Test substance:	other TS
Method:	Test solutions (6 mL) were prepared by adding together A. 5 mL distilled water, 0.5 mL imidazol buffer, 1 mL resazurin solution and 0.5 mL test culture (=reagent control) B. 5.5 mL borate buffer, 1 mL resazurin solution (=blank sample) C. solution of glycerol, 0.5 mL imidazol buffer, 1 mL resazurin solution and 0.5 mL test culture (= test sample) Test concentrations were not specified. Two replicates from each treatment were incubated for 75 minutes at 37 C. At the end of the test, the extinction (615 nm) was measured.
Remark:	literature could not be retrieved.
Result:	Mean of 5 experiments: C50 760000 mg/L
Test substance:	CAS 56-81-5 (glycerine), purity not indicated. 9.12.2001 (12)
Type:	aquatic
Species:	ronema parduzci (Protozoa)
Exposure period:	0 hour(s)
Unit:	mg/l
EC5:	> 10000
Analytical monitoring:	no
Year:	1981
Method:	TEST ORGANISMS - Species: Uronema parduczi Chatton-Lwoff - Laboratory culture: yes - Initial cell concentration: 1.5E3 cells/ml - Feeding before test: Escherichia coli (alive) - Feeding during test: Escherichia coli (inactivated) STOCK AND TEST SOLUTION AND THEIR PREPARATION - Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water. DILUTION WATER

	- bidistilled water GROWTH/TEST MEDIUM CHEMISTRY - medium used for stem-culture: 228 mg/L CaCl ₂ .2H ₂ O, 86 mg/L MgSO ₄ .7H ₂ O, 29 mg/L KH ₂ PO ₄ , 57 mg/L K ₂ HPO ₄ - medium used for pre-and test culture: 290 Ca(NO ₃) ₂ .4H ₂ O, 70 mg/L Mg(NO ₃) ₂ .6H ₂ O, 40 mg/L KNO ₃ TEST SYSTEM - Test cultures are prepared from pre-cultures (22 h, 25 C). Precultures are prepared from stem-cultures (max 72 h, 25 C). - Test solution: 8 mL of test substance solution, 8 mL of test medium, 2 mL bacteria suspension and 2 mL of micro-organisms (15,000 cells/mL). - Temperature: 25 C DURATION OF TEST: 20 hours TEST PARAMETER: cell growth using a Coulter counter OBSERVATION TIMES: 20 hours
Result:	At 10,000 mg/L the effect of glycerine was less than 5%.
Test substance:	CAS 56-81-5 (glycerine), purity not indicated.
Reliability:	(4) not assignable The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (169 substances included). 19.12.2001 (60) (55)
Remark:	A validated method for biological monitoring of glycerol is not available.
Source:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA) 24.09.2001 (5)

As taken from IUCLID Dataset (2000), Glycerol (56-81-5).

Record for 1,2,3-propanetriol:

Spec. Sci. Name Spec. Common Name	Exp. Type Chem. Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff %	Effect Effect Meas.	Conc (Standardized) Appl. Rate	Stat. Signif. Sig. Level
Chlorococcales Green Algae Order	S	FW	1	EC10		PHY ASML	F > 1000000 ug/L	
Microcystis aeruginosa Blue-Green Algae	S U	FW LAB	NR	LOEC	DEC	POP PGRT/	F 2900000 ug/L	SIG

Scenedesmus quadricauda Green Algae	S U	FW LAB	NR	LOEC	DEC	POP PGRT/	F > 10000000 ug/L	SIG
Entosiphon sulcatum Flagellate Euglenoid	S U	FW LAB	3		DEC	POP ABND	F 3200000 ug/L	
Scenedesmus quadricauda Green Algae	S U	FW LAB	7		DEC	POP ABND	F > 10000000 ug/L	
Agmenellum quadruplicatum Blue-Green Algae	S U	SW LAB	28			POP/ GPOP	F 4600000* ug/L	
Amphidinium carterae Dinoflagellate	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	
Anacystis aeruginosa Blue-Green Algae	S U	LAB	8			POP/ GPOP	F 2900000 ug/L	
Anacystis marina Blue-Green Algae	S U	SW LAB	28			POP/ GPOP	F 4600000* ug/L	
Brachiomonas submarina Green Algae	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	
Chilomonas paramecium Cryptomonad		FW LAB	NR		CHG	POP PGRT/	F > 10000000 ug/L	
Chilomonas paramecium Cryptomonad	U	LAB	2			POP/ GPOP	F > 10000000 ug/L	
Chroomonas salina Cryptomonad	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	
Coccolithus huxleyi	S	SW				POP/	F 46000000* ug/L	

Coccolithophorid	U	LAB	28			GPOP		
Dunaliella tertiolecta	S	SW				POP/	F 46000000* ug/L	
Green Algae	U	LAB	28			GPOP		
Entosiphon sulcatum		FW			CHG	POP	F 3200000 ug/L	
Flagellate Euglenoid		LAB	NR			PGRT/		
Entosiphon sulcatum		FW			CHG	POP	F 3200000 ug/L	
Flagellate Euglenoid		LAB	NR			PGRT/		
Entosiphon sulcatum	S	FW			DEC	POP	F 3200000 ug/L	
Flagellate Euglenoid	U	LAB	3			PGRT/		
Hemiselmis virescens	S	SW				POP/	F 46000000* ug/L	
Cryptophyte	U	LAB	28			GPOP		
Isochrysis galbana	S	SW				POP/	F 4600000* ug/L	
Haptophyte	U	LAB	28			GPOP		
Microcystis aeruginosa		FW			DEC	POP	F 2900000 ug/L	
Blue-Green Algae	U	LAB	8			GPOP/		
Nannochloropsis oculata	S	SW				POP/	F 4600000* ug/L	
Yellow Green Algae	U	LAB	28			GPOP		
Pavlova lutheri	S	SW				POP/	F 46000000* ug/L	
Chrysophyte	U	LAB	28			GPOP		
Phaeodactylum tricornutum	S	SW				POP/	F 46000000* ug/L	
Diatom	U	LAB	28			GPOP		
Porphyridium cruentum	S	SW				POP/	F 4600000* ug/L	

Red Algae	U	LAB	28			GPOP		
Porphyridium cruentum	S	SW				POP/	F 46000000*	
Red Algae	U	LAB	28			GPOP	ug/L	
Prymnesium parvum	S	SW				POP/	F 46000000*	
Coccolithid	U	LAB	28			GPOP	ug/L	
Rhodomonas lens	S	SW				POP/	F 46000000*	
Cryptomonad	U	LAB	28			GPOP	ug/L	
Scenedesmus quadricauda	AQUA - NR	FW			DEC	POP	F > 10000000	
Green Algae	U	LAB	10			BMAS/	ug/L	
Scenedesmus quadricauda		FW			CHG	POP	F > 10000000	
Green Algae		LAB	NR			PGRT/	ug/L	
Scenedesmus quadricauda	S					POP/	F > 10000000	
Green Algae	U	LAB	8			GPOP	ug/L	
Tetraselmis maculata	S	SW				POP/	F 4600000*	
Prasinophyte	U	LAB	28			GPOP	ug/L	
Thalassiosira pseudonana	S	SW				POP/	F 46000000*	
Diatom	U	LAB	28			GPOP	ug/L	
Skeletonema costatum	S	SW				POP/	F 46000000*	
Diatom	U	LAB	28			GPOP	ug/L	
Xenopus laevis	S	FW		EC50	INC	DVP	F 10210000	
African Clawed Frog	U	LAB	4			DFRM	ug/L	
Xenopus laevis	S	FW		EC50	INC	DVP	F 10960000	
African Clawed Frog	U	LAB	4			DFRM	ug/L	
Xenopus laevis	S	FW		EC50	INC	DVP	F 11100000	

African Clawed Frog	U	LAB	4			DFRM	ug/L	
Xenopus laevis	S	FW		EC50	INC	DVP	F 11350000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		EC50	INC	DVP	F 11350000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		EC50	INC	DVP	F 12130000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		EC50	INC	DVP	F 5750000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		EC50	INC	DVP	F 6750000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		EC50	INC	DVP	F 7500000 ug/L	
African Clawed Frog	U	LAB	4			DFRM		
Xenopus laevis	S	FW		LC50	INC	MOR	F 10000000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 10000000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 10320000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 17030000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 17160000 ug/L	
African Clawed Frog	U	LAB	4			MORT		

Xenopus laevis	S	FW		LC50	INC	MOR	F 17990000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 18640000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 20680000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW		LC50	INC	MOR	F 8250000 ug/L	
African Clawed Frog	U	LAB	4			MORT		
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 10000000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 11250000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 11250000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 15000000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 15000000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 3630000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 3630000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 3630000 ug/L	SIG
African Clawed	U	LAB	4			LGTH/		<0.05

Frog								
Xenopus laevis	S	FW	WO	LOEC	DEC	GRO	F 8000000 ug/L	SIG
African Clawed Frog	U	LAB	4			LGTH/		<0.05
Daphnia magna		FW		EC50		BEH	F > 10000000 ug/L	
Water Flea			1			EQUL		
Daphnia magna	S	FW		LC50	INC	ITX	F > 10000000 ug/L	
Water Flea	U	LAB	1			IMBL		
Leuciscus idus ssp. melanotus		FW		LC50	INC	MOR	F > 10000000 ug/L	
Carp			2			MORT		
Pagrus major	S	SW		LOEC	DEC	MOR	F 10 % v/v	SIG
Red Sea Bream	U	LAB	0.5		0	HTCH/		<0.05
Pagrus major	S	SW		LOEC	DEC	MOR	F 25 % v/v	SIG
Red Sea Bream	U	LAB	0.5		>60- <80	HTCH/		<0.05
Pagrus major	S	SW		LOEC	DEC	MOR	F 10 % v/v	SIG
Red Sea Bream	U	LAB	0.5		>80- <100	HTCH/		<0.05
Sillago japonica	S	SW		LOEC	DEC	MOR	F 10 % v/v	ASIG
Japanese Whiting	U	LAB	ht		0/	HTCH		<0.05
Sillago japonica	S	SW		LOEC	DEC	MOR	F 10 % v/v	ASIG
Japanese Whiting	U	LAB	ht		>0-<20/	HTCH		<0.05
Sillago japonica	S	SW		LOEC	DEC	MOR	F 10 % v/v	ASIG
Japanese Whiting	U	LAB	ht		>0-<20/	HTCH		<0.05
Sillago japonica	S	SW		LOEC	DEC	MOR	F 10 % v/v	ASIG
Japanese Whiting	U	LAB	ht		>0-<20/	HTCH		<0.05
Paralichthys olivaceus	S	SW		LOEL	DEC	MOR	F 10 %	ASIG
Hirame, Flounder	U	LAB	(1.042 - 1.25)		32.7	SURV		<0.05
Pagrus major	S	SW		NOEC	DEC	MOR	F 20 % v/v	NOSIG

Red Sea Bream	U	LAB	0.5		>80- <100	HTCH/		<0.05
Pagrus major	S	SW		NOEC	DEC	MOR	F 5 % v/v	NOSIG
Red Sea Bream	U	LAB	0.5		>80- <100	HTCH/		<0.05
Pagrus major	S	SW		NOEC	DEC	MOR	F 5 % v/v	NOSIG
Red Sea Bream	U	LAB	0.5		>80- <100	HTCH/		<0.05
Pagrus major	S	SW		NR-LETH	INC	MOR	F 10 % v/v	
Red Sea Bream	U	LAB	ht		100	MORT/		
Paralichthys olivaceus	S	SW		NR-LETH	INC	MOR	F 20 %	
Hirame, Flounder	U	LAB	(1.042 - 1.25)		100	MORT		
Sciaenops ocellatus	S	SW				MOR/	F 0.1 M	
Red Drum	U	LAB	0.014			HTCH		
Sillago japonica	S	SW	WO		INC	ACC	F 10 % v/v	
Japanese Whiting	U	LAB	(0.004 - 0.014)			RSDE		
Danio rerio	S	FW		LD0		MOR	A 0.50 % v/v	
Zebra Danio	U	LAB	2			MORT		
Danio rerio	S	FW			CHG	MOR	A (5-10) % v/v	
Zebra Danio	U	LAB	0.5		>0- <100	SURV		
Oncorhynchus mykiss	S	FW		LC50	INC	MOR	A 54 (51-57) Al ml/L	
Rainbow Trout		LAB	4			MORT		
Carassius auratus	S	FW		LC50		MOR	A > 5000000 ug/L	
Goldfish	M	LAB	1			MORT		
Lemna gibba	S	FW	FD	EC10	DEC	POP	F 30.75 mM	
Inflated Duckweed	U	LAB	7			NCHG		
Lemna gibba	S	FW	FD	EC50	DEC	POP	F 164.25 mM	
Inflated								

Duckweed	U	LAB	7			NCHG		
Aedes aegypti	S	FW		LC50	INC	MOR	A 17.5 % v/v	
Yellow Fever Mosquito	U	LAB	0.167			MORT		
Uronema parduczi		FW			CHG	POP	F > 10000000 ug/L	
Ciliate		LAB	0.833			PGRT/		
Uronema parduczi		FW			CHG	POP	F > 10000000 ug/L	
Ciliate		LAB	NR			PGRT/		

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 56-81-5:

Values used to Generate ECOSAR Profile:

Log Kow: -1.652 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Fish	96-hr	LC50	1.44e+005
Neutral Organics :	Daphnid	48-hr	LC50	58451.754
Neutral Organics :	Green Algae	96-hr	EC50	10797.661
Neutral Organics :	Fish		ChV	9471.097
Neutral Organics :	Daphnid		ChV	2229.923
Neutral Organics :	Green Algae		ChV	1334.352
Neutral Organics :	Fish (SW)	96-hr	LC50	1.78e+005
Neutral Organics :	Mysid	96-hr	LC50	1.57e+006 *
Neutral Organics :	Fish (SW)		ChV	2072.746
Neutral Organics :	Mysid (SW)		ChV	4e+005

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

“Diet has profound effects on animal longevity and manipulation of nutrient sensing pathways is one of the primary interventions capable of lifespan extension. This often is done through caloric restriction (CR) and a variety of CR mimics have been identified that produce life extending effects without adhering to the rigorous CR dietary regimen. Glycerol is a dietary supplement capable mimicking CR by shifting metabolism away from glycolysis and towards oxidative phosphorylation. Glycerol supplementation has a number of beneficial effects, including lifespan extension, improved stress resistance, and enhanced locomotory and mitochondria activity in older age classes. Using rotifers as a model, we show that supplements of 150-300mM glycerol produced 40-50% extension of mean lifespan. This effect was produced by raising glycerol concentration only three times higher than its baseline concentration in rotifer tissues. Glycerol supplementation decreased rotifer reliance on glycolysis and reduced the pro-aging effects of glucose. Glycerol also acted as a chemical chaperone, mitigating damage by protein aggregation. Glycerol treatment improved rotifer swimming performance in older age classes and maintained more mitochondrial activity. Glycerol treatment provided increased resistance to starvation, heat, oxidation, and osmotic stress, but not UV stress. When glycerol was co-administered with the hexokinase inhibitor 2-deoxyglucose, the lifespan extending effect of glycerol was enhanced. Co-administration of glycerol with inhibitors like 2-deoxyglucose can lower their efficacious doses, thereby reducing their toxic side effects.” As taken from Snell TW & Johnston RK. 2014. Exp. Gerontol. 57, 47-56. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24835191>

10.3. Sediment toxicity

“There are no terrestrial and sediment effect data. Glycerol is not expected to partition to soil and sediment and exposure to these compartments is likely to be very low. Calculations using the equilibrium partitioning method in the EUSES program and the tentative aquatic PNEC of 780 mg/l gave a tentative PNEC_{sediment} of 479 mg/kg ww and a tentative PNEC_{soil} of 92.1 mg/kg ww”.

10.4. Terrestrial toxicity

“There are no terrestrial and sediment effect data. Glycerol is not expected to partition to soil and sediment and exposure to these compartments is likely to be very low. Calculations using the equilibrium partitioning method in the EUSES program and the tentative aquatic PNEC of 780 mg/l gave a tentative PNEC_{sediment} of 479 mg/kg ww and a tentative PNEC_{soil} of 92.1 mg/kg ww”.

Record for 1,2,3-propanetriol:

Spec. Sci. Name	Resp. Site	Media Type	Exp. Type	Dose #	Endpoint	Effect	Signif.	Dose
Spec. Common Name	Exp. Dur. (Days)	Test Loc.	Chem. Anal.	Res. Sample Unit	BAF/BCF	Effect Meas.	Sig. Level	Dose Stat. Meth.
Lactuca sativa Lettuce	3	AGR			EC50	REP GERM		NC 76 mM
Varroa destructor	2	NONE LAB	EN U	4	NOEL	MOR MORT	ANOSIG 0.15	A 1 AI ug/cm2

Varroa Mite								

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 56-81-5:

Values used to Generate ECOSAR Profile:

Log Kow: -1.652 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	382.994

“Glycerol metabolism has been well studied biochemically. However, the means by which glycerol functions in plant development is not well understood. This study aimed to investigate the mechanism underlying the effects of glycerol on root development in *Arabidopsis thaliana*. Exogenous glycerol inhibited primary root growth and altered lateral root development in wild-type plants. These phenotypes appeared concurrently with increased endogenous glycerol-3-phosphate (G3P) and H₂O₂ contents in seedlings, and decreased phosphate levels in roots. Upon glycerol treatment, G3P level and root development did not change in glycerol kinase mutant *gli1*, but G3P level increased in *gpdhc1* and *fad-gpdh* mutants, which resulted in more severely impaired root development. Overexpression of the FAD-GPDH gene attenuated the alterations in G3P, phosphate and H₂O₂ levels, leading to increased tolerance to exogenous glycerol, which suggested that FAD-GPDH plays an important role in modulating this response. Free indole-3-acetic acid (IAA) content increased by 46%, and DR5pro::GUS staining increased in the stele cells of the root meristem under glycerol treatment, suggesting that glycerol likely alters normal auxin distribution. Decreases in PIN1 and PIN7 expression, β -glucuronidase (GUS) staining in plants expressing PIN7pro::GUS and green fluorescent protein (GFP) fluorescence in plants expressing PIN7pro::PIN7-GFP were observed, indicating that polar auxin transport in the root was downregulated under glycerol treatment. Analyses with auxin-related mutants showed that TIR1 and ARF7 were involved in regulating root growth under glycerol treatment. Glycerol-treated plants showed significant reductions in root meristem size and cell number as revealed by CYCB1;1pro::GUS staining. Furthermore, the expression of CDKA and CYCB1 decreased significantly in treated plants compared with control plants, implying possible alterations in cell cycle progression. Our data demonstrated that glycerol treatment altered endogenous levels of G3P, phosphate and ROS, affected auxin distribution and cell division in the root meristem, and eventually resulted in modifications of root development.” As taken from Hu J et al. 2014. PLoS One 9(1), e86269. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24465999>

10.5. All other relevant types of ecotoxicity

Memo:	Frog Embryo Teratogenesis Assay - Xenopus (FETAX)

Method:	FETAX is a 96-h whole-embryo developmental toxicity screening assay according to test guideline ASTM E1439-91 (1991). The test was performed in triplo at three different laboratories. Groups of 20 embryos were exposed to 10 concentrations of glycerol in presence and absence of metabolic activation (microsomes from Aroclor 1254 induced rats and an NADPH-generating system) during 96 hours. Exposure medium was FETAX-AB (reconstituted water which includes 100 U/mL penicillin and streptomycin). Controls included were treated with FETAX-AB (4 dishes), FETAX (4 dishes), metabolic activation system (2 dishes) and activated cyclophosphamide (2 dishes with microsomes, 2 with NADPH-generating system and 2 without any activation). Statistical method: Probit analysis (Litchfield-Wilcoxon), trimmed Spearman Karber, Steel and Torrie Criteria for evaluating results: TI (teratogenic index i.e. 96-h LC50/96-h EC50(malformations)) > 1.5 and inhibition of growth > 30% compared to tests with microsomal fraction only.
Result:	Laboratory 1 without rat liver microsomes (Aroclor treated) 96-h LC50 14.84 mg/ml (95% CI 10.41-19.26 mg/ml) 96-h EC50 (malformations) 10.89 mg/ml (95% CI 10.21-11.56 mg/ml) LOEC (growth) 12.67 mg/ml (95% CI 8.09-17.24 mg/ml) TI 1.35 (95% CI 1.02-1.68); TI = teratogenic index (LC50/EC50 malformations) Laboratory 1 with rat liver microsomes (Aroclor treated) 96-h LC50 10.33 mg/ml (95% CI 6.18-14.48 mg/ml) 96-h EC50 (malformations) 10.76 mg/ml (95% CI 10.26-11.25 mg/ml) LOEC (growth) 8.67 mg/ml (95% CI 6.05-11.28 mg/ml) TI 0.97 (95% CI 0.54-1.40); TI = teratogenic index (LC50/EC50 malformations) Laboratory 2 without rat liver microsomes (Aroclor treated) 96-h LC50 19.10 mg/ml (95% CI 17.52-20.69 mg/ml) 96-h EC50 (malformations) 11.48 mg/ml (95% CI 10.81-12.15 mg/ml) LOEC (growth) 10.83 mg/ml (95% CI 10.02-11.65 mg/ml) TI 1.67 (95% CI 1.44-1.90); TI = teratogenic index (LC50/EC50 malformations) Laboratory 2 with rat liver microsomes (Aroclor treated) 96-h LC50 18.85 mg/ml (95% CI 16.72-20.98 mg/ml) 96-h EC50 (malformations) 11.26 mg/ml (95% CI 10.92-11.60 mg/ml)

	LOEC (growth) 10.83 mg/ml (95% CI 9.20-12.47 mg/ml) TI 1.67 (95% CI 1.52-1.83); TI = teratogenic index (LC50/EC50 malformations) Laboratory 3 without rat liver microsomes (Aroclor treated) 96-h LC50 9.42 mg/ml (95% CI 8.27-10.56 mg/ml) 96-h EC50 (malformations) 6.67 mg/ml (95% CI 5.67-7.66 mg/ml) LOEC (growth) 3.63 mg/ml TI 1.41 (95% CI 1.33-1.50); TI = teratogenic index (LC50/EC50 malformations) Laboratory 3 with rat liver microsomes (Aroclor treated) 96-h LC50 13.46 mg/ml (95% CI 11.05-15.87 mg/ml) 96-h EC50 (malformations) 5.84 mg/ml (95% CI 4.79-6.88 mg/ml) LOEC (growth) 2.13 mg/ml (95% CI 0.66-3.60 mg/ml) TI 2.33 (95% CI 1.82-2.85); TI = teratogenic index (LC50/EC50 malformations)
Conclusion:	With metabolic activation: 96-h LC50 14.2 mg/ml (RSD 31%) 96-h EC50 (malformations) 9.29 mg/ml (RSD 29%) LOEC (growth) 7.21 mg/ml (RSD 58%) TI 1.66 (RSD 40%); TI = teratogenic index (LC50/EC50 malformations) Without metabolic activation: 96-h LC50 14.5 mg/ml (RSD 33%) 96-h EC50 (malformations) 9.68 mg/ml (RSD 24%) LOEC (growth) 9.04 mg/ml (RSD 51%) TI 1.48 (RSD 16%); TI = teratogenic index (LC50/EC50 malformations) According to the criteria set, glycerol gave an ambiguous result. Certain criteria suggested that glycerol is non-teratogenic, but others suggested it is. Severe malformations were observed at concentrations approaching the 96h LC50 with metabolic activation. 25.01.2002 (63)

As taken from IUCLID Dataset (2000), Glycerol (56-81-5).

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-2.6417 days (HL = 0.002282 days)

Log BCF Arnot-Gobas method (upper trophic):	-0.049 (BCF = 0.8933)
Log BAF Arnot-Gobas method (upper trophic):	-0.049 (BAF = 0.8933)
log Kow used:	-1.76 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List state that glycerol is not bioaccumulative in the environment:

Empirical Log Kow	-1.76
Log Kow predicted by KowWin	-1.65
Log BAF T2MTL predicted by Gobas	0.0000540371955876
Log BCF 5% T2LTL predicted by Gobas	0.0005144646215092
Log BCF Max predicted by OASIS	0.947549472119754
Log BCF predicted by BCFWIN	0.5

Data accessed May 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

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SCIENTIFIC OPINION

Scientific Opinion on the evaluation of the substances currently on the list in the annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils – Part III of III¹

EFSA Panel on Contaminants in the Food Chain (CONTAM)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Shipping of edible fats and oils into Europe is permitted in bulk tanks, in which substances, included in a positive list, had been previously transported. The European Commission requested EFSA to evaluate the list of substances in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils, taking into account its review of the Scientific Committee on Food criteria for acceptable previous cargoes and criteria proposed by the Codex Committee for Fats and Oils. This is the third and last scientific opinion of the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) on this topic, in which sixteen of these substances or groups of substances have been evaluated. The CONTAM Panel concluded that sodium silicate (water glass) solution, iso-octanol, iso-nonanol, iso-decanol, 1,3-propanediol, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-butyl acetate, propylene tetramer, paraffin wax, candelilla wax, white mineral oils and glycerol would not be of health concern as previous cargoes. The CONTAM Panel concluded that carnauba wax was not acceptable as a previous cargo because of its insolubility in water and high melting point, which raise concerns regarding the efficiency of tank cleaning. There was insufficient information available on the composition of montan wax for the CONTAM Panel to conclude that it would be of no health concern when used as previous cargo and hence it does not meet the criteria for acceptability as previous cargo. The CONTAM Panel made several recommendations regarding the way in which the substances are described in the Annex to Commission Directive 96/3/EC, to correct inaccuracies and to better reflect current transport practices.

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KEY WORDS

acceptable previous cargo, edible fats and oils, sea transport, criteria for acceptability of previous cargoes

¹ On request from the European Commission, Question No EFSA-Q-2010-01464, adopted on 22 November 2012.

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SUMMARY

The worldwide trade of edible fats and oils in bulk requires their transport by road, railroad, inland waterways and sea. The carriage by sea of edible fats and oils into Europe is also permitted in bulk tanks that have previously been used to transport substances included in a positive list of acceptable previous cargoes. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) reviewed the Scientific Committee on Food (SCF) criteria for acceptable previous cargoes and criteria proposed by the Codex Committee for Fats and Oils in 2009. In addition, the CONTAM Panel identified the importance of taking into account possible impurities and reaction products with edible fats and oils of the chemicals shipped as previous cargoes, as these might be more toxic than the chemical itself. Since usually no specifications of the impurities are available for the often rather crude substances shipped in bulk, those potentially present were determined primarily by assessing information on the source or starting substances, making worst case assumptions in each case. In November 2009, the CONTAM Panel published an opinion on a limited number of substances that had been proposed at Codex level for addition to the list of Codex acceptable previous cargoes, which were evaluated against the criteria in the previously mentioned opinion of the CONTAM Panel.

Following a request from the European Commission, the CONTAM Panel was asked to deliver a scientific opinion on the evaluation of the substances listed in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils. This was to ensure that substances currently on the list of acceptable previous cargoes had been evaluated against the same criteria as recently agreed by EFSA.

This is the third and last scientific opinion of the CONTAM Panel on the evaluation of the substances listed in the Annex to Commission Directive 96/3/EC. The CONTAM Panel considered that sodium silicate (water glass) solution, 1,3-propanediol, isobutylacetate, sec-butyl acetate, tert-butyl acetate, n-butyl acetate, propylene tetramer, paraffin wax, candelilla wax, white mineral oils and glycerol when used as previous cargoes would not raise any concerns regarding their acute or chronic toxicity, genotoxicity, carcinogenicity or reproductive toxicity. In addition there were no concerns regarding possible allergenicity or adjuvant effects from such transport. In the case of iso-octanol, iso-nonanol and iso-decanol, because of data gaps in the respective toxicological profiles, the CONTAM Panel used 'read-across' from the assessment profile of oxo-alcohols C9-C13 category and concluded that the iso-alcohols under consideration are of low toxicity following acute and chronic exposure, they are not genotoxic or allergenic and therefore they are of no toxicological concern when used as previous cargoes.

The CONTAM Panel noted that four of these substances are authorized for use in food either as flavouring (isobutyl acetate, sec-butyl acetate, n-butyl acetate) or glazing (candelilla wax) agents. For two substances, acceptable daily intakes of 'not specified' or 'not limited' have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or SCF because of low toxicological concern. These are sodium silicate (water glass) solution and glycerol. n-Butyl acetate has an ADI established by the SCF greater than 0.1 mg/kg body weight (b.w.) per day. In the case of sec-butyl acetate, it is a Cramer Class I substance for which exposures below a threshold of toxicological concern of 1 800 µg/person per day (30 µg/kg b.w. per day) would not be expected to be of safety concern, and the available information does not indicate any toxicological concern at the exposure levels that might occur from transport as a previous cargo. The remaining substances (iso-octanol, iso-nonanol and iso-decanol, 1,3-propanediol, tert-butyl acetate, propylene tetramer) are of relatively low toxicity and the margin of exposure that would occur comparing the maximum assumed carryover from their transport as previous cargo and the respective no-observed-adverse-effect level would indicate no concern for human health.

For all these substances no reaction products with fats and oil of toxicological concern were identified or anticipated. The CONTAM Panel noted that the only impurities of potential concern are aromatic hydrocarbons, which may be present in paraffin wax and white mineral oils. While in the case of white mineral oils they are controlled to very low levels, in the case of paraffin waxes the CONTAM Panel

concluded that this entry should be restricted to paraffin waxes that have been treated to remove aromatic hydrocarbons and which otherwise meet relevant standards to be considered as ‘food grade’.

Regarding mineral oil hydrocarbons the CONTAM Panel notes that some aliphatic hydrocarbons bioaccumulate in the body, such as branched and cyclic species in the mass range of 16 - 35 carbon atoms. However, since exposure to mineral oil hydrocarbons via contamination of edible fats and oils from previous cargoes occurs only rarely and mostly at levels lower than those observed anyway in edible oils, it will contribute little to overall exposure.

In the case of montan wax, there was insufficient information available on the composition and toxicological profile of this substance for the CONTAM Panel to conclude that it does not contain components that would be of concern to human health when used as previous cargo. The CONTAM Panel therefore concludes that it does not meet the criteria for acceptability as a previous cargo for edible fats and oils.

Although the CONTAM Panel considered that there would be no health concerns arising from the use of carnauba wax as a previous cargo, using normal assumptions regarding worst case carryover, the Panel concluded that it does not meet the criteria for its acceptability as previous cargo because of its insolubility in water and high melting point and hence doubts concerning the efficiency of tank cleaning.

In addition, the CONTAM Panel noted a number of inaccuracies in the chemical identification and inconsistencies in the chemical specification of substances with respect to current transport practices, in the Annex to Commission Directive 96/3/EC. The CONTAM Panel therefore made a number of recommendations regarding the way in which the substances are described in this Annex, to correct such inaccuracies and inconsistencies.

The CONTAM Panel also made recommendations on the information that should be provided by interested parties when new substances are to be evaluated as previous cargoes.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

General hygiene requirements relating to transport of food applicable to all food business operators laid down in Regulation (EC) No 852/2004⁴ (Annex II, Chapter IV) state, amongst others, that "receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination."

Information showed that the application of this principle to the bulk transport was not practical and imposed an unduly onerous burden on food business when applied to bulk transport in sea-going vessels of liquid fats and oils and of raw sugar. This led to the adoption of two derogations^{5,6} providing equivalent protection to public health.

Equivalent protection to public health is guaranteed on technical (e.g. tank design) and procedural (e.g. intermediate cleaning) conditions, on record keeping (e.g. on effectiveness of cleaning and on the nature of the previous cargoes) and, in the case of bulk transport of liquid fats and oils in sea-going vessels, on a list of acceptable previous cargoes. The presence of substances on the list of acceptable previous cargoes for fats and oils in the Annex to Commission Directive 96/3/EC is based on three opinions of the former Scientific Committee on Food (SCF).^{7,8,9}

On 26 May 2009, the Panel on Contaminants in the Food Chain (CONTAM Panel) issued a scientific opinion on the criteria for acceptable previous cargoes for edible fats and oils. In this opinion, the CONTAM Panel reviewed the 5 criteria for the assessment of acceptability as previous cargoes for edible fats and oils previously used by the SCF and evaluated the appropriateness of four criteria developed for the same purpose by the Codex Committee for Fats and Oils (CCFO).

The CONTAM Panel noted that by application of CCFO criterion 2 some substances will turn out to be unacceptable as previous cargoes. This could include substances with ADI (or TDI) < 0.1 mg/kg b.w. or substances with genotoxic activity. The Panel considers that the exclusion of such substances as previous cargoes is appropriate.

The criteria in this Scientific Opinion were subsequently applied in the CONTAM Scientific Opinion on the evaluation of substances as acceptable previous cargoes for edible fats and oils, adopted on 29 November 2009. In this opinion, a limited number of substances that had been proposed at Codex level for addition to the list of acceptable previous cargoes were evaluated against the criteria in the previously mentioned Scientific Opinion.

In order to assure that the substances currently on the list of acceptable previous cargoes are evaluated against the same criteria, an additional Scientific Opinion covering an evaluation of the substances currently on the list of acceptable previous cargoes against the criteria used in the Opinion on the

⁴ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (OJ L 139, 30.4.2004, p. 1).

⁵ Commission Directive 96/3/EC of 26 January 1996 granting a derogation from certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk liquid oils and fats by sea (OJ L 21, 27.01.1996, p. 42).

⁶ Commission Directive 98/28/EC of 29 April 1998 granting a derogation from certain provisions of Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport by sea of bulk raw sugar (OJ L 140, 12.05.1998, p. 10).

⁷ SCF, 1996. Scientific Committee on Food. Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes, expressed on 20 September 1996 - Fortieth Series (1997) Catalogue No: GT 07 97652-EN-DE-FR. http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_40.pdf

⁸ SCF, 2003. Updated opinion of the Scientific Committee on Food on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes, expressed on 4 April 2003. Health and Consumer Protection Directorate-General, European Commission, Brussels. http://ec.europa.eu/food/fs/sc/scf/out189_en.pdf

⁹ SCF, 1997. Scientific Committee on Food. Amendment of its previous opinion of 20 September (SCF 1996). Opinion on Methyl esters of fatty acids in previous cargoes, expressed on 12-13 June 1997. Minutes of the 107th Meeting of the Scientific Committee for Food http://ec.europa.eu/food/fs/sc/oldcomm7/out13_en.html

evaluation of substances as acceptable previous cargoes for edible fats and oils carried out by EFSA would be needed.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the evaluation of the substances currently on the list in the Annex to Commission Directive 1996/3/EC as acceptable previous cargoes for edible fats and oils. The evaluation should be based on the SCF criteria and the criteria proposed by the CCFO as reviewed by the Panel on Contaminants in Food Chain in 2009¹⁰ for acceptable previous cargoes for edible fats and oils.

¹⁰ <http://www.efsa.europa.eu/en/scdocs/scdoc/1110.htm>

ASSESSMENT

1. Introduction

General hygiene requirements relating to transport of food applicable to all food business operators are laid down in Annex II, Chapter IV of Regulation (EC) No 853/2004¹¹ and state, amongst others, that ‘receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination’. However, the application of this principle to bulk transport is not practical and imposes an unduly onerous burden on food businesses when applied to bulk transport in sea-going vessels of liquid fats and oils. Commission Directive 96/3/EC⁵ permits sea transport of fats and oils in bulk tanks, which have previously been used to transport substances included in a positive list of acceptable previous cargoes.

The majority of the global trade in oils and fats is done under contracts of the Federation of Oils, Seeds and Fats Associations (FOSFA), a professional international contract-issuing and arbitral body concerned exclusively with the world trade in oilseeds, oils and fats, which provides a wide range of standards covering different methods of transportation and different terms of trade. FOSFA does not require dedicated containers and allows transport in tanks that have previously been used to transport substances from an approved positive list. A FOSFA list of banned previous cargoes also exists (FOSFA, 2008).

In 1996, the Scientific Committee on Food (SCF) assessed the risk to human health arising from potential contamination of fats and oils shipped in tanks, which may have been used to transport the substances as given in the Annex to Commission Directive 96/3/EC⁵ (SCF, 1997a). A number of substances were evaluated and a set of criteria for acceptable previous cargoes (SCF criteria) was proposed. In 2003, the SCF issued an update of its previous opinion in the light of new toxicological information, where available (SCF, 2003).

Based on the evaluations carried out by the SCF in 1996 and 2003, the list of substances acceptable as previous cargoes set out in the Annex to Commission Directive 96/3/EC⁵ was amended by Commission Decision 2004/4/EC.¹² However, the substances in the list were only considered to be acceptable as long as the legal provisions were applied, especially regarding the cleaning and condition of the tanks and accurate documented evidence relating to the nature of the three previous cargoes, and to the efficacy of the cleaning process between cargoes, to be kept by the captain of the vessel.

The Codex Alimentarius Commission (CAC) also sets international food standards to protect the health of consumers and ensure fair practices in the food trade. Under the Codex system, the Codex Committee for Fats and Oils (CCFO) has been established to elaborate standards for fats and oils of animal, vegetable and marine origin, including margarine and olive oil. It has adopted the Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk, which includes a Draft Codex List of Acceptable Previous Cargoes and a Proposed Draft List of Acceptable Previous Cargoes. In addition, a set of criteria (CCFO criteria) has been developed to determine the acceptability of substances as previous cargoes, based on the criteria proposed by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (FAO/WHO) Joint Technical Meeting (FAO/WHO, 2007). Both the draft lists of acceptable previous cargoes and the criteria were adopted by the CAC (Geneva, 4-9 July 2011) (FAO/WHO, 2011).

¹¹ Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. OJ L 139, 30.4.2004, p. 206-320.

¹² Commission Directive 2004/4/EC of 15 January 2004 amending Directive 96/3/EC granting a derogation from certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk liquid oils and fats by sea. OJ L 15, 22.1.2004, p. 25-30.

In 2009, the European Commission requested the European Food Safety Authority (EFSA) to review the SCF criteria for acceptable previous cargoes for edible fats and oils, in the light of the CCFO criteria (CCFO, 2009). The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) issued an opinion in May 2009 and concluded that the criteria for evaluation of acceptable previous cargoes as proposed by the CCFO were not in conflict with any of the five criteria developed by the SCF (EFSA, 2009a). Most of the SCF criteria were either explicitly or implicitly covered by the CCFO criteria. The last SCF criterion, dealing with the availability of analytical methods is not explicitly addressed in the CCFO criteria and the CONTAM Panel considered that this criterion is still important, though rather in the sense that the development of a corresponding method is considered to be feasible rather than the immediate availability of such a method (as most substances used as previous cargoes are not routinely analyzed in fats and oils). The Panel also considered relevant the inclusion of criteria covering possible allergenicity and the potential for reaction of compounds with oils and fats.

The criteria in the Scientific Opinion of 2009 were subsequently applied by the CONTAM Panel for the evaluation of the acceptability as previous cargoes of the substances included in the Codex Proposed Draft List of Acceptable Previous Cargoes (EFSA, 2009b).

The European Commission asked EFSA for a scientific opinion on the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils. The evaluation should be based on the review of the criteria performed by the CONTAM Panel in 2009, in order to ensure that the substances currently on the list are evaluated against the same criteria.

The outcome of the evaluation of the substances is presented in three scientific opinions, for practical purposes. A first opinion was published in December 2011 reporting the evaluation of 13 substances in the list (EFSA, 2011). A second opinion reporting the evaluation of 35 substances (or groups of) in the list was published in May 2012 (EFSA, 2012a). In this third and last output, the evaluation of 16 substances (or groups of) listed in Table 1 is described. The entries in Table 1 are as listed in the Annex to Commission Directive 96/3/EC.⁵ In reviewing these substances, the CONTAM Panel concluded that modifications to some of these entries would improve accuracy and these are discussed in the Opinion (see Table 4).

Substances transported in bulk as previous cargoes are often rather crude and usually no specific information is available on the impurities present. Hence, for many substances, the CONTAM Panel had to determine which impurities might be present primarily by assessment of the source or starting material and likely method of preparation of the substance to be transported. Chemicals transported as previous cargoes may vary in composition, depending on the starting materials and the method of preparation, details for which were obtained, in part, from information obtained from FOSFA. The CONTAM Panel based its evaluations on worst case assumptions on these aspects.

Table 1: Substances on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils and re-evaluated in the present opinion.

Substance (synonyms)	CAS Number
Sodium silicate (water glass)	1344-09-8
iso-Octanol (isooctyl alcohol)	26952-21-6
iso-Nonanol (isononyl alcohol)	27458-94-2
iso-Decanol (isodecyl alcohol)	25339-17-7
1,3-Propylene glycol (trimethylene glycol; 1,3-propanediol)	504-63-2
iso-Butyl acetate	110-19-0
sec-Butyl acetate	105-46-4
tert-Butyl acetate	540-88-5
n-Butyl acetate	123-86-4
Propylene tetramer	6842-15-5
Montan wax	8002-53-7
Paraffin wax	8002-74-2 / 63231-60-7
Carnauba wax — (Brazil wax)	8015-86-9
Candelilla wax	8006-44-8
White mineral oils	8042-47-5
Glycerine (glycerol; glycerin)	56-81-5

2. Previous risk assessments

2.1. Scientific Committee on Food (SCF)

In 1996, the SCF issued an opinion on the potential risk to human health arising from the transport of fats and oils in ships' tanks from substances proposed as acceptable previous cargoes (SCF, 1997a). The Committee was asked to examine the substances given in the Annex to Commission Directive 96/3/EC⁵ and other substances that may be proposed for addition to the list. The SCF was asked to take into account the information provided by industry concerning (i) the likelihood and potential levels of contamination in the light of the information regarding cleaning procedures, dilution and limits of detection of analytical methods and (ii) the additional processing of fats and oils. The SCF focused its attention on the evaluation of the toxicological properties of the substances without considering other aspects such as the ecotoxicological characteristics, the microbial status or nutritional relevance. The Committee's view on the acceptability of the substances in the list of acceptable previous cargoes from Commission Directive 96/3/EC⁵ was based on the criteria shown in Table 2.

Table 2: Criteria for the inclusion of substances in the list of acceptable previous cargoes according to the SCF (SCF, 1997a, 2003).

SCF Criteria ^(a)
1. No toxicological concerns, particularly with regard to their genotoxic and carcinogenic potential, for which a threshold is difficult to establish.
2. Efficacy of procedures used to clean ships' tanks between cargoes
3. Dilution factor in relation to the potential amount of residue of the previous cargo and any impurity which the previous cargo might have contained and the quantity of oil or fat transported.
4. Subsequent application of refining processes and solubility relevant to the occurrence of possible contaminating residues.
5. Availability of analytical methods to verify the presence of trace amounts of residues or the absence of contamination of oils and fats.

(a): The SCF criteria have no numbering in the original reference. In the present opinion they have been included for an easier referral throughout the document.

The substances in the list were only considered to be acceptable as long as the provisions of the Hygiene of Foodstuffs Directive 93/43/EEC,¹³ later replaced by Regulation (EC) 852/2004,¹⁴ were applied, and especially regarding the cleaning and condition of the tanks, as well as the requirement included in Commission Directive 96/3/EC,⁵ where accurately documented evidence relating to the three previous cargoes, and the efficacy of the cleaning process between cargoes, should be kept by the captain of the vessel.

Some of the substances evaluated were accepted as previous cargoes by the SCF because they are food or food components. A number of other substances were considered acceptable from a toxicological point of view.

For others, although the available toxicological information was insufficient to enable a full evaluation, the SCF was able to accept a number of compounds provisionally on the basis of their unlikely genotoxic potential, their easy removal by tank cleaning procedures, and the very low residues expected as a result of these factors and their likely dilution in a subsequent cargo of edible fats or oils (e.g. iso-decanol, iso-nonanol, iso-octanol, montan and paraffin wax, white mineral oils and methyl tertiary butyl ether (MTBE)).

Ten substances were considered as not acceptable due to inadequate toxicological and/or technical data (2,3-butanediol, 1,3-propylene glycol, methyl esters of fatty acids (laurate, palmitate, stearate, and oleate) and nonane) or because their genotoxic and carcinogenic potential were a reason for concern (iso-butanol, cyclohexanol and cyclohexanone).

Later, the SCF was requested to update the list of substances from its previous opinion in the light of new toxicological information, if available (SCF, 2003). Priority was given to those substances provisionally accepted as previous cargoes. As in its previous opinion, the SCF focused on the potential toxicological concerns, without considering other aspects. Neither the specifications of the transported fats and oils nor the purity of the previous cargo were taken into account. The criteria used for re-evaluation were the same as those described in its opinion from 1996 (Table 2). The re-evaluation led to the full acceptance of some substances previously considered as not acceptable (e.g. methyl esters of the following fatty acids: laurate, palmitate, stearate and oleate) or provisionally acceptable (e.g. MTBE) in view of the new toxicological information. Others were still considered to be not acceptable as previous cargoes since the new information did not allow for a re-evaluation of their carcinogenicity or genotoxicity (e.g. 2,3-butanediol, isobutanol, cyclohexanol and cyclohexanone). Finally, some were considered to be still only provisionally acceptable, as there was insufficient new information on their toxicity to allow re-evaluation (iso-decanol, iso-nonanol, iso-octanol, montan and paraffin wax and white mineral oils).

Details of the SCF conclusions are given in the corresponding Section for each substance under evaluation.

2.2. European Food Safety Authority (EFSA)

At the request of the European Commission, the EFSA reviewed the criteria for acceptable previous cargoes for edible fats and oils set by the SCF (Table 2). In doing so, the CONTAM Panel assessed the appropriateness of the four CCFO criteria (Table 3), one by one, by comparing them with those set by the SCF for acceptable previous cargoes for edible fats and oils in 1996.

¹³ Council Directive 93/43/EEC on the hygiene of foodstuffs of 14 June 1993. OJ L 175, 19.7.1993, p. 1-11.

¹⁴ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1.

Table 3: Criteria proposed for immediate previous cargoes by the CCFO during their 21st meeting (CCFO, 2009) and adopted by the CAC (FAO/WHO, 2011).

CCFO Criteria (adopted at Step 5)	
1.	The substance is transported/stored in an appropriately designed system; with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, followed by effective inspection and recording procedures.
2.	Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg b.w./day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case by case basis.
3.	The substance should not be or contain a known food allergen, unless the identified food allergen can be adequately removed by subsequent processing of the fat or oil for its intended use.
4.	Most substances do not react with edible fats and oils under normal shipping and storage conditions. However, if the substance does react with edible fats and oils, any known reaction products must comply with criteria 2 and 3.

ADI: acceptable daily intake; b.w.: body weight; CAC: Codex Alimentarius Commission; CCFO: Codex Committee for Fats and Oils; TDI: tolerable daily intake.

Criterion 2 was based on the fact that it could be estimated, as a worst case, that the residue of the substance remaining in the tanks after cleaning could give rise to a maximum of 100 mg residue/kg of fat or oil (FAO/WHO, 2007). Average consumption of fats and oils, based on the WHO-GEMS/Food Consumption Cluster diets is 25 g/day for a single type of fat or oil. Using a factor of 2.5 to take account of high consumers, an ADI of 0.1 mg/kg body weight (b.w.) per day would be the minimum requirement to ensure sufficient protection of all consumers, including children and high-intake consumers.

The CONTAM Panel concluded that the criteria for evaluation of acceptable previous cargoes for edible fats and oils as proposed by the CCFO are not in conflict with any of the five criteria developed by the SCF. SCF criteria 1 to 4 are either explicitly or implicitly covered by the CCFO criteria. SCF criterion 5 dealing with the availability of analytical methods is not explicitly addressed in the CCFO criteria. The CONTAM Panel considers that SCF criterion 5 is still important. The CCFO criteria also cover food allergens and the potential for compounds to react with edible fats and oils. The CONTAM Panel considers these additions relevant.

In addition, the CONTAM Panel made the following remarks:

- The CCFO criteria specifically apply to the immediate previous cargo. The CCFO criterion 1, which addresses among other issues, documentation procedures, does not specify for how many previous cargoes records should be kept. This might be particularly important in the event that earlier previous cargoes comprise substances for which an acceptable daily intake (ADI) (or tolerable daily intake (TDI)) has not been established. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk.
- With respect to CCFO criterion 2, the CONTAM Panel agreed with the proposed threshold of an ADI (or TDI) of ≥ 0.1 mg/kg b.w. For substances for which there is no numerical ADI (or TDI) a case by case evaluation is needed. The Panel also considered the situation of second and third previous cargoes and concluded that for non-genotoxic substances their transport as second and third previous cargoes is not of concern, taking into account their very limited carry over. However, the CONTAM Panel noted that genotoxic substances would not be acceptable as previous cargoes. Also in relation to CCFO criterion 2, the CONTAM Panel noted that as consequence of the above some substances will turn out to be unacceptable as

previous cargoes. This could include substances with an ADI (or TDI) < 0.1 mg/kg b.w. or substances with genotoxic activity. The Panel was of the opinion that the exclusion of such substances as previous cargoes is appropriate.

- CCFO criterion 3 is sufficient to cover ‘known food allergens’. However, the CONTAM Panel considered that the scope of the CCFO criterion is too narrow, and should apply to all known allergens, not just to known food allergens, given the fact that the same cargo may be sold for cosmetic use.
- The CONTAM Panel endorsed CCFO criterion 4 without any further remarks.

3. Evaluation of the substances currently on the list in the annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils

The CONTAM Panel has evaluated the acceptability of the substances listed in Table 1 (as amended, see Table 4) as previous cargoes for edible fats and oils. The evaluation is based on its review of the criteria for acceptable previous cargoes as described in Section 2.2. (EFSA, 2009a) and the experience gained in its subsequent evaluation of 13 substances as previous cargoes which highlighted the importance of addressing any impurities that might be present (EFSA, 2009b):

- The substance is transported/stored in an appropriately designed system; with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, followed by effective inspection and recording procedures. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk. The CONTAM Panel noted that the choices made with respect to design of the transport system and the cleaning methods are the responsibility of those managing the transport of previous cargoes. It was the nature and amount of substances that might be carried over into a subsequent cargo of edible fats and oils that was taken into account by the CONTAM Panel in its evaluation of previous cargoes.
- Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg b.w. per day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case by case basis. For non-genotoxic substances their transport as second and third previous cargoes is not of concern, taking into account their very limited carry over. However, genotoxic substances would not be acceptable as previous cargoes.
- The substance should not be or contain a known allergen, unless the identified allergen can be adequately removed by subsequent processing of the fat or oil for its intended use. This criterion covers all allergens, not only food allergens.
- If the substance reacts with edible fats and oils, any known reaction products must comply with the above two criteria. Reactions may be promoted by the acidity from free fatty acids (crude oils) and may occur over many months; they do not need to result in high yields to be potentially relevant. Transesterifications are known to occur under such conditions (Biedermann et al., 2008). Prominent reactive functions of lipids are hydroxyl groups on the alkyl chain of fatty acids or non-esterified positions of the glycerol moiety and epoxides from epoxidized fatty acids (Fankhauser-Noti et al., 2006).
- The development of analytical methods of sufficient sensitivity to verify the presence of trace amounts of residues or the absence of contamination of fats and oils should be feasible, e.g. for control authorities. Such methods are seldom routinely available, since most substances used as previous cargoes are not commonly analyzed in fats and oils. The CONTAM Panel therefore evaluated the feasibility of developing such methodology as part of its assessment of

each substance. In those cases where, due to the nature or composition of the substance (or group of) to be evaluated as previous cargo, the feasibility of developing suitable analytical methods was considered questionable, this was indicated when discussing the substance (or group of) in the respective chapter and was used as an argument for the rejection of a substance as previous cargo.

- It is unrealistic to assume that chemical analysis would regularly be applied to check the suitability of a material used as previous cargo or the efficiency of a cleaning procedure for a substance. Therefore the substances were evaluated under worst case assumptions with regard to cleaning efficiency and material composition (in particular the potential presence of toxic impurities or the formation of reaction products with edible fats and oils).
- Potentially relevant impurities in the previous cargo should be taken into account since they may be toxicologically more important than the substance itself. As most products exist in different grades, a reasonable worst-case product within the specification provided was assumed, the concentration of the impurity estimated from available literature and evaluated in the same way as a listed substance. Impurities are often specified for fine chemicals and highly purified products. However, these are unlikely to be shipped in bulk. Those more commonly encountered are likely to be of intermediate to low purity grade and no specific information about impurities is publicly available (methods of synthesis are usually confidential). Due to this lack of information, the source and most probable way (or ways) of synthesis of the substance was investigated to determine potentially relevant impurities, such as unreacted starting substances or products of side reactions.

The current evaluation of the substances as acceptable previous cargoes is based on available studies/information from literature searches carried out, up to the time of the evaluation, on public databases, e.g. PubMed, International Uniform Chemical Information Database (IUCLID), European Chemicals Agency (ECHA), evaluations made by national and international bodies, e.g. WHO and Organisation for Economic Co-operation and Development (OECD) and on information requested from FOSFA.

The safety of the substances as identified chemically in the Annex (Table 1, with any clarifications necessary as indicated in Table 4) was evaluated first. If the substance was considered acceptable as a previous cargo from a toxicological point of view, it was further evaluated in accordance with the additional criteria listed above (EFSA, 2009a,b).

As part of the evaluation of safety for human health, responses of the immune system have been considered. This is necessary for allergens, but it is also relevant for substances which are not allergens themselves but can promote allergy, so-called adjuvants. Adjuvant activity has been shown e.g. for various natural lipids such as pollen-associated oxylipins (Traidl-Hoffmann et al., 2009), for plant lectins (reviewed by Lavelle et al., 2001), for saponins from a variety of plants (Lacaille-Dubois, 2005; Sun et al., 2009), and for inulin and certain other carbohydrates (Petrovsky and Cooper, 2011). It has been determined on a case-to-case basis whether any documented adjuvant activity is sufficiently strong to be of concern in the context of transport as a previous cargo.

3.1. SODIUM SILICATE (water glass) (CAS No 1344-09-8)

Sodium silicate, also called water glass, is traded as a solid (powder) or as a viscous liquid when dissolved in water. In reality, solid sodium silicate is a metasilicate of oligomeric structure containing on average 3-5 silicate moieties. Sodium silicate solutions are strongly basic and barely soluble in edible fats and oils.

Sodium silicate is prepared by heating sodium carbonate with silicium dioxide (quartz sand).

Sodium silicate has many different uses. Large amounts are used in the building industry as an additive to special cements (e.g. to improve tightness of concrete), but also for ceramics, as a binder for mineral dyes, for bleaching pulp, repairing cars, etc.

3.1.1. Previous evaluations

The SCF evaluated sodium silicate (water glass) in 1996 as a previous cargo for edible fats and oils and considered this substance acceptable in view of the fact that it was approved as a food additive by the European Union (E550) (Annex 1 of Directive 95/2/EC¹⁵), with an ADI 'not specified' (SCF, 1997a). In the 2003 SCF evaluation of acceptable previous cargoes, sodium silicate was not further evaluated as it was already considered acceptable (SCF, 2003).

In 1974, an ADI 'not limited' was also assigned by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to sodium silicate (JECFA, 1974).

In 2004, OECD reviewed the toxicological data of soluble silicates including sodium silicate and concluded that soluble silicates possess properties indicating a hazard for human health (irritancy/corrosivity) but, provided that adequate risk reduction measures are in place (classification and labelling), they were currently considered of low priority for further work (OECD, 2004a).

3.1.2. Current evaluation

Sodium silicate is a solid at normal temperature and pressure, with a melting point above 800 °C. Hence, in this form it is not a suitable cargo for the type of tanker used to transport edible fats and oils by sea. When used as a previous cargo to edible fats and oils it has to be transported as a solution, to enable effective transfer and tank cleaning (see Documentation provided to EFSA).

3.1.2.1. Expected impurities

Sodium silicate is often made starting from crude minerals, and for many applications purity is not critical. It will contain other alkali or earth alkali ions and also other anions. These tend to be easily removed from the tank by washing with water. Hence, these substances are unlikely to be of any concern at the levels that would occur in edible fats and oils when sodium silicate is transported as the previous cargo.

3.1.2.2. Reactivity and reaction products

Sodium silicate solution is strongly basic. When mixed into fats and oils at low amounts (below 100 mg/kg), it will interact immediately with free fatty acids in the oil and be neutralised. The resulting salts of organic acids are not reactive. This is also why it does not cause saponification when mixed with fats and oils at the concentrations expected when used as a previous cargo.

3.1.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Silicon is an essential trace element that participates in important metabolic processes including bone, cartilage and connective tissue formation. The silicon is present almost entirely as free soluble monosilicic acid (Carlisle, 1986, as cited in OECD, 2004a). No reliable toxicokinetic, metabolic or mechanistic studies are available for soluble silicates. Since concentrated silicate solutions are only stable at pH >11.5 and lowering the pH leads to the formation of an insoluble silica gel, after ingestion gel formation is expected to occur due to the hydrochloric acid of the stomach. The degree of gel formation will depend on the amount of ingested silicate solution and the neutralising and buffering capacity of the gastrointestinal tract. Gastrointestinal absorption of insoluble silica will be insignificant as compared to that of soluble anions. Absorbed soluble silicates are excreted via urine and to a lesser

¹⁵ European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18. 3. 1995, p. 1.

extent via the faeces. Markedly increased and rapid urinary excretion of silica was observed when soluble sodium silicates were administered by various routes to different animal species (OECD, 2004a). The excretion rate of sodium silicate administered to rats via stomach tube (Benke and Osborn, 1979, as cited in OECD, 2004a) was independent of the doses applied, indicating that the limiting factor is the rate of production of soluble or absorbable silicon in the gastrointestinal tract.

Acute toxicity

Exposure to silicate solutions involves not only exposure to silica in the form of its various silicate anions but also to alkalinity. Both distribution of the various silicate anion species and alkalinity depend on the silica to alkali-oxide ratio and the concentration of a given solution.

Sodium and potassium silicates can be irritating or corrosive to the skin of animals as well as of humans, depending on their molar ratio and concentration. Any effects on the skin decrease with increasing molar ratio, superimposed by increasing irritancy with increasing concentrations (OECD, 2004a).

The oral toxicity of sodium silicate in rats decreased at increasing molar ratio $\text{SiO}_2:\text{Na}_2\text{O}$. The LD_{50} ranged from 500 mg/kg b.w. for molar ratio 0.5 to 8 650 mg/kg b.w. for 3.38, thus showing the inverse correlation between molar ratio and toxicity (Schleyer and Blumberg, 1982 and references therein, as cited in OECD, 2004a). Clinical signs observed near to or exceeding the LD_{50} values (Saiwai et al., 1980, as cited in OECD, 2004a) consisted of apathy, staggering gait, dyspnoea, piloerection, abdominal discomfort, and unconsciousness. Autopsy revealed acute gastro-enteritis, vascular congestion, mottled livers, changes in pH of body fluids, shock, chemical irritation and/or corrosion of the viscera. In another study in rats the acute oral LD_{50} was 1 960 mg/kg in groups receiving the 2.0 molar ratio and 2 710 mg/kg in groups receiving the 2.4 ratio sodium silicate (Rhone-Poulenc, 1971, as cited in Elmore, 2005). Although it is not possible to attribute unequivocally any observed toxicity of a silicate solution to either silicate, alkalinity or a combination of both, the observed toxicological signs are indicative of effects due to high alkalinity.

Subacute, subchronic and chronic toxicity studies

Soluble silicates have been tested in drinking water in a number of repeated dose studies. Sodium silicate had a no-observed-adverse-effect level (NOAEL) of 159 mg/kg b.w. per day (highest tested dose) in rats exposed for 180 days (Smith et al., 1973, as cited in OECD, 2004a). The NOAEL for sodium metasilicate in rats exposed for 3 months was 227 and 237 mg/kg b.w. per day for males and females, respectively (Ito et al., 1975, as cited in OECD, 2004a). When rats were administered sodium metasilicate pentahydrate in the diet at 1 259 mg/kg b.w. per day for 8 weeks no effects on body or organ weights were observed but plasma calcium and magnesium and liver zinc were significantly reduced (Kayongo-Male and Jia, 1999, as cited in OECD, 2004a).

Genotoxicity

In vitro, soluble silicates did not induce gene mutations in a series of *in vitro* bacterial assays including *Salmonella typhimurium* Ames test (Saiwai et al., 1980; Ito et al., 1986, as cited in OECD, 2004a). An aqueous sodium silicate solution (36 % active ingredient) induced no chromosomal aberrations in V79 cells, either in the absence or in the presence of metabolic activation (Schulz, 2006, as cited in OECD, 2004a, amended in 2006). *In vivo*, sodium metasilicate did not induce chromosomal aberrations in bone marrow cells of mice (Saiwai et al., 1980, as cited in OECD, 2004a). Although the reliability of these studies cannot be fully evaluated, the lack of structural alerts and these negative results indicate that sodium silicate is unlikely to present any genotoxic potential.

Carcinogenicity

There were no carcinogenicity studies available.

Developmental and reproductive toxicity

The available data on toxicity to reproduction are limited and do not allow any firm conclusions to be drawn. In a developmental toxicity study with mice, sodium metasilicate in aqueous solution was administered at doses of 12.5, 50 or 200 mg/kg b.w. per day from day 0 until day 17/18 of gestation by gavage. No treatment-related effects were observed either in mothers or in pups (Saiwai et al., 1980, as cited in OECD, 2004a).

3.1.2.4. Allergenicity

There is a single report on immediate type allergic contact reactions in a worker exposed to 20 % aqueous sodium silicate (Tanaka et al., 1982). A mouse study on allergy-prone BALB/c mice reported an allergic reaction at high concentrations (4-6 % sodium metasilicate), but no effects on cell proliferation in the auricular lymph nodes were observed at concentrations up to 6 % (Karrow et al., 2002, as cited in OECD, 2004a). Available data give no indication that sodium silicate is an allergen or an adjuvant at concentrations expected from its use as a previous cargo.

3.1.3. Conclusions

Current shipping practices mean that when sodium silicate is transported prior to edible fats and oils, the design of the tanker will be such that sodium silicate has to be transported as a solution. Hence, the CONTAM Panel recommends that the entry for the substance in the Annex to Commission Directive 96/3/EC be amended to 'Sodium silicate (water glass) solution (CAS No 1344-09-8)'.

Toxicological effects of sodium silicate following acute and repeat dosing are mostly due to high alkalinity. However, following ingestion it will be diluted and buffered by the neutralising capacity of the gastrointestinal tract. Thus the CONTAM Panel considered that the levels that would occur following oral ingestion of fats and oils transported subsequent to sodium silicate as a previous cargo would not give rise to any toxicological concern. Although there are no carcinogenicity studies available sodium silicate did not show any genotoxic activity in a variety of *in vitro* and *in vivo* assays thus indicating no genotoxic potential. Available data give no indication that sodium silicate is an allergen or an adjuvant at concentrations expected from its use as a previous cargo. Exposure to sodium silicates can be irritating or corrosive to the skin, however the potential levels arising in fats and oils following its transport as previous cargo would be of no concern. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern.

The CONTAM Panel therefore concludes that sodium silicate solution meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.2. **ISO-OCTANOL (isooctyl alcohol) (CAS No 26952-21-6), ISO-NONANOL (isononyl alcohol) (CAS No 27458-94-2) and ISO-DECANOL (isodecyl alcohol) (CAS No 25339-17-7)**

Iso-octanol, iso-nonanol and iso-decanol are grouped as a family of saturated alcohols. They consist of complex mixtures of straight chain and branched alcohols and are usually not completely separated from each other, e.g. iso-nonanol may contain some iso-octanol as well as some iso-decanol.

Iso-octanol, iso-nonanol and iso-decanol are slightly viscous, high boiling liquids, less dense than water and insoluble in water.

These iso-alcohols are also called oxo-alcohols, as they are produced by hydroformylation (also called 'oxo' process); this process involves reaction of an olefin with carbon monoxide and hydrogen to produce an aldehyde that then undergoes hydrogenation to an alcohol. The olefins are obtained by condensation of propylene and butenes with phosphoric acid at 200 °C under pressure to give a mixture of branched olefins. A recent development in the oxo technology uses a modified rhodium catalyst in order to enable working with a broader range of olefins.

These iso-alcohols are traded as mixtures of isomers.

Iso-octanol, iso-nonanol and iso-decanol are used as intermediates for the production of plasticizers, such as diisononyl- and diisodecyl phthalate (DINP and DIDP) or corresponding adipates, as ingredient in synthetic lubricants, agents in uranium refining, specialty solvents and antifoaming agents in textile processing. Iso-octanol is also used as an intermediate for non-ionic detergents and surfactants, synthetic drying oils, cutting and lubricating oils, hydraulic fluids, resin solvents, emulsifiers and antifoaming agents.

3.2.1. Previous evaluations

The SCF evaluated iso-decanol, iso-nonanol and iso-octanol as previous cargoes in 1996 and they were included in the list of Annex 2 of its Opinion as substances provisionally acceptable because of a lack of toxicological data and uncertainty as to their composition. It was also noted that they can be easily removed if vegetable oil is refined (SCF, 1997a).

In 2003 the SCF re-evaluated a series of provisionally accepted previous cargoes, including iso-decanol, iso-nonanol and iso-octanol, on the basis of further information provided by FOSFA. However, the information available was considered inadequate or needed additional clarification. Therefore, the SCF decided to maintain its previous opinion unchanged (SCF, 2003).

In 2006, OECD published an initial assessment report on the environmental fate and human health effects of oxo-alcohols (C9 to C13) including a mixture of alcohols C8-C10-iso, iso-nonanol and iso-decanol (OECD, 2006). A detailed assessment of the available toxicological database was carried out. It was concluded that the chemicals of this category are of a low order of toxicity, do not possess mutagenic activity, there is no evidence of carcinogenic potential or adverse effects on fertility and reproduction and therefore they are of low priority for further work.

3.2.2. Current evaluation

3.2.2.1. Expected impurities

Iso-octanol, iso-nonanol and iso-decanol are mixtures in themselves and mostly of technical quality. The starting substances are volatile and their byproducts would easily be removed when not integrated into the product. The residual amounts of intermediate alkenes and aldehydes would not be of concern at the levels present in products used as previous cargoes. Hydroformylation is not expected to result in byproducts of concern.

3.2.2.2. Reactivity and reaction products

Iso-octanol, iso-nonanol and iso-decanol may react with lipids by interesterification, but this does not result in any products of concern.

3.2.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

The C8-C10 oxo-alcohols are readily absorbed from the gastrointestinal tract and are rapidly eliminated from the blood. The main metabolic pathway involves initial oxidation to the corresponding aldehyde, catalysed primarily by cytosolic alcohol dehydrogenases (ADH), with a lesser contribution from P450 and other oxidases. The aldehydes are then converted to the respective carboxylic acids, by aldehyde dehydrogenases (ALDH). The carboxylic acids are subsequently metabolized to carbon dioxide via mitochondrial beta-oxidation pathways and the tricarboxylic acid cycle, in the same way as dietary fatty acids (OECD, 2006). This stepwise removal of C2 units is more efficient for linear acids than for the corresponding branched acids. The latter can also be metabolised by microsomal ω - or ω -1 oxidation followed by β -oxidation, which is relatively efficient for such compounds (Verhoeven et al., 1998). For unsaturated carboxylic acids, cleavage of C2-units continues

until a double bond is reached. These double bonds will be in the *cis*-configuration, and can be isomerised to the *trans*-configuration by enoyl-CoA isomerase. β -Oxidation then continues with the *trans*-isomer (JECFA, 1999). The alcohols (both parent substances and their primary metabolites) or their oxidation products can be conjugated with sulphate or glucuronic acid, catalysed by sulphotransferases and UDP-glucuronosyltransferases respectively, the extent of these reactions increasing with the degree and complexity of branching (Williams, 1959; Bevan, 2001; OECD, 2006).

The CONTAM Panel undertook a limited exercise on the prediction of the main metabolites of two examples of oxo-alcohols, representing the potential for most branching amongst the compounds likely to be present in the technical mixtures being assessed as previous cargoes (See Figure 1a and 1b).

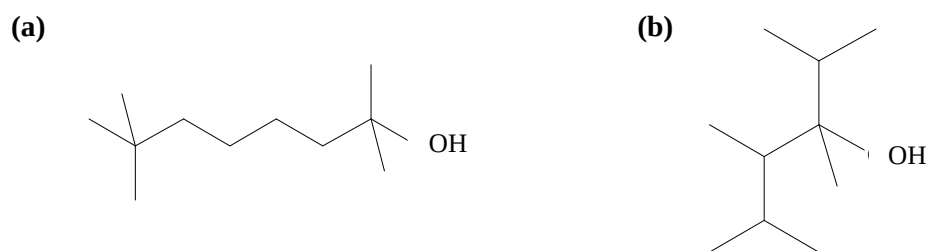


Figure 1: Two examples of oxo-alcohols representing the potential for most branching amongst the compounds likely to be present in the technical mixtures being assessed as previous cargoes.

Using the METEOR software 13.0.0 (Lhasa Ltd), plausible metabolic transformations of the compounds depicted in Figure 1a and Figure 1b included terminal methyl oxidation, primary alcohol oxidation and glucuronide conjugation of the resulting carboxylic acids. These pathways were supported by reference to several known examples in each case (Wim Mennes, 2012, personal communication).

The CONTAM Panel concluded that the potential for retention or bioaccumulation for the parent alcohols and their biotransformation products is likely to be limited.

Acute toxicity

In the OECD assessment (2006) it was concluded that the sub-category of oxo-alcohols C9 to C13 is practically non-toxic with oral LD₅₀s ranging from > 2 000 to 5 400 mg/kg b.w. Acute oral LD₅₀s of 6 500 and 3 950 mg/kg b.w. were reported in rats orally administered iso-decanol (Nishimura et al., 1994), and iso-nonanol (Anonymous 1986a, as cited in ECHA, online) respectively. Oral LD₅₀s of 1 480 and 1 670 mg/kg b.w. were reported in rats and mice respectively, upon oral administration of iso-octanol.^{16,17} Dermal LD₅₀s ranged from > 2 600 to 5 010 mg/kg b.w. and inhalation exposures conducted at saturated vapour pressures generally produced no deaths (OECD, 2006). Members of this category were moderately irritating to the skin and irritating to the eyes of rabbits and, in addition, the alcohols C9-C11-iso, C9 rich, produced moderate upper airway sensory irritation in male mice exposed to vapours. There is no indication of skin sensitizing potential for the oxo-alcohols C9-C13 category (OECD, 2006).

Subacute, subchronic and chronic toxicity studies

In a 14-day study in rats, designed to evaluate potential effects on liver and testes, iso-decanol and iso-nonanol given by gavage produced minimal or no effects on the liver, and no testicular effects at doses of 168 and 144 mg/kg b.w. per day, respectively (Rhodes et al., 1984; OECD, 2006). No oral repeated dose toxicity studies were identified for iso-octanol. In general, the available data suggest that

¹⁶ ChemID Plus, online. Available at: <http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp>

¹⁷ RTECS, online. Available at: <http://www.cdc.gov/niosh/rtecs/>

the members of the oxo-alcohols C9-C13 category present low order of subchronic toxicity (OECD, 2006).

Genotoxicity

Bacterial mutagenicity studies (using *Salmonella typhimurium* as well as *Escherichia coli*) for four members of the oxo-alcohols C9-C13 category showed a consistent lack of mutagenic activity (OECD, 2006). In particular, iso-nonanol (Anonymous 1986d, as cited in ECHA online) and iso-octanol (Henkel KgaA, 1982a; HLS, 1996k, both as cited in OECD, 2006) were negative in the Ames test with and without metabolic activation. Iso-decanol was also tested in an *in vitro* chromosomal aberration assay with V79 Chinese hamster lung fibroblasts, and no mutagenic effects were noted with or without metabolic activation (OECD, 2006). In addition to these *in vitro* results, two category members and the analogue linear alcohol 1-dodecanol were negative in an *in vivo* mouse bone marrow micronucleus test (OECD, 2006) thus indicating that the members of the oxo-alcohols C9-C13 category are unlikely to present any genotoxic potential either *in vitro* or *in vivo*.

Carcinogenicity

No carcinogenicity studies have been conducted on oxo-alcohols C9-C13 category members. The potential for initiation, promotion or co-carcinogenicity has been investigated for several aliphatic alcohols including the analogue linear alcohol 1-dodecanol. Even taking into account the limitations and low reliability of these experiments, the data show that none of the aliphatic alcohols tested have a potential to induce local skin tumours upon repeated dermal application at or above the maximum tolerated (irritant) dose (Sicé, 1966; Bingham and Falk, 1969; Van Duuren and Goldschmidt, 1976, as cited in OECD, 2006).

In other assays, oxo-alcohols C10-C12 members were repeatedly injected into the peritoneal cavity or implanted in the bladder of mice. Although there are limitations to these studies, no induction of tumours was observed (Bryan and Springberg, 1966; Stoner et al., 1973, both as cited in OECD, 2006) and in one study (Ando et al., 1972, as cited in OECD, 2006) a prolongation of survival time was reported.

Based on the lack of genotoxic effects both *in vitro* and *in vivo* and on the absence of any structural alerts, members of the oxo-alcohols C9-C13 category are unlikely to possess genotoxic carcinogenic potential.

Developmental and reproductive toxicity

In a comparative developmental toxicity study with rats, iso-decanol (a mixture of different isomers) was administered by gavage at doses of 0, 1, 5 and 10 mmol/kg (0, 158, 790, and 1 580 mg/kg b.w. per day) during gestation day (GD) 6 to 15. Iso-decanol elicited maternal toxicity at 10 mmol/kg and caused a low incidence of retardations and rare malformations at that dose level. Body weight, uterus weight, and fetal weights were all significantly lower than controls in the highest dose group. Overt maternal mortality (4/10) was observed only in this dose group thus accounting for the effects observed. Some maternal signs of toxicity, but no fetal effects, were observed at 5 mmol/kg. The NOAELs for maternal and fetal effects were 1 mmol/kg and 5 mmol/kg, respectively (Hellwig and Jäckh, 1997).

In another study maternal toxicity, including decreased feed intake and body weight gain and abnormal clinical signs, was observed at 790 mg/kg b.w. given by gavage to rats from gestation day 6 to 15. No embryo-fetal toxicity was observed (Eastman Kodak Co., 2009).

No effects to parents or offspring were observed in a combined repeated dose developmental/reproductive toxicity study with the analogue 1-dodecanol at doses up to 2 000 mg/kg

b.w. per day (OECD, 2006) thus supporting the conclusion that members of the oxo-alcohols C9-C13 category are not reprotoxic.

3.2.2.4. Allergenicity

Available data give no indication that iso-decanol, iso-nonanol or iso-octanol are allergens or adjuvants.

3.2.3. Conclusion

Iso-octanol, iso-nonanol and iso-decanol were classified by the SCF in its more recent re-evaluation (SCF, 2003) as provisionally acceptable as previous cargoes because the information available was considered inadequate or limited. The CONTAM Panel used a 'read across' approach (OECD, 2006) to fill data gaps on the toxicological profile of iso-octanol, iso-nonanol and iso-decanol. They are of a low order of toxicity following acute and repeated exposures upon oral, dermal or inhalational exposure. The lack of effects found in the limited studies available suggests that they are not genotoxic. They are not allergenic and there are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern.

The CONTAM Panel therefore concludes that iso-decanol, iso-nonanol or iso-octanol meet the criteria for acceptability as previous cargoes for edible fats and oils.

3.3. 1,3-PROPYLENE GLYCOL (trimethylene glycol; 1,3-propanediol) (CAS No 504-63-2)

The term 'propylene glycol' is misleading: when used without specification of the position of the hydroxyl groups, 1,2-propylene glycol is meant, which is produced in far larger quantities than the 1,3-analogue. For this reason, '1,3-propanediol' is the preferred name of the substance considered here. 1,2-Propylene glycol was evaluated for its acceptability as a previous cargo for edible fats and oils by the CONTAM Panel in 2011 and it was concluded that it met the criteria for acceptability (EFSA, 2011).

1,3-Propanediol is a flammable, colourless, stable liquid. 1,3-Propanediol is produced commercially from acrolein (addition of water in acidic medium followed by hydrogenation), by hydroformylation of ethylene oxide or biochemical fermentation from glycerol (a by-product of the biodiesel chain) or by starch fermentation. 1,3-Propanediol is then separated and purified by several means, including ultrafiltration and/or distillation.

Between 75 and 90 % of 1,3-propanediol is used in a polymerization process to manufacture polytrimethylene terephthalate (PTT). 1,3-Propanediol may also be a component of de-icing fluids, engine coolants, heat transfer fluids, chemical intermediates, personal care products, or process solvents.

3.3.1. Previous evaluations

In the SCF's 1996 opinion on acceptable previous cargoes (SCF, 1997a), 1,3-propanediol was considered not acceptable as a previous cargo because of inadequate toxicological data on a substance that is structurally of concern.

In 1998, the SCF considered 1,3-propanediol for use as a co-monomer in polyesters. On the basis of new mutagenicity and developmental toxicity studies, the SCF concluded that the use of 1,3-propanediol was acceptable and should be classified in SCF List 3 (defined as substances for which an ADI or a TDI could not be established, but where the present use could be accepted) with a restriction of not more than 0.05 mg/kg in food (SCF, 1998).

In 2003 the SCF, on the basis of new information from a sub-chronic toxicity study showing low oral toxicity of 1,3-propanediol and provided that residues would be low after tank cleaning, considered that 1,3-propanediol was acceptable as a previous cargo (SCF, 2003).

3.3.2. Current evaluation

3.3.2.1. Expected impurities

After production, 1,3-propanediol is purified by distillation. As the boiling point is far above that of acrolein (211-217 °C *versus* 53 °C), an efficient separation and removal of acrolein is expected.

3.3.2.2. Reactivity and reaction products

1,3-Propanediol slowly reacts with lipids by transesterification, but no products of concern are expected when it is transported as a previous cargo.

3.3.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Pollitt et al. (1987) proposed that 1,3-propanediol can be metabolized to 3-hydroxypropionaldehyde and then to 3-hydroxypropionic acid or to malonaldehyde. Malonaldehyde is expected to be a short-lived metabolic intermediate which can be further converted to malonic semialdehyde and then to malonic acid (Gingell et al., 2000).

Acute toxicity

1,3-Propanediol was administered to rats by gavage at doses ranging between 9.0 and 18.7 mL/kg b.w. (Spanjers and Til, 1979, as cited in ACC 1,3-Propanediol Panel, 2007). Sluggishness, ataxia and sedation were the only signs observed within a few hours. The LD₅₀ was 14.9 mL/kg b.w., corresponding to 15.8 g/kg b.w. In another experiment with rats administered by gavage the LD₅₀ was 10 mL/kg b.w. corresponding to 10.5 g/kg b.w. (Coombs and Clark, 1977, as cited in ACC 1,3-Propanediol Panel, 2007).

Subacute, subchronic and chronic toxicity studies

1,3-Propanediol was orally administered to rats by gavage at doses up to 1 000 mg/kg b.w. per day for 13 weeks (Gingell et al., 2000). All animals survived and showed no evidence of any systemic toxicity. In particular, no effects on haematology or serum chemistry parameters were reported, spermatogenic end-points were unaffected and no apparent pathological or functional effects were observed in the liver. The NOAEL for this study was therefore 1 000 mg/kg b.w. per day, the highest dose tested (Kirkpatrick, 1999, as cited in ACC 1,3-Propanediol Panel, 2007; Gingell et al., 2000). A NOAEL of 1 000 mg/kg b.w. per day (highest dose tested) was also identified in a 2-week oral toxicity study with rats (Mertens, 1997, as cited in ACC 1,3-Propanediol Panel, 2007).

Genotoxicity

1,3-Propanediol was negative in the Ames test (Degussa, 1994a, as cited in SCF, 2003; Wollny, 1994a, as cited in ACC 1,3-Propanediol Panel, 2007) and in a gene mutation assay in cultured mammalian cells (Degussa, 1994b, as cited in SCF, 2003; Wollny, 1994b, as cited in ACC 1,3-Propanediol Panel, 2007). Contrasting data were reported for clastogenic effects in mammalian cells in culture (Degussa, 1994c, as cited in SCF, 2003; Volkner, 1994, as cited in ACC 1,3-Propanediol Panel, 2007; Gudi and Brown, 2001, as cited in ACC 1,3-Propanediol Panel, 2007).

In 1984 one study was published (Summerfield and Tappel, 1984) indicating that administration of 1,3-propanediol at 500 mg/kg in the diet of rats for up to 15 weeks caused DNA-protein and interstrand DNA cross-links, as detected by high-performance liquid chromatography, in liver and, to a limited extent, in testis. This was associated with the fact that liver but not testis can metabolize 1,3-propanediol to malondialdehyde, a cross-linking agent. In a well conducted mouse micronucleus study (Krauser, 1995, as cited in ACC 1,3-Propanediol Panel, 2007), male and female mice received a single oral dose of 1,3-propanediol (at 1 000, 1 470 or 2 150 mg/kg b.w.) and were euthanized at

24 and 48 hours after treatment. The assay was conducted on two separate occasions. All animals, in both assays, survived to scheduled termination and no treatment-related toxic signs were observed. In the initial assay, 1,3-propanediol was not considered to induce chromosome mutations in mice by damage to chromosomes or the mitotic apparatus, but a statistically significant increase in micronucleated polychromatic erythrocytes (PCEs) was observed at 48 hours in the sexes combined, as compared to the negative control (23/1000 cf 11/1000, $P < 0.05$). Hence, it was not possible to exclude a weak clastogenic effect. However, in the second assay, there was no increase in the number of micronucleated PCEs following exposure to 1,3-propanediol. In conclusion, based on the overall weight of evidence, 1,3-propanediol does not have genotoxic potential.

Carcinogenicity

No data are available on the carcinogenicity of 1,3-propanediol.

Developmental and reproductive toxicity

In 90-day oral toxicity studies with rats there were no effects of 1,3-propanediol at any dose up to 1 000 mg/kg per day on reproductive organs or on spermatogenic endpoints (Kirkpatrick, 1999, as cited in ACC 1,3-Propanediol, 2007; Gingell et al., 2000).

In a developmental toxicity study with rats, 1,3-propanediol orally administered at doses up to 1 000 mg/kg b.w. per day (Mitterer, 1992) did not induce any treatment-related adverse effect. The NOAEL for maternal and fetal toxicity was therefore 1 000 mg/kg b.w., the highest dose tested (ACC 1,3-Propanediol Panel, 2007).

3.3.2.4. Allergenicity

In the guinea pig maximization test and in the guinea pig Landsteiner/Draize test, 1,3-propanediol did not act as a sensitizer (Coombs and Clark, 1977; Til and Keizer, 1979, both as cited in ACC 1,3-Propanediol Panel, 2007). In a study with human volunteers, 1,3-propanediol did not produce adverse skin responses in any of the study participants (Anonymous, 2006b, as cited in ECHA, online).

Available data give no indication that 1,3-propanediol is an allergen or an adjuvant.

3.3.3. Conclusions

The term 'propylene glycol' is misleading: when used without specification of the position of the hydroxyl groups, 1,2-propylene glycol is meant, which is produced in far larger quantities than the 1,3-analogue. For this reason, '1,3-propanediol' is the preferred name of the substance considered here. The CONTAM Panel therefore recommends that the entry for the substance in the Annex to Commission Directive 96/3/EC is amended to '1,3-propanediol (1,3-propylene glycol; trimethylene glycol) (CAS No 504-63-2)'.

1,3-Propanediol is of low systemic toxicity when administered by the oral route. There are no carcinogenicity data but the available evidence indicates that 1,3-propanediol does not have any genotoxic potential. 1,3-Propanediol is not an allergen. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern.

Therefore, the CONTAM Panel concludes that 1,3-propanediol meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.4. ISO-BUTYL ACETATE (CAS No 110-19-0)

Chemically, the term iso-butyl refers to a number of branched butyl groups (including 1-methyl propyl, also called sec-butyl, and 1,1-dimethyl ethyl, also called tert-butyl). Isobutyl is a trivial name

standing for 2-methyl propyl. For this reason 'isobutyl acetate' is the preferred name, i.e. without the hyphen. The IUPAC name of isobutyl acetate is 2-methylpropyl acetate.

Isobutyl acetate is used as a solvent for coatings, thinners, sealants, adhesives, printing inks, caulks, leather treatment, cleaners, cosmetics (nail polishes), and as a process solvent in numerous applications. It is also used in perfumes and as a flavouring agent in various foods and non-alcoholic beverages.

It is produced from the esterification of isobutanol with acetic acid in the presence of a strong acid (usually sulphuric acid).

All of the butyl acetate isomers have been found to occur naturally in a range of fruits, such as bananas (Macku and Jennings, 1987; Bisesi, 1994, as cited in WHO, 2005) and nectarines (Takeoka et al., 1988, as cited in WHO, 2005).

3.4.1. Previous evaluations

The SCF evaluated isobutyl acetate in 1996 as a previous cargo for edible fats and oils and considered this substance was acceptable on the basis that it was 'generally used as a flavouring in food on FEMA and GRAS lists' (SCF, 1997a). In the 2003 SCF evaluation of acceptable previous cargoes, isobutyl acetate was not further evaluated as it was already considered acceptable (SCF, 2003).

Isobutyl acetate was given Generally Recognized as Safe (GRAS) status by the Flavor and Extract Manufacturers Association of the US (FEMA) in 1965.

In a monograph on fragrance raw materials, published in 1978, Opdyke states that the Council of Europe listed isobutyl acetate as an acceptable flavouring substance, with an ADI of 1.0 mg/kg b.w. (Opdyke, 1978).

The SCF (1992a) considered isobutyl acetate acceptable as a flavouring substance for use in food, on the basis of an evaluation by the Expert Committee on Flavourings of the Council of Europe. The Expert Committee classified it as category A, which may be used in foodstuffs, and established practical upper levels of 150 mg/kg in food and 10 mg/kg in beverages (CoE, 1992).

JECFA (1998) evaluated isobutyl acetate as a flavouring agent and concluded that there was 'no safety concern at current levels of intake', estimated to be 1 200-1 300 µg/person per day. This was because isobutyl acetate is a Cramer class I substance, and it can be predicted to be metabolised to innocuous products. Hence, exposures below a threshold of toxicological concern (TTC) of 1 800 µg/person per day (30 µg/kg b.w. per day) would not be expected to be of safety concern.

OECD (2003) concluded that isobutyl acetate was currently of low priority for further work.

WHO (2005) published a Concise International Chemical Assessment of isobutyl acetate, in which it was concluded that there was insufficient information, including an absence of data on potential carcinogenicity, to enable derivation of tolerable intakes or concentrations.

Isobutyl acetate is approved under US Food and Drug Administration (FDA), Title 21 US CFR citations for food additives permitted for direct addition to food for human consumption (FDA, 2011a).

3.4.2. Current evaluation

3.4.2.1. Expected impurities

Technical grades of butyl acetates contain butyl alcohol as an impurity (Syracuse Research Corp., 1979, as cited in WHO, 2005). This would not be of toxicological concern.

3.4.2.2. Reactivity and reaction products

Isobutyl acetate may be hydrolysed to isobutanol and acetic acid or transesterified with lipids. Neither these nor any other reaction products of concern are expected when isobutyl acetate is transported as a previous cargo.

3.4.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

There is little specific information on the absorption and disposition of isobutyl acetate. Given its physicochemical characteristics (log P_{ow} 2.3; molecular weight 116.2) it is likely to be readily absorbed from the gastrointestinal tract and distributed throughout the body. Isobutyl acetate partitions somewhat more into tissues than blood, with tissue/blood ratios of 2-5 for most tissues, but approx 20 for fat (Kaneko et al., 1994). Metabolism is by hydrolysis to acetic acid and isobutanol, most likely by carboxylesterases, which are found in a variety of tissues, including liver and gastrointestinal tract (Longland et al., 1977; Dahl et al., 1987). As the hydrolysis rate of n- and isobutyl acetate are similar (Dahl et al., 1987), it is likely that isobutyl acetate, by analogy with n-butyl acetate, will have a very short half-life in rats, as will its hydrolysis products, in the order of minutes (Teeguarden, 2005, as cited in ECHA online). Acetic acid is oxidised via the citric acid cycle to carbon dioxide and water. Isobutanol is rapidly metabolised by alcohol dehydrogenase (mainly class 1) to isobutyraldehyde and then by aldehyde dehydrogenase to isobutyric acid. These are further oxidised to carbon dioxide (JECFA, 1999; WHO, 2005). Small amounts of isobutanol may be excreted unchanged or conjugated with glucuronic acid (WHO/IPCS, 1987; OECD, 2004b).

Acute toxicity

Isobutyl acetate is of low acute oral toxicity, with LD₅₀ values in rats and rabbits of the order of several grams per kg b.w. (Smyth et al., 1962; Munch, 1972, as cited in OECD, 2007).

The irritant properties of isobutyl acetate to the skin have not been well characterised. In an early study (1962) it was reported not to be irritating to skin following uncovered application, whereas in a later study (1978), with limited information available, it was reported to be irritating when applied under occlusion. Isobutyl acetate caused only minor, reversible irritation to the eyes in a well conducted study.¹⁸ Isobutyl acetate was tested as a 2 % preparation in petrolatum in human volunteers, in a 48-h closed-patch test. It was not irritating to skin in this test (Opdyke, 1978). This substance has not been classified as irritating to either skin or to eyes in the ECHA database.¹⁹

Subacute, subchronic and chronic toxicity studies

No data are available on the repeat dose toxicity of isobutyl acetate.

The toxicity of its major metabolite, isobutanol, has been investigated by the oral and inhalation route. Groups of 30 Crj: CD(SD) rats of both sexes received isobutanol (purity not stated) by oral gavage daily at doses of 0, 100, 316 and 1 000 mg/kg b.w. per day for 90 days. Effects were seen only at the highest dose. There was an early reduction in body weight in males and in food consumption in both sexes. Clinical signs, including hypoactivity, ataxia and salivation, were observed in males and females for the first few weeks. Serum potassium was reduced by 11-15 % at week 4 or 5. No histopathological changes were observed. The NOAEL was 316 mg/kg b.w. per day (OECD, 2007).

¹⁸ European Chemicals Agency (ECHA). Isobutyl acetate. CAS No 110-19-0. Available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8c29e4-9219-2519-e044-00144f67d249/AGGR-ec680302-4ca3-4ee8-a91e-723cf0590511_DISS-9d8c29e4-9219-2519-e044-00144f67d249.html#section_1.1

¹⁹ European Chemicals Agency (ECHA). Summary Of Classification and Labelling. Available at: <http://clp-inventory.echa.europa.eu/SummaryOfClassAndLabelling.aspx?SubstanceID=24499&HarmOnly=no?fc=true&lang=en>; accessed 17/05/2012

In an inhalation study, groups of 10 or 20 male and female Sprague-Dawley (SD) rats were exposed (whole body) to isobutanol (purity > 99 %) at concentrations of up to 2 500 ppm (ca. 7 700 mg/m³) (ca. 2 200 mg/kg b.w. per day) for 13 weeks, for 6 h per day, 5 days per week. At the highest concentration, there were slight increases in total erythrocyte count, haemoglobin, and haematocrit in females. There were no other toxicologically relevant treatment-related effects (Li et al., 1999).

In a study of a structural analogue, isobutyl isobutyrate (purity ≥ 98 %) was administered by oral gavage at doses of 0, 10, 100, and 1 000 mg/kg b.w. per day to groups of 15 Wistar rats/sex daily for 18 weeks. No treatment-related effects of toxicological significance were observed in any dose group. The NOAEL was 1 000 mg/kg b.w. per day, the highest dose tested.¹⁸

Genotoxicity

Isobutyl acetate was negative in an Ames/Salmonella test with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, either with or without metabolic activation by hepatic post-mitochondrial supernatant (S9 preparation) (OECD, 2007).

Isobutanol, a major metabolite, had no effect on the frequency of micronuclei and there was no evidence of clastogenicity or 'impairment of chromosome distribution' in bone marrow of mice administered doses of up to 2 000 mg/kg b.w. by oral gavage (OECD, 2007).

Carcinogenicity

No studies on the potential carcinogenicity of isobutyl acetate were identified.

Developmental and reproductive toxicity

No studies on the reproductive or developmental toxicity of isobutyl acetate could be identified.

Studies on the major metabolite, isobutanol, following inhalation exposure, are available. In a 2-generation study of possible effects on reproduction, groups of 30 male and 30 female, Crl:CD(SD)IGS BR rats were exposed (whole body) to isobutanol (purity > 99 %) at concentrations of 500 ppm, 1 008 ppm and 2 522 ppm (approx 0, 1 515, 3 030 or 7 575 mg/m³) for 6 h/day, 7 days/week, for 10 weeks prior to mating. Females were exposed during gestation and lactation (from postnatal day 5). F1 pups were exposed directly from the day of weaning, on postnatal day 29, for 10 weeks prior to mating. No treatment-related effects were observed on parents or offspring at any exposure level. The no-observed-adverse-effect concentration (NOAEC) was 7 600 mg/m³ (ca. 3 100 mg/kg b.w. per day), the highest concentration tested (WHO, 2005; OECD, 2007).

In developmental toxicity studies, groups of pregnant Wistar rats (25 per dose group) and Himalayan rabbits (15 per group) were exposed by inhalation (whole body) to concentrations of 0, 500, 2 500 and 10 000 mg/m³ isobutanol for 6 hours per day during gestation (rats: GD6-GD15; rabbits: GD7-GD19). Body weight gain was slightly reduced in rabbits exposed to the highest concentration. No treatment-related effects were observed in rat dams. There was no evidence for either developmental or fetotoxic effects in either species. The NOAEC for developmental toxicity was 10 000 mg/m³ (ca. 4 100 and 900 mg/kg b.w. per day for rats and rabbits, respectively), the highest concentration tested, in both rats and rabbits (OECD, 2007).

3.4.2.4. Allergenicity

A maximization test was carried out on 28 volunteers with 2 % isobutyl acetate in petrolatum. No dermal sensitization was observed (Epstein, 1976, as cited in OECD, 2003). Isobutyl acetate did not cause sensitization in the guinea pig maximization test (OECD TG 406) (Huels, 1988, as cited in OECD, 2003 and WHO, 2005).

Available data give no indication that isobutyl acetate is an allergen or an adjuvant.

3.4.3. Conclusions

The CONTAM Panel notes that the name iso-butyl acetate is rarely used to refer to this substance. It is more normally referred to as isobutyl acetate. The preferred IUPAC name is 2-methylpropyl acetate. The CONTAM Panel therefore recommends that the entry for the substance in the Annex to Commission Directive 96/3/EC⁵ to be amended to 'Isobutyl acetate (2-methylpropyl acetate) (CAS No 110-19-0)'.

The toxicity of acetate, one of the major metabolites of isobutyl acetate, was addressed in a previous Opinion of the CONTAM Panel, when it was considered that there would be no health concerns following the maximum potential carryover into edible fats and oils when it is transported as a previous cargo (EFSA, 2012a).

In its evaluation of previous cargoes in 1996, the SCF concluded that isobutanol, the other major metabolite, was 'not acceptable', because 'limited toxicological data indicates a suspicion of carcinogenic concerns.' In a re-evaluation in 2003, the SCF maintained its previous opinion that this substance was not acceptable as a previous cargo because the Committee was aware of a number of issues that still needed clarification. The CONTAM Panel considered isobutanol as a previous cargo for edible fats and oils in 2009, and concluded that it was acceptable, based on the low level of toxicity observed in a more recent chronic toxicity study, as well as its volatility and ease of tank cleaning (EFSA, 2009b).

Although the toxicological database for isobutyl acetate is limited, available data on this substance and on its hydrolysis products, acetic acid and isobutanol, suggest that isobutyl acetate is of relatively low systemic toxicity. It is not genotoxic or allergenic. There are no reaction products or impurities expected to be of toxicological concern.

The CONTAM Panel therefore concludes that isobutyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.5. SEC-BUTYL ACETATE (CAS No 105-46-4)

sec-Butyl acetate, s-butyl acetate, 2-butanol acetate or 1-methylpropyl acetate is a racemic mixture. It is a solvent commonly used in lacquers and enamels.

The first method of production of sec-butyl acetate was the esterification of sec-butanol and acetic anhydride. sec-Butyl acetate is now prepared by direct esterification of acetic acid with secondary butyl alcohol at 100-110 °C in the presence of H₂SO₄ in a continuous process. sec-Butyl acetate is purified by distillation.

3.5.1. Previous evaluations

The SCF evaluated sec-butyl acetate in 1996 as a previous cargo for edible fats and oils and considered this substance was acceptable, with the explanation 'Limited toxicological data but no indication of a hazard. Easily removed by tank cleaning.' (SCF, 1997a). In the 2003 SCF evaluation of acceptable previous cargoes, sec-butyl acetate was not further evaluated as it was already considered acceptable (SCF, 2003).

WHO (2005) concluded that there was insufficient information on sec-butyl acetate to enable a tolerable intake to be established.

EFSA (2008a) evaluated sec-butyl acetate as a flavouring agent and concluded that it was a Cramer class I substance, and that it can be predicted to be metabolised to innocuous products. Hence, exposures below a TTC of 1 800 µg/person per day (30 µg/kg b.w. per day) would not be expected to be of safety concern.

3.5.2. Current evaluation

3.5.2.1. Expected impurities

sec-Butyl acetate is synthesised from substances which are not expected to contain impurities of concern. Also, the esterification step is not expected to result in impurities of concern.

3.5.2.2. Reactivity and reaction products

sec-Butyl acetate may be hydrolysed to sec-butanol and acetic acid or transesterified with lipids. Neither these nor any other reaction products are expected to be of concern when sec-butyl acetate is transported as a previous cargo.

3.5.2.3. Toxicological profile

There is very little specific information on the toxicity of sec-butyl acetate.

Absorption, distribution, metabolism and excretion

There is little specific information on the absorption and disposition of sec-butyl acetate. Given its physicochemical characteristics (log P_{ow} 1.51; molecular weight 116.2) it is likely to be readily absorbed from the gastrointestinal tract and distributed throughout the body. Metabolism is by hydrolysis to acetic acid and sec-butanol, most likely by carboxylesterases, which are found in a variety of tissues, including liver and gastrointestinal tract (Longland et al., 1977; Dahl et al., 1987). As the hydrolysis rates of n- and sec-butyl acetate are similar (Dahl et al., 1987), it is likely that sec-butyl acetate, by analogy with n-butyl acetate, will have a very short half-life in rats, as will its hydrolysis products, in the order of minutes (Teeguarden et al., 2005, as cited in ECHA online). Acetic acid is oxidised via the citric acid cycle to carbon dioxide and water. sec-Butanol is rapidly metabolised by alcohol dehydrogenase (mainly class 1) to 2-butanone (methyl ethyl ketone), which is then either excreted unchanged in breath and urine or further metabolised to 3-hydroxy-2-butanone and 2,3-butanediol (WHO, 2005). There is evidence that sec-butyl acetate can also be hydroxylated by at least one form of P450 to yield an unstable hemiketal (2-hydroxy-2-acetoxybutane), which is cleaved non-hydrolytically to 2-butanone (Peng et al., 1995; WHO, 2005).

The toxicity of methyl ethyl ketone (MEK) was reviewed in a previous opinion of the CONTAM Panel (EFSA, 2012a), when it was considered to meet the criteria for acceptability as a previous cargo for edible fats and oils.

Acute toxicity

sec-Butyl acetate is of low acute toxicity, with an oral LD_{50} value in rats of 3 200-6 400 mg/kg b.w. (WHO, 2005).

Some information sheets suggest that sec-butyl acetate is mildly irritating to the eyes, but no specific information is provided (e.g. ICSC, 1994). In their review of 2005, WHO was unable to identify any data on the irritation or sensitization potential of sec-butyl acetate. sec-Butyl acetate has not been classified in the ECHA database for irritation to skin or eyes.²⁰

Subacute, subchronic and chronic toxicity studies

No data were identified on the repeat dose toxicity of sec-butyl acetate. Nor could any data be found for the effects of the major metabolite, sec-butanol.

²⁰ European Chemicals Agency (ECHA). Summary of Classification and Labelling. Available at: <http://clp-inventory.echa.europa.eu/SummaryOfClassAndLabelling.aspx?SubstanceID=120101&HarmOnly=no?DisclaimerAgr=Agr&Index=105-46-4&ExecuteSearch=true&fc=true&lang=en> (accessed 17/05/2012)

Genotoxicity

No information on the genotoxicity of sec-butyl acetate could be identified.

The major metabolite sec-butanol has been tested for genotoxicity in a number of organisms *in vitro*. It did not cause mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, *Escherichia coli* WP2uvrA/pKM101, nor did it cause gene conversion in yeast (*Saccharomyces cerevisiae* JD1), either with or without metabolic activation by hepatic postmitochondrial supernatant (S9 preparation) from Aroclor 1254-treated rats (Brooks et al., 1988, as cited in WHO, 2005).

Carcinogenicity

No information could be found on the carcinogenicity of either sec-butyl acetate or of its major metabolite, sec-butanol.

Developmental and reproductive toxicity

No studies on the developmental or reproductive toxicity of sec-butyl acetate could be identified. The reproductive toxicity of its major metabolite, sec-butanol, has been evaluated in a two-generation study in rats. Groups of 30 male and 30 female Wistar rats received sec-butanol in their drinking water at concentrations of 0, 0.3, 1 and 3 % (equivalent to 0, 450, 1 500 and 4 500 mg/kg b.w. per day). The highest dose was reduced to 2 % (3 000 mg/kg b.w. per day) in the second generation, because of toxicity. In a developmental phase of the study, fetuses were examined on gestation day 20. Exposure to sec-butanol at 3 000 mg/kg b.w. per day resulted in a significant reduction in fetal weight and retardation of skeletal maturation. There were no skeletal or visceral malformations. There were no effects on fertility. The NOAEL for developmental toxicity was 1 500 mg/kg b.w. per day (Gallo et al., 1977, as cited in WHO, 2005).

The developmental toxicity of sec-butanol has been investigated in rats following exposure by inhalation. Groups of 15 pregnant SD rats were exposed to sec-butanol by inhalation for 7 h/day from gestation day 1-19, at concentrations of 0, 3 500, 5 000 and 7 000 ppm (i.e. 0, 11 000, 15 000 or 22 000 mg/m³). There was a reduction in body weight gain in dams in all groups exposed to sec-butanol. At the highest concentration, there was a reduction in the number of live fetuses and an increase in the number of resorptions. Fetal body weight was reduced at $\geq 15\,000\text{ mg/m}^3$. There was no increase in the incidence of malformations. The NOAEC for developmental toxicity was 11 000 mg/m³ (ca. 5 200 mg/kg b.w. per day) (Nelson et al., 1989, as cited in WHO, 2005).

3.5.2.4. Allergenicity

Available data give no indication that sec-butyl acetate is an allergen or an adjuvant.

3.5.3. Conclusion

There are no data on repeated-dose toxicity of sec-butyl acetate. It is a Cramer class I substance, with a TTC of 1 800 µg/person per day. It is rapidly metabolised to acetate and sec-butanol, which can be further metabolised to MEK. The CONTAM Panel has previously evaluated acetic acid and MEK for their suitability as previous cargoes for edible fats and oils and concluded that they meet the criteria for acceptability (EFSA, 2012a). Sec-Butanol is not genotoxic. The information available on sec-butanol does not indicate any toxicological concern at the exposure levels that might occur from the transport of sec-butyl acetate as a previous cargo to edible fats and oils. sec-Butyl acetate is not allergenic. There are no reaction products or impurities of toxicological concern.

The CONTAM Panel therefore concludes that sec-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.6. TERT-BUTYL ACETATE (CAS No 540-88-5)

Tert-butyl acetate or t-butyl acetate is used as a solvent in the production of lacquers, enamels, inks, adhesives, thinners and industrial cleaners.

Tert-butyl acetate is synthesised by reaction of acetic acid with isobutylene.

3.6.1. Previous evaluations

The SCF evaluated tert-butyl acetate in 1996 as a previous cargo for edible fats and oils and considered this substance was acceptable, with the explanation 'Limited toxicological data but no indication of a hazard. Easily removed by tank cleaning.' (SCF, 1997a). In the 2003 SCF evaluation of acceptable previous cargoes, tert-butyl acetate was not further evaluated as it was already considered acceptable (SCF, 2003).

WHO (2005) concluded that there was insufficient information on tert-butyl acetate to enable a tolerable intake to be established.

3.6.2. Current evaluation

3.6.2.1. Expected impurities

Available information does not suggest that there are any impurities of concern in tert-butyl acetate.

3.6.2.2. Reactivity and reaction products

tert-Butyl acetate may be hydrolysed to tert-butanol and acetic acid or transesterified with lipids. Neither these nor any other reaction products are expected to be of concern when tert-butyl acetate is transported as a previous cargo.

3.6.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

There is little specific information on the absorption and disposition of tert-butyl acetate. Given its physicochemical characteristics (log P_{ow} 1.64; molecular weight 116.2) it is likely to be readily absorbed from the gastrointestinal tract and distributed throughout the body. Elimination becomes saturated at high exposure levels in experimental animals. Saturation of elimination of the major metabolite, tert-butanol, persists for longer than for that of the parent compound (WHO, 2005). Metabolism is by hydrolysis to acetic acid and tert-butanol, most likely by carboxylesterases, which are found in a variety of tissues, including liver and gastrointestinal tract (Longland et al., 1977; Dahl et al., 1987). The hydrolysis rate is somewhat slower than that of n-butyl acetate, most likely a consequence of steric hindrance (Dahl et al., 1987). Hydrolysis by rat or human blood *ex vivo* was up to two orders of magnitude slower than that of n-butyl acetate (WHO, 2005). A second major route of metabolism involves hydroxylation of the tertiary-butyl moiety to yield 2-hydroxymethylisopropyl acetate. Hydroxylation of the tert-butyl side chain appears to predominate at lower exposures whilst hydrolysis predominates at higher exposures. Oxidation of the acetate side chain can also occur to a minor extent (Cruzan and Kirkpatrick, 2006). tert-Butanol is not a substrate for alcohol dehydrogenase and is metabolised only slowly, either by glucuronide conjugation of the hydroxyl group, with excretion in the urine, or oxidation of one or more of the alkyl substituents, which can be catalysed by P450 enzymes. The oxidation products include acetone, which is excreted in the urine and expired air, either unchanged or following metabolism to carbon dioxide (Cederbaum et al., 1983; WHO/IPCS, 1987). Acetic acid is oxidised via the citric acid cycle to carbon dioxide and water.

Acute toxicity

tert-Butyl acetate is of low acute toxicity by the oral route, with reported LD₅₀ values in rats from 3 300-4 500 mg/kg b.w. (WHO, 2005).

tert-Butyl acetate is slightly irritating to the skin and slightly irritating to the eyes. The effects are readily reversible. tert-Butyl acetate has not been classified in the ECHA database for irritation to skin or eyes.

Subacute, subchronic and chronic toxicity studies

Groups of 30 male and 30 female CD-1 mice were exposed by inhalation (whole-body) to tert-butyl acetate (> 99 % purity) at target concentrations of 0, 100, 400 and 1 600 ppm (actual concentrations were 0, 101, 400 and 1 698 ppm) for 6 h/day, 7 days/week for 90 days. Mid- and high-dose animals showed transient clinical signs, hyperactivity, excessive grooming, impaired equilibrium and laboured respiration (at 1 600 ppm). Both males and females in the high-dose group showed a slight increase in liver weights. Circulating T4 levels were statistically significantly reduced in the 1 600 ppm group males. The NOAEC in this study was 100 ppm (ca. 240 mg/kg b.w. per day).²¹

Groups of 10 male and 10 female SD rats were exposed by inhalation (whole-body) to tert-butyl acetate (> 99 % purity) at target concentrations of 0, 100, 400 and 1 600 ppm (actual concentrations were 0, 101, 400 and 1 600 ppm) for 6 h/day, 7 days/week for 13 weeks. Absolute and relative adrenal gland and liver weights were increased in both sexes at the highest dose. Relative kidney weights were increased in a dose-dependent manner in all treated groups of males, and was slightly increased in high-dose females. Renal histopathology revealed an increased incidence of hyaline droplets (primarily alpha-2u-globulin accumulation), 100 % at the lowest dose and tubular basophilia (increasing with dose) in males of all treated groups. No such effects were seen in females.²¹ The NOAEC was 400 ppm (ca. 780 mg/kg b.w. per day), on the basis that the renal effects observed in males were a consequence of accumulation of alpha-2u-globulin, and therefore not relevant to humans. Exposure of rats to tert-butanol, a major metabolite of tert-butyl acetate, via the drinking water also resulted in a male-specific increase in the incidence of hyaline droplets in the kidney (NTP, 1995).

The chronic toxicity of tert-butanol administered in drinking water has been investigated in rats and mice (NTP, 1995). Groups of 60 male and 60 female B6C3F1 mice received drinking water with concentrations of tert-butanol of 0, 5.0, 10 and 20 mg/mL, corresponding to received doses of 0, 540, 1 040 and 2 070 mg/kg b.w. in males and 0, 510, 1 020 and 2 110 mg/kg b.w. in females, for 2 years. Survival in the high-dose group was reduced. Final body weight of high-dose females was significantly lower than in controls. The incidence of follicular cell hyperplasia of the thyroid gland was statistically significantly increased in all male dose groups, but there was little dependency on dose (5/60 [13 %] in controls; 18/59 [26 %] at 5 mg/mL; 15/59 [25 %] at 10 mg/mL; 18/57 [32 %] at 20 mg/mL). The incidence of this lesion was statistically significantly and dose-dependently increased in mid- and high-dose females. The incidence of chronic inflammation of the bladder was statistically significantly increased in high dose males and females, and there was also an increase in the incidence of transitional epithelial hyperplasia in the high-dose males. The NOAEL in this study was 510 mg/kg b.w. per day, based on an increased incidence of follicular cell hyperplasia of the thyroid gland in females at the LOAEL, and assuming that the changes observed in low-dose males were spurious, as reflected by the absence of any difference in incidence between the low- and mid-dose groups.

Groups of 60 male and 60 female Fischer F334/N rats received drinking water with concentrations of tert-butanol in males of 0, 1.25, 2.5 and 5.0 mg/mL, corresponding to received doses of 0, 90, 200 and 420 mg/kg b.w. and in females of 0, 2.5, 5.0 and 10 mg/mL, corresponding to received doses of 0,

²¹ European Chemicals Agency (ECHA). tert-Butyl acetate. CAS No 540-88-5. Available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d9b886e-dba3-571e-e044-00144f67d249/DISS-9d9b886e-dba3-571e-e044-00144f67d249_DISS-9d9b886e-dba3-571e-e044-00144f67d249.html

180, 330 and 650 mg/kg b.w., for 2 years. Ten rats per group were evaluated after 15 months. Final body weights were reduced in high-dose males and females, as was survival. Urine volume was reduced in mid- and high-dose females after 15 months. At the interim time point (15 months), relative kidney weights were increased in mid- and high-dose males and in all female dose groups. At 2 years, in males there was increased mineralisation of the kidney and renal tubule hyperplasia, which was statistically significant in the high-dose group. The severity and incidence of nephropathy and the incidence of transitional cell hyperplasia in the kidney were increased at the high dose in both males and females (NTP, 1995). The NOAEL for this study was 200 mg/kg b.w. per day in males and 330 mg/kg b.w. per day in females, if one considers that effects seen at lower doses in males were due to accumulation of alpha-2u-globulin and hence not relevant to humans.

Genotoxicity

tert-Butyl acetate was negative in tests for mutagenicity with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 and with *E. coli* WP2uvrA/pKM101, with and without metabolic activation by hepatic postmitochondrial supernatant (S9 preparation) from Aroclor 1254-treated rats (McGregor et al., 2005). tert-Butyl acetate was negative in a test for induction of chromosomal aberrations in isolated human lymphocytes at concentrations up to 10 mM, with and without metabolic activation by hepatic postmitochondrial supernatant (S9 preparation) from Aroclor 1254-treated rats (WHO, 2005). Groups of male and female SD rats were exposed to tert-butyl acetate, at concentrations of 480, 1 900 and 7 700 mg/m³ for 6 hours and then killed 24 h or 48 h later. There was no statistically significant effect on the frequency of micronucleated immature erythrocytes (WHO, 2005).

Carcinogenicity

tert-Butyl acetate has not been tested for its potential carcinogenicity.

One of its major metabolites, tert-butanol has been evaluated for carcinogenicity in rats and mice (NTP, 1995). Details of these studies have been described above. In mice exposed to tert-butanol for 2 years, the only tissue in which there was any increase in the incidence of tumours, compared to the controls, was the thyroid gland of females, in which there was an increase in the incidence of follicular cell adenomas in the high-dose group, 2 110 mg/kg b.w. per day. In rats exposed to tert-butanol for 2 years, there was no effect of treatment on tumour incidence in females, and in males the only tissue in which there was an increase in the incidence of tumours compared to the controls was the kidney. There was some indication for an increase in the incidence of renal tubular adenomas, although this reached statistical significance only in the mid-dose group, 200 mg/kg b.w. per day. There was also some evidence for a marginal increase in the incidence of renal tubular carcinomas (NTP, 1995).

There is evidence that the renal tumours observed only in male rats were secondary to interaction with alpha-2u-globulin, followed by its accumulation in proximal tubular cells. In the absence of any human counterpart to this protein at levels sufficient to support a toxicologically relevant interaction, this carcinogenic response is considered not relevant to humans (Cruzan and Kirkpatrick, 2006; TERA, 2009). The follicular cell adenomas in female mice were accompanied by follicular cell hyperplasia. In shorter term studies there was evidence for effects on the liver, consistent with enzyme induction, and reduced levels of circulating T4, suggesting that the adenomas in the thyroid may have been secondary to hepatic enzyme induction with increased clearance of thyroid hormones (Cruzan and Kirkpatrick, 2006).

Developmental and reproductive toxicity

In a 1-generation study of effects on reproduction, groups of 10 male and 10 female, SD rats were exposed (whole body) to tert-butyl acetate (purity > 99 %) at concentrations of 0, 100, 400 and 1 600 ppm for 6 h/day, 7 days/week, for 10 weeks prior to mating. Females were exposed during gestation and lactation (from postnatal day 5). F1 pups were exposed directly from the day of weaning,

on postnatal day (PND) 22, for 5 days. Body weight and body weight gain were reduced in high dose F0 males. The only effect observed in F1 pups was a slight, transient reduction in mean body weight gain in both males and females in the high dose group exposed on PND 22-26. The NOAEC for reproductive and neonatal toxicity was 1 600 ppm, the highest concentration tested.²¹

The developmental toxicity of tert-butyl acetate was investigated in rats. Groups of 22 pregnant SD rats were administered tert-butyl acetate (purity > 99 %) in corn oil by gavage from GD6-19 at doses of 0, 400, 800, 1 000 and 1 600 mg/kg b.w. per day. tert-Butyl acetate was toxic to dams at the highest dose, with increases in the incidences of clinical signs (reflecting mainly central nervous system (CNS) depression) and possibly mortality (2 animals died from unexplained causes), reduced body weight gain and food intake, increases in the weights of adrenal glands (absolute and relative) and liver (relative) and a decrease in absolute thymus weight. There was a statistically significant increase in the number of skeletal variations in offspring in the mid- and high-groups. Variations were typical of those occurring with embryo/fetotoxicity, particularly reduced ossification. There was a decrease in fetal body weight in the high dose group, and increased incidences of supernumerary ribs and a delay in fetal ossification in the mid- and high-dose groups. tert-Butyl acetate was not teratogenic in this study. The NOAEL for maternal toxicity was 800 mg/kg b.w. per day. The NOAEL for developmental toxicity was 400 mg/kg b.w. per day (Yang et al., 2007).

In a further study by the same laboratory to clarify the maternal toxicity of tert-butyl acetate, groups of 22 pregnant SD rats were administered tert-butyl acetate (purity > 99 %) in corn oil by gavage from GD6-19 at doses of 0, 400, 800, 1 000 and 1 600 mg/kg b.w. per day. One dam in the high dose-group died. Clinical signs indicative of effects on the CNS were observed at doses $\geq$ 800 mg/kg b.w. per day. Reductions in mean body weight, body weight gain and/or food consumption were also observed in these dose groups. The NOAEL for maternal toxicity was 400 mg/kg b.w. per day. Fetal body weight was statistically significantly reduced in the mid- and high-dose groups.²¹

The overall NOAEL for maternal toxicity was 400 mg/kg b.w. per day, based on clinical signs and decreased body weight gain at 800 mg/kg b.w. per day and above. Fetotoxicity appeared to be secondary to maternal toxicity, with an overall NOAEL of 400 mg/kg b.w. per day.

3.6.2.4. Allergenicity

In the Buehler test with guinea pigs, no indications of sensitization were observed (Anonymous, 1997a, as cited in ECHA, online). Available data give no indication that tert-butyl acetate is an allergen or an adjuvant.

3.6.3. Conclusion

The toxicological database on tert-butyl acetate is somewhat limited. The available data on tert-butyl acetate and on acetate and tert-butanol, its major metabolites, do not give rise to concerns regarding systemic toxicity, developmental toxicity or genotoxicity. Any carcinogenic risk would likely be from a non-genotoxic mode of action and would not be of concern at the levels of exposure that might occur from the use of tert-butyl acetate as a previous cargo for edible fats and oils. tert-Butyl acetate is not allergenic. There are no reaction products or impurities of toxicological concern.

The CONTAM Panel therefore concludes that tert-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.7. n-BUTYL ACETATE (CAS No 123-86-4)

n-Butyl acetate, often termed butyl acetate, is a liquid boiling at 127 °C. It is widely used as a solvent in lacquers, adhesives, cleaning agents and pharmaceutical solvents.

It is prepared by esterification of n-butyl alcohol with acetic acid (with sulphuric acid as catalyst) or acetic anhydride.

3.7.1. Previous evaluations

The SCF evaluated n-butyl acetate as a previous cargo in 1996 and considered it acceptable (SCF, 1997a). This conclusion was based on the fact that n-butyl acetate had a temporary ADI of 0-6 mg/kg b.w. and was temporarily acceptable as an extraction solvent. A temporary ADI was established by the SCF in 1981, based on the results of short term studies. The Committee required information on the levels of residues in extracted food by 1983. In the 2003 SCF evaluation of acceptable previous cargoes, n-butyl acetate was not further evaluated as it was already considered acceptable (SCF, 2003).

n-Butyl acetate was given GRAS status by FEMA in 1965.

The Council of the European Communities approved the use of n-butyl acetate as an extraction solvent for food, and it was included in Part 1 of the Annex to Directive 88/344/EEC.²²

The SCF issued its second report on extraction solvents in 1992. No further information on levels in food was available, so the SCF extended the temporary ADI of 0-6 mg/kg b.w. The Committee reiterated its request for analytical data on residues in food or for other reassurance that the ADI would not be exceeded (SCF, 1992b).

The Expert Committee on Flavourings of the Council of Europe classified n-butyl acetate as category A, i.e. substances which may be used in foodstuffs (CoE, 1992).

JECFA (1998) evaluated n-butyl acetate as a flavouring agent and concluded that there was 'no safety concern at current levels of intake', estimated to be 170-1 200 µg/person per day. This was because n-butyl acetate is a Cramer class I substance, and can be predicted to be metabolised to innocuous products. Hence, exposures below a TTC of 1 800 µg/person per day (30 µg/kg b.w. per day) would not be expected to be of safety concern.

n-Butyl acetate is included in the EU register of flavouring substances used in or on foodstuffs, according to Commission Decision 2009/163/EC, amending Decision 1999/217/EC, with FL No. 09.004, and a note that no further evaluation is needed.²³

n-Butyl acetate is on the approved list of substances²⁴ for making food contact materials, without limit (other than the generic limit of 60 mg/kg food).

n-Butyl acetate has been evaluated under the OECD Screening Information Data Set (SIDS) programme on High Production Volume (HPV) chemicals (OECD, 2001a). OECD concluded that n-butyl acetate was currently of low priority for further work.

n-Butyl acetate is approved under US Food and Drug Administration (FDA), Title 21 US CFR citations for resinous and polymeric coatings for polyolefin films, without any specific limitations (FDA, 2011b).

3.7.2. Current evaluation

3.7.2.1. Expected impurities

n-Butyl acetate is synthesised from substances, the impurities of which are not expected to be of concern. Also the esterification step is not expected to result in impurities of concern.

²² Council Directive of 13 June 1988 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. OJ L 157, 24.6.1988, p. 28.

²³ Database of Flavouring Substances. DG Health and Consumers. European Commission. Available at: http://ec.europa.eu/food/food/faef/flavouring/index_en.htm (accessed 18/05/2012).

²⁴ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1.

3.7.2.2. Reactivity and reaction products

n-Butyl acetate may be hydrolysed to n-butanol and acetic acid or transesterified with lipids. Neither these nor any other reaction products are expected to be of concern when n-butyl acetate is transported as a previous cargo.

3.7.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

There is little specific information on the absorption and disposition of n-butyl acetate following oral administration. Given its physicochemical characteristics ($\log P_{ow}$ 2.3; molecular weight 116.2) it is likely to be readily absorbed from the gastrointestinal tract and distributed throughout the body. n-Butyl acetate partitions more readily into tissues than blood, with tissue/blood ratios of 2-3 for most tissues, but 17 for fat (Kaneko et al., 1994). Metabolism is by hydrolysis to acetic acid and n-butanol, most likely by carboxylesterases, which are found in a variety of tissues, including liver and gastrointestinal tract (Longland et al., 1977; Dahl et al., 1987). Hydrolysis half-lives on addition to whole blood from female rats and male human volunteers were 12 min and 4 min, respectively. As anticipated from its rapid hydrolysis, n-butyl acetate has a very short half-life in rats, as does its hydrolysis products, in the order of minutes (Teeguarden et al., 2005, as cited in ECHA online). Acetic acid is oxidised via the citric acid cycle to carbon dioxide and water. n-Butanol is rapidly metabolised by alcohol dehydrogenase (mainly class 1) to butyraldehyde and then by aldehyde dehydrogenase to butyric acid. These are further oxidised to carbon dioxide. A small amount of n-butanol is excreted as the glucuronide conjugate (WHO/IPCS, 1987; WHO, 2005). Several studies on n-butyl acetate administered by inhalation or by intraperitoneal injection have confirmed the rapid elimination of this compound, with a half-life in rats of the order of minutes²⁵ (WHO, 2005). There is evidence that the microsomal P450 system, in particular CYP2E1, may play a role in the metabolism of n-butyl acetate (Peng et al., 1995). Barton et al. (2000) have developed a pharmacokinetic model for the disposition of n-butyl acetate and its major metabolites following inhalation exposure.

Acute toxicity

n-Butyl acetate is of low acute toxicity by the oral route, with LD₅₀ values of approx. 3-13 g/kg b.w. in mice, rats, guinea pigs and rabbits (OECD, 2001a; WHO, 2005). Rats appear to be slightly less sensitive than some other species.

n-Butyl acetate is not irritating to the skin and at worst only slightly irritating to the eyes (OECD, 2001a; WHO, 2005). n-Butyl acetate has not been classified in the ECHA database for irritation to skin or eyes.²⁶

Subacute, subchronic and chronic toxicity studies

No repeat dose toxicity studies on n-butyl acetate administered by the oral route could be identified.

Groups of 15 male and 15 female SD rats were exposed by inhalation (whole body) to n-butyl acetate (purity > 99 %) for 6 h/day, 5 days/week for 13 weeks, at concentrations of 0, 500, 1 500 and 3 000 ppm (to provide target concentrations of 0, 2 400, 7 200 and 14 000 mg/m³). Groups of 5 animals/sex were killed after 30 days for interim assessment. Exposure to n-butyl acetate had no effects on mortality. Animals in the high-dose group, and minimally in the mid-dose group, showed minor clinical signs indicative of sedation. Males and females in the mid- and high-dose groups

²⁵ European Chemicals Agency (ECHA). n-Butyl acetate. CAS No 123-86-4. Available at: [http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d933481-e0e5-623f-e044-00144f67d249/DISS-9d933481-e0e5-623f-e044-00144f67d249.html](http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d933481-e0e5-623f-e044-00144f67d249/DISS-9d933481-e0e5-623f-e044-00144f67d249_DISS-9d933481-e0e5-623f-e044-00144f67d249.html)

²⁶ European Chemicals Agency (ECHA). Summary of Classifications and Labelling. Available at: <http://clp-inventory.echa.europa.eu/SummaryOfClassAndLabelling.aspx?SubstanceID=56478&HarmOnly=no?DisclaimerAgr=Agree&Index=123-86-4&ExecuteSearch=true&fc=true&lang=en> (accessed 20/05/2012).

showed a statistically significant reduction in body weight, body weight gain and food intake. Changes in the weight of a number of organs were observed in mid and/or high-dose males and/or females and included decreased liver and kidney weights, decreased spleen weights, increased adrenal and lung weights, decreased brain weights and increased relative testes weight. The only lesions observed, either macroscopically or histopathologically, were in the stomach and nasal passages, indicative of local irritation and degeneration, apparent in both mid- and high-dose groups. This may be due to local formation of n-butanol and acetic acid. The NOAEC was 2 400 mg/m³ (ca. 700 mg/kg b.w. per day) (David et al., 2001).

Studies of rats exposed to n-butyl acetate by inhalation (whole body) at concentrations of up to 3 000 ppm showed no evidence of neurotoxicity, as assessed by a functional observational battery, motor activity, neurohistopathology and schedule-controlled operant behaviour (WHO, 2005).

Genotoxicity

n-Butyl acetate has been tested relatively extensively for genotoxicity *in vitro*. n-Butyl acetate was negative in an Ames/Salmonella test with *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation by hepatic postmitochondrial supernatant (S9 preparation) from rats and/or hamsters treated with Aroclor 1254 (OECD, 2001a; WHO, 2005; CCRIS, 2009). n-Butyl acetate was also negative in tests for genotoxicity using *Escherichia coli* strain WP2 uvrA, with and without metabolic activation by hepatic postmitochondrial supernatant (S9 preparation) from rats treated with a combination of phenobarbital and beta-naphthoflavone, *Saccharomyces cerevisiae* strain D61.M without metabolic activation, in a test for mitotic aneuploidy, and in Chinese hamster lung fibroblasts, without metabolic activation, in a test for clastogenicity (OECD, 2001a; WHO, 2005; CCRIS, 2009).

n-Butanol, a major metabolite of n-butyl acetate, was negative in tests for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA102, UTH8413, and UTH8414, with and without metabolic activation with hepatic postmitochondrial supernatant (S9 preparation) from Aroclor 1254 treated rats (CCRIS, 1996), clastogenicity (sister chromatid exchanges) in Chinese hamster ovary (CHO) cells and did not produce any effects on the chromosomes of cultured human lymphocytes (WHO/IPCS, 1987). n-Butanol was also negative in a bone marrow micronucleus test in NMRI mice at doses of up to 2 000 mg/kg b.w.²⁵ (OECD, 2001a).

Carcinogenicity

n-Butyl acetate has not been tested for its carcinogenic potential.

According to WHO/IPCS (1987), two long-term studies of n-butanol in rats have been recorded by the US National Cancer Institute, but these were not adequate for assessment of the carcinogenicity of the substance.

Developmental and reproductive toxicity

The reproductive toxicity of n-butyl acetate has been investigated in rats following exposure by inhalation. Groups of CrI:CD(SD) rats (30/sex/group) were exposed (whole body) to n-butyl acetate (purity > 99 %), by inhalation for 6 h/day, 7 days per week, for at least 70 days prior to mating. Target concentrations were 750, 1 500 and 2 000 ppm for all three generations. Exposure of the F0 and F1 males continued throughout mating, until termination. Exposure of dams continued until GD 20 and recommenced on lactation day 4. During lactation days 1-4, dams were administered n-butyl acetate by oral gavage at doses of 0, 1 125, 2 250 and 3 000 mg/kg per day (as 3 equal doses, 2 hours apart). F1 animals were exposed by inhalation from PND 22. Adults in the F0, F1 and F2 generations all showed signs of systemic toxicity in the mid- and high-dose groups, with reduced body weight, body weight gain and food consumption. Site of contact degeneration of the olfactory epithelium was apparent on exposure to ≥ 750 ppm. The NOAEC for systemic toxicity in adult rats was 750 ppm.

Exposure to n-butyl acetate had no effect on reproduction. Pups born to dams in the mid- and high-dose groups had lower mean body weights and body weight gains and some delay in attainment of post-weaning developmental landmarks, secondary to the reduction in body weights. The NOAEC for effects on fertility was 2 000 ppm (ca. 4 000 mg/kg b.w. per day), the highest concentration tested, whilst that for developmental toxicity was 750 ppm (ca. 1 500 mg/kg b.w. per day).²⁵

Groups of pregnant SD rats (19-21 rats/dose group) were exposed (whole body) to n-butyl acetate by inhalation at concentrations of 0, 500, 1 000, 2 000 and 3 000 ppm 6 h/day from day 6 to 20 of gestation. Maternal toxicity was evident at concentrations of $\geq 1\ 000$ ppm, with reduced food consumption, and at $\geq 2\ 000$ ppm there was a reduction in body weight gain. Exposure of dams to n-butyl acetate had no effects on development other than to cause a reduction in fetal weight at the highest dose, which was maternally toxic. The NOAEC for maternal toxicity was 500 ppm (ca. 1 000 mg/kg b.w. per day), whilst the NOAEC for developmental toxicity was 2 000 ppm (ca. 4 000 mg/kg b.w. per day).²⁵

The developmental toxicity of n-butyl acetate has also been studied in rats and rabbits following inhalation exposure at a single high concentration. Groups of female SD rats (37-43 per group) were exposed (whole body) to 1 500 ppm (7 200 mg/m³) n-butyl acetate (purity > 99 %) for 7 h/day on GD 7-16, GD 1-16, or for 5 days/week for 3 weeks prior to mating and then on GD 1-16. Food consumption of dams was decreased in all exposed groups, with reduced body weight gain noted in the groups exposed for longer. Relative kidney and lung weights were increased in all groups. There were no effects on mating or reproductive performance. Fetal body weights and crown-rump length were reduced in all exposed groups. There was no treatment-related effect on the incidence of malformations (OECD, 2001a; WHO, 2005). The NOAEC for teratogenicity was 1 500 ppm, the only concentration tested (ca. 3 400 and 800 mg/kg b.w. per day for rats and rabbits, respectively).

Groups of 30 artificially inseminated New Zealand White rabbits (21-25 pregnant animals/group at termination) were exposed (whole body) to 1 500 ppm (7 200 mg/m³, ca. 800 mg/kg b.w. per day) n-butyl acetate (purity > 99 %) by inhalation for 7 h/day on GD 7-19 or GD 1-19. There was no effect on either maternal or fetal body weight. There was no effect of exposure on reproductive performance. There was an increase in the incidences of misaligned sternebrae, retinal folds and clear gall bladders in fetuses of dams exposed from GD1-19. There were no major malformations (OECD, 2001a).

3.7.2.4. Allergenicity

n-Butyl acetate is mildly to moderately irritating (OECD, 2001a; WHO, 2005). n-Butyl acetate showed no sensitization potential in the guinea pig maximization test and in a mouse ear swelling test (Gad et al., 1986). Similarly negative results with regard to dermal sensitization were obtained in humans using a repeated-insult patch test (Roed-Peterson, 1980; Eiermann et al., 1982).

The available data give no indication that n-butyl acetate is an allergen or an adjuvant at concentrations expected from its use as a previous cargo.

3.7.3. Conclusion

The SCF established a temporary ADI of 0-6 mg/kg for n-butyl acetate, on the basis of limited data. The toxicological database has several data gaps (no repeat dose studies by the oral route, no studies of chronic toxicity or carcinogenicity). However, there were sufficient data on its major metabolites, acetate and n-butanol, for the CONTAM Panel to conclude previously that these are not of concern, when used as previous cargoes. n-Butyl acetate is not genotoxic. The CONTAM Panel considers that the available information on the acute effects of n-butyl acetate and on its subchronic, reproductive and developmental toxicity following exposure by the inhalation route, together with information on its major metabolites, was sufficient to conclude that the risk from short-term exposure to n-butyl acetate when used as a previous cargo would not give rise to any toxicological concern. There are no concerns regarding the allergenicity of n-butyl acetate. There are no reaction products or impurities of toxicological concern.

The CONTAM Panel therefore concludes that n-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.8. PROPYLENE TETRAMER (CAS No 6842-15-5)

In the catalogues available on the internet, propylene tetramer has a variety of descriptions. The majority of the sources indicate a linear dodecane with a terminal double bond, which, however, cannot be obtained from propylene. Often dodeca-1,4,7,10-tetraene is given as a synonym, but this is not consistent with the name 1-dodecene and again cannot be obtained from propylene. As it is made by condensation of propylene, it is expected to have the structure of a tetramethyl octane, possibly with a terminal double bond depending on the method of production.

According to the Substance Registry Service of the US-EPA, the systematic name is 1-propene, tetramer with a molecular formula of $(C_3H_6)_4$.

According to the information provided to EFSA (...) 'the material which is shipped seems to be a blend of isomers of which about 65 % are 1-propene (tetramer). Another Material Safety Data Sheet (MSDS) lists a detailed breakdown of the isomers which are present: > 71 % C12 alkene, < 22 % C10/C11 olefins and < 15 % C13-C15 alkenes with a total of > 98 % olefins' (see documentation provided to EFSA).

Propylene tetramer is described as a liquid with a melting point of -31 °C.

Propylene tetramer is produced by polymerizing propylene with a phosphate catalyst. This yields a broad mixture from which the tetramer is isolated by fractionation.

As condensation of propylene cannot produce hydrocarbons with 10, 11, 13 or 14 carbon atoms, either highly impure propylene is used or addition reactions interfere. It is concluded that propylene tetramer is a complex and probably variable mixture.

Propylene tetramer is used as a starting substance to make dodecyl phenol, tridecyl alcohol, branched dodecylbenzene sulphonic acids used as surfactants, emulsifiers and to produce plasticizers or lubricating oil additives.

3.8.1. Previous evaluations

The SCF evaluated propylene tetramer as a previous cargo in 1996 and considered it acceptable (SCF, 1997a). This conclusion was based on the fact that some toxicological data were available. It was not of structural concern. Subject to the examination of ongoing genotoxicity studies this substance was acceptable as a previous cargo. Low residue levels were expected, as it is easily removed by tank cleaning and it will easily be removed from vegetable oil if it is refined. In the 2003 SCF evaluation of acceptable previous cargoes, propylene tetramer was not further evaluated as it was already considered acceptable, and no reference was made to the results of ongoing genotoxicity studies (SCF, 2003).

Propylene tetramer is approved under US Food and Drug Administration (FDA), Title 21 US CFR citations for indirect food additives: 'paper and paperboard components', and 'adjuvants, production aids, and sanitizers' (FDA, 2011c, d).

Within the OECD SIDS programme on HPV chemicals, it was concluded that for olefins, amongst which are those comprising propylene tetramer, 'the weight of evidence indicates alpha and internal olefins with carbon numbers between C6 and C24 have a similar and low level of mammalian toxicity, and the toxicity profile is not affected by changes in the location of the double bond or the addition of branching to the structure'. It was further concluded that these compounds are currently of low priority for further work (OECD, 2001b, 2004c).

3.8.2. Current evaluation

3.8.2.1. Expected impurities

Propylene tetramer is a crude mixture largely of olefins (see above).

3.8.2.2. Reactivity and reaction products

Propylene tetramer is not expected to react with edible fats and oils.

3.8.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

There is little specific information on the absorption and disposition of propylene tetramer or its components following oral administration. Given the physicochemical characteristics of the main olefins present (average molecular weight 168 to 169; highly lipid soluble), they are likely to be absorbed from the gastrointestinal tract to a reasonable extent and distributed throughout the body. Metabolism appears to involve initial microsomal epoxidation by P450 enzymes to an unstable epoxide, which is then either hydrolysed to the corresponding diol, catalysed by epoxide hydrolase, or conjugated with glutathione. The glutathione conjugates are further metabolised to form mercapturic acids, which are excreted in the urine (White et al., 1986; OECD, 2001b). In general, alkenes accumulate in the body with increasing number of carbon atoms, starting at around C6-C7, with the highest concentrations in rats having been observed in brain, liver, kidneys and perirenal fat (OECD, 2004c; EFSA, 2012b).

Acute toxicity

The alkenes comprising propylene tetramer are of low acute toxicity. The oral LD₅₀ of individual alkenes and mixtures of alkenes is > 5 g/kg b.w. in rats and, when tested, in mice. In most cases, the LD₅₀ was > 10 g/kg b.w. (OECD, 2001b, 2004c).

Some olefins appear to be mildly irritating to rabbit skin when tested as pure chemicals, and slightly to severely irritating when tested as mixtures. Olefins are either non-irritating or slightly irritating to rabbit eyes, regardless of whether tested individually or as mixtures (OECD, 2001b, 2004c). None of the olefins is classified on the ECHA database for irritation to skin or eyes.²⁷

Subacute, subchronic and chronic toxicity studies

In general, olefins are not very toxic on repeat dose administration by the oral route. The most common effect observed is on the kidney of male rats, due to binding with alpha-2u-globulin and its accumulation in proximal tubular cells. As there is no equivalent protein in humans at a level sufficient to support such a reaction, the male rat specific renal effects are not considered relevant for human risk assessment (OECD, 2001b, 2004c; EFSA, 2012b). In repeat dose studies in rats with a variety of alkenes administered orally as either the individual compounds or as mixtures, the lowest NOAEL for systemic toxicity other than alpha-2u-dependent renal toxicity in male rats, was for 1-octene administered by gavage. A NOAEL of 50 mg/kg b.w. per day was identified on the basis of marginal effects on kidney weight and serum creatinine in females at 500 mg/kg b.w. per day (OECD, 2001b, 2004c).

Genotoxicity

The olefins found in propylene tetramer and mixtures thereof have been tested for possible genotoxicity *in vitro* and to a lesser extent *in vivo*. The main test systems used were the

²⁷ European Chemicals Agency (ECHA). Summary of Classification and Labelling. Available at: <http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database> (accessed 21/05/2012).

Ames/Salmonella assay for bacterial mutagenicity and the mouse micronucleus test *in vivo*. A number of additional test systems were employed with some of the compounds or mixtures. None of the olefins was genotoxic (OECD 2001b; 2004c).

Carcinogenicity

No studies of the potential carcinogenicity of propylene tetramer could be located.

Developmental and reproductive toxicity

1-Hexene and 1-tetradecene have been tested for effects on reproduction in combined studies of reproductive/developmental toxicity in which rats were treated prior to mating and during mating, gestation and lactation by oral gavage with doses of up to 1 000 mg/kg b.w. per day. Neither compound had any effect on reproduction or on development. Hence, the NOAEL was 1 000 mg/kg b.w. per day, the highest dose tested, for both of these compounds (OECD, 2001b). In their evaluation of higher olefins, OECD (2004c) summarised oral studies of reproductive and developmental toxicity of several mixtures of alkenes. These were: alkenes, C6 (internal branched /linear stream); C16/18 internal linear and branched; C18 internal linear and branched. In all cases, the NOAEL for reproductive and developmental toxicity was 1 000 mg/kg b.w., the highest dose tested.

3.8.2.4. Allergenicity

In the Buehler test in guinea pigs, propylene tetramer was not found to be a sensitizer (Cushman et al., 1992). Similarly, in the Buehler test in guinea pigs, 1-dodecene was not found to be a sensitizer (Morris, 1992, as cited in ECB, 2002a). No information has been found regarding adjuvanticity. The available data give no indication that propylene tetramer is an allergen or an adjuvant at concentrations expected from its use as a previous cargo.

3.8.3. Conclusions

Although specific studies on propylene tetramer itself are somewhat limited, data are available on many of its main components and mixtures of these. In general, the toxicological profile of alkenes depends on carbon length, and is similar for those with similar carbon length. The CONTAM Panel considers that propylene tetramer would not be of toxicological concern at the levels that would occur when used as a previous cargo for edible fats and oils. Although there are no studies of carcinogenicity, the CONTAM Panel concludes that in the absence of genotoxicity or of pathological changes in subchronic studies indicative of a potential carcinogenic hazard, there was no concern for possible carcinogenicity from the use of propylene tetramer as a previous cargo. Propylene tetramer is not allergenic. There are no reaction products or impurities of toxicological concern.

The CONTAM Panel therefore concludes that propylene tetramer meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.9. MONTAN WAX (CAS No 8002-53-7)

Montan wax is a hard, brittle, lustrous wax with a melting point around 80 °C extracted with toluene from lignites/brown coal, principally from Central Europe.

Montan wax is formed from resins, waxes and fats of plants. It consists of about 50 % esters of C22-34 fatty acids with C24-C28 alcohols, about 20 % fatty acids and resins of phenols, ketones and asphaltenes.

Montan wax is used for technical purposes, such as sealing concrete, in cleaning agents, lubricants, adhesives, and for electrical insulation in cables.

Montan wax should be distinguished from a variety of products called ‘montan acid esters’ or ‘montanic acid esters’, which are purified (e.g. by bleaching) and modified products. Fatty acids from

montan waxes are esterified, e.g., with ethylene glycol or fatty alcohols. Other isolates from montan wax are partially saponified. This entry does not deal with these purified products.

3.9.1. Previous evaluations

The SCF evaluated montan wax (CAS no. 8002-53-7) as a previous cargo in 1996 and considered it provisionally acceptable, noting its solubility (SCF, 1997a). This conclusion was based on the fact the SCF had concluded that montan wax esters (E192) were temporarily acceptable as a food additive, used as a glazing agent for food (SCF, 1992c). In the 2003 SCF evaluation of acceptable previous cargoes, the Committee reconsidered montan wax and concluded that the information available was inadequate. The SCF therefore maintained their opinion as provisionally acceptable (SCF, 2003).

No previous evaluations of natural montan wax have been carried out, by JECFA, SCF, EFSA or other regulatory agencies. Natural montan wax is not authorised as a food additive in the EU,¹⁵ although montan acid esters are authorised for the surface treatment of certain fruits, with the E number E 912. Montan wax is approved for use as a food contact additive in plastics under Regulation (EU) 10/2011,²⁴ with no restrictions other than the generic overall migration limit of 60 mg/kg food. Montan wax is also on the US FDA list of approved indirect additives used in food contact materials (FDA, 2011e).

3.9.2. Current evaluation

3.9.2.1. Expected impurities

As montan wax is a complex mixture with a composition depending on its source, the toxicological evaluation should consider the whole mixture, taking into account variability in composition. Anticipated impurities have not been specifically considered in this opinion.

3.9.2.2. Reactivity and reaction products

The large majority if not all of the components in montan wax are not expected to react in edible fats and oils.

3.9.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Montan wax has a high melting point relative to other waxes, is insoluble in water and is hydrophobic in nature. Overall the CONTAM Panel considered that absorption of montan wax from the gastrointestinal tract will be limited. Any alkane constituents of the absorbed wax will be slowly metabolised to the corresponding fatty alcohols and then fatty acids, with some metabolism also occurring in the small intestine, and enter normal biochemical pathways (EFSA, 2012b).

Acute toxicity

Montan wax can be anticipated to be of low acute oral toxicity. An LD₅₀ of > 12 000 mg/kg b.w. has been reported.²⁸ Montan wax has been reported to be only very slightly irritating to skin and eyes, based on the results of *in vitro* tests.²⁸

Subacute, subchronic and chronic toxicity studies

A 90-day toxicity study in Fisher 344 rats has been carried out with montan wax, administered in the diet at levels of 0, 0.56, 1.67 or 5 % (about 260, 835 or 2 500 mg/kg b.w. per day) (Ikeda et al., 2008). Haematological changes occurred in all treated rats, and aspartate aminotransferase (AST) and

²⁸ European Chemicals Agency (ECHA). Montan wax. CAS No. 8002-53-7. Available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb31e9c-8883-2565-e044-00144f67d031/DISS-9eb31e9c-8883-2565-e044-00144f67d031_DISS-9eb31e9c-8883-2565-e044-00144f67d031.html (consultation date: 2012-01-20).

alanine aminotransferase (ALT) in serum were elevated. Liver, spleen, lung and kidney weights relative to body weight were also increased. Diffuse multiple granulomatous change occurred in the liver in all treated rats, together with severe hepatocyte damage and lymphocytic infiltration. A NOAEL could not therefore be identified in this study.

No chronic toxicity study is available on montan wax.

Genotoxicity

Montan wax was non-mutagenic in a bacterial mutagenicity study using *Salmonella typhimurium* with and without metabolic activation.²⁸

Carcinogenicity

No carcinogenicity study is available on montan wax.

3.9.2.4. Allergenicity

Montan wax was tested in the mouse local lymph node assay (LLNA) according to OECD Guideline 429. Montan wax did not induce skin sensitization in this test (Anonymous, 2009, as cited in ECHA, online). No other information has been found regarding sensitization, adjuvanticity or irritancy of Montan wax. The available data give no indication that montan wax is an allergen or an adjuvant at concentrations expected from its use as a previous cargo.

3.9.3. Conclusion

No ADI or TDI has been established for montan wax by the SCF, JECFA or EFSA. Data recently provided to ECHA indicate that montan wax is not mutagenic in a bacterial mutagenicity test, and the CONTAM Panel considers that it is not likely to be a significant sensitizer, adjuvant or irritant. In a subchronic toxicity study in rats, haematological changes and hepatotoxicity were observed at the lowest dose tested, of approximately 260 mg/kg b.w. per day, and hence no NOAEL could be identified. There are no data on chronic toxicity or carcinogenicity.

Montan wax is an ill-defined material for which it cannot be excluded that it contains components of concern.

The CONTAM Panel therefore concludes that, given the deficiencies in the available data on montan wax, it does not meet the criteria for acceptability as a previous cargo.

3.10. PARAFFIN WAX (CAS No 8002-74-2 / 63231-60-7)

Waxes are mixtures of mineral hydrocarbons having a melting point above ambient temperature. There are hard waxes (like those used in candles), but also soft waxes, e.g. used for cosmetics. For chemists, the term 'paraffin' suggests that the wax consists of saturated, open-chain hydrocarbons, i.e. that it neither contains saturated cyclic components (naphthenes) nor aromatic components. The term is also used, however, to distinguish waxes of mineral sources from waxes of other origin and may, therefore, not in itself exclude appreciable amounts of aromatic components.

CAS No 8002-74-2 has entries as 'paraffin wax', but also as 'paraffin wax and other hydrocarbon waxes', which means that aromatic hydrocarbons can be present. CAS No 63231-60-7 refers to microcrystalline wax. Microcrystalline waxes differ from paraffin waxes in the size and structure of crystals: microcrystalline wax is almost amorphous, whereas paraffin wax is macrocrystalline. To achieve these properties, they contain a higher proportion of saturated hydrocarbons other than n-alkanes. Microcrystalline waxes used in foods (E 905) are hydrogenated to remove the aromatic compounds. However, microcrystalline waxes are not per se food grade.

Waxes are obtained by solvent crystallisation from mineral oil fractions. To be solids they must have a high concentration of n-alkanes (usually > 90 %) and be of molecular masses above about C20. Crystallisation reduces the content of aromatics to at most a few percent. Other characteristics may vary, such as the molecular mass distribution. The higher the molecular mass, the higher may be the percentage of the branched and cyclic hydrocarbons still resulting in a melting point above ambient.

Waxes are used e.g. for candles, tyres and other rubber articles, treatment of surfaces to improve water-resistance, cosmetics and electrical insulation. This includes both the paraffin waxes and the microcrystalline waxes.

3.10.1. Previous evaluations

The SCF evaluated paraffin wax as a previous cargo in 1996 and considered it provisionally acceptable (SCF, 1997a), noting that ‘Existing SCF opinion on mineral hydrocarbons - waxes states that there are insufficient data to establish the safety of paraffin waxes. (SCF, 37th report, 1997). However given the nature of the toxicity of paraffin waxes it would not be expected that very low residues would give rise to problems. The normal cleaning process involving heating of the tank should ensure the removal of paraffin waxes to acceptable residual levels’. In the 2003 SCF evaluation of acceptable previous cargoes, the Committee reconsidered paraffin wax and concluded that the information available was inadequate. SCF therefore maintained their opinion on paraffin wax as provisionally acceptable previous cargo (SCF, 2003).

Mineral hydrocarbons including paraffin wax and microcrystalline wax (E 905) have been evaluated several times for the safety for use as food additives by both the Scientific Committee on Food (SCF) in 1990 and 1995 (SCF, 1992d, 1997b) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on several occasions, most recently in 2009 (JECFA, 2010).

JECFA noted at its 39th meeting that long-term toxicity studies had indicated that petroleum-derived paraffin waxes and microcrystalline waxes were non-toxic and non-carcinogenic (JECFA, 1992, 1993a). The Committee therefore established an ADI ‘not specified’ for these waxes for the following uses: chewing-gum base, protective coating, defoaming agent, and surface finishing agent. The ADI ‘not specified’ for paraffin waxes was withdrawn in 1995, and an ADI of 0-20 mg/kg b.w. was established for hydrotreated, high-melting point microcrystalline wax and clay-treated microcrystalline wax at the 44th JECFA meeting, based on new short-term feeding studies showing no adverse effects up to the highest dose tested of 2 % microcrystalline wax in the diet (JECFA, 1995, 1996). Based on the same studies, the SCF likewise established an ADI of 0-20 mg/kg b.w. (SCF, 1997b). Only microcrystalline wax is authorised as a food additive within the EU.

In the USA petroleum wax including microcrystalline wax is classified as Generally Recognized As Safe (GRAS) by the US FDA, and is permitted in chewing gum base, on cheese and raw fruits and vegetables and as a defoamer in food (FDA, 1977).

Microcrystalline wax is approved for use as a food contact additive in plastics under Commission Regulation 10/2011²⁴ with the name waxes, refined, derived from petroleum based or synthetic hydrocarbon feedstocks and with no restrictions other than the generic overall migration limit of 60 mg/kg food.

3.10.2. Current evaluation

In view of concerns regarding the toxicity and carcinogenicity of aromatic hydrocarbons, the current evaluation is restricted to paraffin waxes that have been treated to remove aromatic hydrocarbons and which otherwise meet relevant standards to be considered as ‘food grade’.

3.10.2.1. Expected impurities

Waxes of mineral origin predominantly consist of n-alkanes. Branched and cyclic hydrocarbons may reach a few percent of the wax (mostly < 10 %). The food grade waxes being considered here as

previous cargoes for edible fats and oils will always contain less than 3 % highly alkylated aromatic hydrocarbons. No other impurities of concern are anticipated.

3.10.2.2. Reactivity and reaction products

Mineral waxes do not react with edible fats and oils.

3.10.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

In a 90-day study in which rats were fed five different microcrystalline or lower melting point paraffin waxes (LMPW) at a level of 2 mg/kg in the diet, mineral hydrocarbons were found in liver, mesenteric lymph nodes, and perirenal fat of all groups with the exception of animals fed microcrystalline waxes (Smith et al., 1996). The SCF concluded from this study that microcrystalline wax is not absorbed after oral administration but is excreted unchanged in the faeces (SCF, 1997b), in contrast to mineral hydrocarbon oils and lower melting point paraffin waxes. It has been reported that absorption of aliphatic hydrocarbons was inversely proportional to the number of carbon atoms and ranged from 60 % for C14 compounds to 5 % for C28 compounds, while no absorption was detected for carbon numbers greater than C30 (Albro and Fishbein, 1970). The results of a number of extraction and migration tests on waxes and wax-bearing products were used by JECFA as indirect evidence that hydrocarbon waxes consumed in the diet are unlikely to be absorbed or metabolized in detectable or significant amounts (JECFA, 1993a). Any absorbed paraffin wax (high carbon number alkanes) will be slowly metabolised to the corresponding fatty alcohols and then fatty acids, with some metabolism also occurring in the small intestine, and enter normal biochemical pathways (EFSA, 2012b).

Acute toxicity

Paraffin waxes are anticipated to be of low acute oral toxicity. An LD₅₀ of greater than 5 000 mg/kg b.w. has been reported²⁹ (US-EPA, 2011). They have been reported to be only very slightly irritant to skin and eyes.²⁹

Subacute, subchronic and chronic toxicity studies

Both JECFA and the SCF have extensively reviewed the results of subacute, subchronic and chronic oral toxicity studies and human data on a number of mineral and synthetic oils and waxes including paraffin waxes (JECFA, 1993a; SCF, 1997b). The SCF (1997b) reported some of these substances 'not only accumulate with repeated dosing, but also give rise to effects which are not confined solely to localised foreign body reactions and provide clear evidence of toxicity in animals. [...]'. The following effects were observed: increased organ weights, especially liver and lymph nodes; altered serum enzyme levels; increased monocyte and neutrophil counts; reduced red blood cells, haemoglobin, haematocrit, mean corpuscular hemoglobin concentration (MCHC). The main histopathological findings were granulomata in the liver and focal collections of vacuolated macrophages (histiocytosis) in the lymph nodes. In animals dosed with certain of the waxes, an inflammatory lesion at the base of the mitral valve in the heart was observed'.

In the most recent of these studies (Smith et al., 1996), a range of mineral oils and waxes were administered in the diet to groups of F344 rats (20/sex) at levels of 0.002, 0.02, 0.2 or 2 % for 90 days (equivalent to 2, 20, 200 or 2 000 mg/kg b.w. per day). The results confirmed the toxicity of the mineral oils and low melting point wax tested, as described by the SCF (1997b), however no treatment-related effects were associated with administration of the high melting point wax and high sulphur wax (both microcrystalline waxes) other than minor haematological changes in females only, receiving the high sulphur wax. A NOAEL of 0.002 % in diet (about 1.5 mg/kg b.w. per day) was

²⁹ European Chemicals Agency (ECHA). Paraffin waxes and hydrocarbon waxes. CAS No 8002-74-2. Available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb27f4b-c95d-44a9-e044-00144f67d031/DISS-9eb27f4b-c95d-44a9-e044-00144f67d031_DISS-9eb27f4b-c95d-44a9-e044-00144f67d031.html (consultation date: 2012-01-23).

identified for the low melting point wax, while for the microcrystalline waxes the NOAEL was ≥ 2 % in diet (about 1 500 mg/kg b.w. per day).

In a 60-day study with Fischer-344 (F-344) and SD rats, 2 % LMPW (low melting point paraffin wax) was administered in the diet for 60 day (Hoglen et al., 1998). The results of this study indicated that 2 % LMPW altered the morphology and functional capacity of Kupffer cells of F-344 rats, but not of SD rats. The authors suggested that these effects may ultimately lead to the formation of hepatic granuloma.

No treatment-related effects were reported in a chronic toxicity/carcinogenicity study in which SD rats were fed 5 types of refined waxes (three of the waxes were microcrystalline and the other two were refined paraffin waxes, not further specified) at a dietary concentration of 10 % (about 5 000 mg/kg b.w. per day) for two years (Shubik et al., 1962).

Genotoxicity

The results of a range of *in vitro* genotoxicity studies conducted with paraffin wax and reported in the ECHA database,²⁹ including a bacterial mutagenicity study with *Salmonella typhimurium* and *Escherichia coli*, a mouse lymphoma mutation assay and a chromosome aberration test in CHO cells, all conducted with and without metabolic activation, indicate no genotoxic potential. In genotoxicity studies, high viscosity and medium viscosity white oils also do not show genotoxic effects (EFSA, 2009c).

Carcinogenicity

In a chronic toxicity/carcinogenicity study in which rats were fed 5 types of refined waxes (three of the waxes were microcrystalline and the other two were refined paraffin waxes, not further specified) at a dietary concentration of 10 % (about 5 000 mg/kg b.w. per day) the incidence of tumours was comparable in test animals and controls (Shubik et al., 1962). The polycyclic aromatic hydrocarbon (PAH) content of the waxes tested was determined analytically, and the two refined paraffin waxes were found to have detectable levels of PAHs, while the three microcrystalline waxes did not. As reported by the authors, however, all five waxes were negative in the 2-year oral carcinogenicity study and also in a skin painting study in mice (Shubik et al., 1962). Both JECFA and the SCF concluded that (microcrystalline) paraffin wax is not carcinogenic, based on the results of this study. EFSA has also concluded that, in chronic toxicity/carcinogenicity studies conducted with high viscosity and medium viscosity white oils, no carcinogenic effects were observed in any of the studies in F344 rats or in skin painting studies in mice (EFSA, 2009c).

Developmental and reproductive toxicity

No data are available on the reproductive and developmental toxicity of paraffin wax or microcrystalline wax. There are also no data on high viscosity or medium viscosity white oils. EFSA concluded, however, that studies on low viscosity white mineral oils can be used to support the lack of reproductive or developmental effects for white oils (EFSA, 2009c). The CONTAM Panel considered that this could also be applied to the paraffin waxes.

3.10.2.4. Allergenicity

In the guinea pig maximization test, no skin reactions were observed in groups treated with 100 % or 50 % paraffin wax in liquid paraffin (Anonymous, 1997b, 2007, both as cited in ECHA, online). Negative results for paraffin wax have been recorded also in a human patch-test study (Dooms-Goossens and Degreef, 1983, as cited in ECHA, online).

Clinical studies in humans with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16 %) and microcrystalline (4.35-15 %) waxes recorded, at most, slight erythema, and none of the test substances caused skin sensitization (Elder, 1984). No data

on adjuvanticity or irritancy have been found. The available data give no indication that paraffin wax is an allergen or an adjuvant at concentrations expected from its use as a previous cargo.

3.10.3. Conclusion

Paraffin wax may contain aromatic hydrocarbons, some of which are genotoxic carcinogens. Hence, the CONTAM Panel concluded that this entry to the Annex should be restricted to paraffin waxes that have been treated to remove aromatic hydrocarbons and which also meet other relevant standards to be considered as 'food grade'. Accordingly, the CONTAM Panel recommends that the entry for these waxes in Annex to Commission Directive 96/3/EC be amended to 'Paraffin Wax (CAS No 8002-74-2 / 63231-60-7) (food grade)'.

An ADI of 0-20 mg/kg b.w. has been established by both JECFA and the SCF for high molecular mass food-grade microcrystalline wax, with specifications as laid down according to Commission Directive 2008/84/EC³⁰ and JECFA. The CONTAM Panel (EFSA, 2012b) noted that this ADI was established from toxicological studies in which no effects were observed at any tested dose. Food grade paraffin wax is not genotoxic or allergenic. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated.

The CONTAM Panel therefore concludes that paraffin wax (food grade, CAS No. 8002-74-2 / 63231-60-7) meets the criteria for acceptability as a previous cargo.

3.11. CARNAUBA WAX (Brazil wax) (CAS No 8015-86-9)

Carnauba wax is one of the hardest and highest-melting point natural waxes, with a melting point range between 82 and 86 °C. It is modestly soluble in solvents, virtually insoluble in water, i.e. difficult to remove from a container in solid form. The cleaning of a vessel might be inefficient.

Carnauba wax is mechanically obtained from the leaves of the Brazilian palm trees *Copernicia prunifera* and *Copernicia cerifera*. For purification it is melted and filtered.

Carnauba wax contains about 85 % esters of long chain fatty acids, hydroxy fatty acids and cinnamic acid with long chain alcohols and diols. The remaining material consists of long chain free acids, long chain fatty alcohols and saturated hydrocarbons.

Carnauba wax is used for polishing surfaces (e.g. shoes, furniture, floors, cars), as a release agent for bakery ware and sugar products, in chewing gums, as coatings of fruits, in cosmetics or to protect printed surfaces.

3.11.1. Previous evaluations

The SCF evaluated carnauba wax as a previous cargo in 1996 and considered it acceptable (SCF, 1997a). This conclusion was based on the fact that carnauba wax was temporarily acceptable as a food additive, E903, for use as a glazing agent for food (SCF, 1992d, 1997c). The SCF noted the insolubility of carnauba wax. In the 2003 SCF evaluation of acceptable previous cargoes, carnauba wax was not further evaluated as it was already considered acceptable (SCF, 2003).

The SCF has evaluated carnauba wax as a food additive on several occasions (SCF, 1992d, 1997c, 2001). The SCF did not establish an ADI for carnauba wax, and in its 1992 and 1997 opinions considered its use as a glazing agent as temporarily acceptable. In 2001, based on new toxicological and exposure data the SCF accepted the use of carnauba wax as a glazing agent up to a maximum use level of 200 mg/kg of food and withdrew its temporary status (SCF, 2001).

An ADI of 0-7 mg/kg b.w. for carnauba wax was established by JECFA in 1993 (JECFA, 1993b).

³⁰ Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253/1. p. 175.

In USA carnauba wax is classified as GRAS and is permitted with no other limitation than good manufacturing practice (GMP) in a variety of food products (FDA, 1983a).

Carnauba wax is approved for use as a food contact additive in plastics under Commission Regulation (EU) 10/2011,²⁴ with no restrictions other than the generic overall migration limit of 60 mg/kg food.

EFSA re-evaluated carnauba wax (E 903) as a food additive in 2012 (EFSA, 2012c). The Panel on Food Additives and Nutrients added to Food (ANS Panel) did not establish an ADI due to the lack of long-term toxicity data. It noted, however, that available toxicity studies consistently reported no adverse effects associated with carnauba wax intake, and that the available data suggests no concern for genotoxicity. In addition, the exposure estimates to carnauba wax indicated sufficient 'margins of safety', and therefore concluded that its use as a food additive within the currently authorised uses would not be of safety concern.

3.11.2. Current evaluation

3.11.2.1. Expected impurities

Carnauba wax is not expected to contain impurities of concern when transported as a previous cargo.

3.11.2.2. Reactivity and reaction products

Carnauba wax is not expected to produce reaction products with edible fats and oils which are of concern when it is transported as a previous cargo.

3.11.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Carnauba wax has a high melting point relative to other waxes, is insoluble in water and is hydrophobic in nature. There are no specific experimental data on the absorption, distribution, metabolism and elimination of carnauba wax (EFSA, 2012c). Overall the CONTAM Panel considered, however, that absorption of carnauba wax from the gastrointestinal tract will be low, if any, and that the wax is unlikely to be susceptible to metabolism by digestive enzymes or the intestinal microbiota. Any degradation products, e.g. long-chain aliphatic esters which are the main components of carnauba wax, will be incorporated into normal cellular metabolic pathways and eliminated thereafter.

Acute toxicity

Carnauba wax is of low acute toxicity, an oral LD₅₀ of greater than 1 100 mg/kg b.w. has been reported (Liebert, 1984). Carnauba wax is not anticipated to have irritant properties.

Subacute, subchronic and chronic toxicity studies

As reported by the SCF (2001) and EFSA (2012c), a 90-day oral study in Wistar rats was carried out with carnauba wax (Rowland et al., 1982) at levels of up to 10 % in the diet, in which no treatment-related effects were reported. A NOAEL of 8 800 mg/kg b.w. per day, the highest dose tested, was identified in this study. A further 90-day feeding study in Fischer F-344 rats was designed to investigate whether components of carnauba wax could be absorbed and accumulate in the liver and other organs of this strain, as seen with high molecular mass mineral oils and other waxes (Edwards, 1998). Groups of 20 male and 20 female rats were fed diets containing carnauba wax corresponding to intakes of 0, 15, 150 and 1 500 mg/kg b.w. per day. No treatment-related effects were identified, there were no dose-related histopathological changes in liver and other tissues, and a NOAEL of 1 500 mg/kg b.w. per day, the highest dose tested, can be identified in this study (Edwards, 1998). A 6-month feeding study has also been carried out in Beagle dogs, using dietary levels of up to 1 % in

the diet (equivalent to up to 250 mg/kg b.w. per day) (Parent et al., 1983a). No treatment-related effects were identified. No studies on the chronic toxicity of carnauba wax are available (EFSA, 2012c).

Genotoxicity

As reported by JECFA (1993b) and the SCF (2001), carnauba wax was not mutagenic in *in vitro* tests with *Salmonella typhimurium* and *Saccharomyces cerevisiae*, with and without metabolic activation. As reported by SCF (2001), there was no evidence of clastogenicity of carnauba wax in *in vitro* chromosome aberration tests using human lymphocytes (Edwards, 1996, 1998). The SCF and, more recently, the EFSA ANS Panel (EFSA, 2012c) concluded that carnauba wax was not genotoxic *in vitro*, based on the results of these studies. There are no *in vivo* genotoxicity data available on carnauba wax.

Carcinogenicity

No studies on the carcinogenicity of carnauba wax are available (EFSA, 2012c).

Developmental and reproductive toxicity

No treatment-related effects were reported in a reproductive toxicity study with carnauba wax in which Wistar rats were administered levels of 0, 0.1, 0.3 or 1 % in the diet for 4 weeks prior to mating and through gestation and lactation (Parent et al., 1983b). This study was used by JECFA as the basis for setting the ADI of 7 mg/kg b.w. per day for carnauba wax (rounded up), by applying a 100 uncertainty factor to the NOAEL of approximately 670 mg/kg b.w. per day. As reported by the SCF (2001), no developmental toxicity was evident in a study in which rats were fed 0, 0.1, 0.3 or 1 % carnauba wax in the diet for two weeks before mating and throughout gestation.

3.11.2.4. Allergenicity

One report of a test-proven case of sensitization to carnauba wax has been published (Chowdhury, 2002). In addition, Jacob et al. (2008) report one case of supposed sensitization to carnauba wax, based on reaction to a product containing propolis and carnauba wax and test-proven sensitization to cinnamic acid/cinnamaldehyde, which is a component of both the mentioned substances. However, no testing with carnauba wax was performed. Chowdhury (2002) state that sensitization to carnauba wax is very rare. No other information on sensitizing properties, adjuvanticity or irritancy has been found. The CONTAM Panel considers that taking into account the scarcity of reports of sensitization in the literature as well as the relevant dilution factor, carnauba wax when used as a previous cargo is not likely to be a significant sensitizer, adjuvant or irritant.

3.11.3. Conclusion

JECFA has established an ADI of 0-7 mg/kg b.w. for carnauba wax, while the SCF concluded that its use as a glazing agent up to a maximum use level of 200 mg/kg of food was acceptable. The EFSA ANS Panel noted that available toxicity studies consistently reported no adverse effects associated with carnauba wax intake, and that the available data suggests no concern for genotoxicity. In addition, the exposure estimates to carnauba wax indicated sufficient 'margins of safety', and therefore concluded that its use as a food additive within the currently authorised uses would not be of safety concern. The CONTAM Panel considered, based on the outcome of these expert evaluations, the likely limited absorption of carnauba wax and the toxicological profile of its main component groups of chemicals, that this wax will not pose any toxicological concern when used as a previous cargo, based on normal assumptions regarding worst case carryover. There is no evidence that it is genotoxic and there is no allergenic potential of concern. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated.

The CONTAM Panel noted however, the insolubility of carnauba wax in water, its high melting point (82 to 86 °C) and the fact that heating of ships' tanks is normally to a maximum of 80 °C. The CONTAM Panel therefore has concerns regarding the feasibility of tank cleaning following transport of carnauba wax as a previous cargo, such that carryover may exceed the worst case normally assumed.

The CONTAM Panel concludes that carnauba wax does not meet the criteria for acceptability as a previous cargo because of doubts concerning the efficiency of tank cleaning following transport of carnauba wax as a previous cargo.

According to the information provided to EFSA, carnauba wax does not appear to be transported as a previous cargo.

3.12. CANDELILLA WAX (CAS No 8006-44-8)

Candelilla wax is yellowish to brown, hard (melting point around 70 °C), brittle, aromatic and opaque to translucent. It is insoluble in water, but soluble in many organic solvents.

Candelilla wax is obtained from the leaves and stems of a shrub from the family Euphorbiaceae growing in Mexico and the south-western United States by boiling with dilute sulphuric acid. The resulting 'cerote' is skimmed and purified.

Candelilla wax consists mainly of n-alkanes C29-33 (about 50 %), wax esters, sterol esters, free acids and resins.

Candelilla wax is used for making varnish. It is an approved food additive (glazing agent for fruits and sweets, E 902), and is also used in cosmetics. One of its major uses is as a binder for chewing gums. It is often used to replace the more expensive beeswax.

3.12.1. Previous evaluations

The SCF evaluated candelilla wax as previous cargo in 1996 and considered it acceptable (SCF, 1997a). This conclusion was based on the fact that candelilla wax was temporarily acceptable as a food additive, E902, for use as a glazing agent for food (SCF, 1992e). In the 2003 SCF evaluation of acceptable previous cargoes, candelilla wax was not further evaluated as it was already considered acceptable (SCF, 2003).

The SCF evaluated the safety of candelilla wax as a food additive in 1990 (SCF, 1992e). The Committee did not establish an ADI for candelilla wax, but took into consideration the available toxicity data, its long established use without apparent adverse effects and the expected limited intake when used as a glazing agent, and accepted the temporary continued use of candelilla wax (SCF, 1992e).

JECFA evaluated the use of candelilla wax as a glazing agent, chewing gum base component, surface finishing agent and carrier for flavours in 1993 and again in 2005 (JECFA, 1993c, 2006). JECFA estimated dietary exposure to candelilla wax using the conservative assumption that an individual would consume all the foods (and tablets or capsules) containing candelilla wax at the highest percentile in each food category and that all those foods contained candelilla wax, and calculated that the dietary exposure would be < 650 mg per person per day. JECFA concluded that the functional uses indicated did not raise any toxicological concerns provided the dietary exposure was less than 650 mg per person per day.

EFSA re-evaluated candelilla wax (E 902) as a food additive in 2012 (EFSA, 2012d). The Panel on Food Additives and Nutrients added to Food (ANS Panel) did not establish an ADI due to the lack of long-term toxicity data. It noted, however, that the available toxicity studies consistently reported no adverse effects associated with intake of the main components constituting candelilla wax, and that the

available data suggest that candelilla wax is not genotoxic. In addition, the exposure estimates (using the maximum permitted levels of carnauba wax) indicated a sufficient ‘margin of safety’ and therefore concluded that its use as a food additive within the currently authorised uses would not be of safety concern.

In the USA candelilla wax is classified as GRAS and is permitted with no other limitation than GMP in a variety of food products (FDA, 1983b).

Candelilla wax is approved for use as a food contact additive in plastics under Commission Regulation (EU) 10/2011,²⁴ with no restrictions other than the generic overall migration limit of 60 mg/kg food.

3.12.2. Current evaluation

3.12.2.1. Expected impurities

Candelilla wax does not include impurities that would be of concern when it is used as a previous cargo.

3.12.2.2. Reactivity and reaction products

Candelilla wax does not contain substances which could react with edible fats and oils to produce compounds of concern.

3.12.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

No specific data on absorption, distribution, metabolism or excretion of candelilla wax were available to the Panel for evaluation. Overall, the CONTAM Panel considered that absorption of candelilla wax from the gastrointestinal tract will be low, if any, and that degradation by digestive enzymes or the intestinal microbiota would be limited. The components of candelilla wax, e.g. straight-chain hydrocarbons (C29-C33 n-alkanes) together with esters of acids and alcohols with even-numbered carbon chains (C28-C34) (JECFA, 2006), are also expected to have limited absorption and if absorbed, will be incorporated into normal cellular metabolic pathways and eliminated thereafter (EFSA, 2012d).

Acute toxicity

Candelilla wax is of low acute toxicity. As reported by the EFSA ANS Panel in 2012, the SCF reviewed a number of acute oral toxicity studies on candelilla wax (no further details) and reported that ‘none of the studies reported any adverse treatment-related toxicological findings’ (SCF, 1992e). Liebert (1984) reported that the acute oral toxicity of candelilla wax following gavage administration has been investigated in SD and Hooded Long Evans rats and other rats of undefined strain without report of any toxic effects. JECFA reported an oral LD₅₀ of > 5 000 mg/kg b.w. for candelilla wax in rats (JECFA, 1993c).

Subacute, subchronic and chronic toxicity studies

JECFA in their evaluation of candelilla wax described four unpublished short-term studies (8-27 weeks in duration) in rats and one study in dogs (6 months in duration) (JECFA, 1993c). The studies in rats used a mixture of candelilla wax and either gum base or a butadiene-styrene polymer. The composition of the mixtures administered is not clear from the JECFA report, but the intakes of candelilla wax could have been up to 2 400 mg/kg b.w. per day in rats. No treatment-related effects were reported in any of these studies (JECFA, 1993c). In the 6-month study in dogs, candelilla wax was given in a gum base in the diet at levels equivalent to up to 600 mg candelilla/kg b.w. per day; again, no treatment-related effects were reported.

As also reported by JECFA (1993c), in a study in which groups of 30 rats were fed diets containing 0, 0.8, 2.0 or 5.0 % mixture of gum base containing 25 % candelilla wax for 89 weeks, no significant differences were reported between the groups regarding food intake, urinalysis, haematology or histopathology of major organs (Harrisson, 1953).

Genotoxicity

As reported by JECFA (1993c), candelilla wax was not mutagenic in four bacterial mutagenicity studies using *Salmonella typhimurium*, at test concentrations of up to 10 000 µg/plate, with and without metabolic activation. Candelilla wax also gave negative results in two other studies respectively using *Escherichia coli* and *Saccharomyces cerevisiae* D4 (JECFA, 1993c). No other *in vitro* or *in vivo* genotoxicity studies appear to have been carried out with candelilla wax. Available studies on the main components of candelilla wax (e.g. straight-chain hydrocarbons (C29-C33 n-alkanes) together with esters of acids and alcohols with even-numbered carbon chains (C28-C34) (JECFA, 2006)) do not give rise to concern regarding genotoxicity.

Carcinogenicity

As reported by JECFA (1993c), a mixture of gum base containing 25 % candelilla wax was administered to mice at levels of 0, 0.8 or 5.0 % in the diet, corresponding to 0, 1 200 or 7 500 mg/kg b.w. per day of gum mixture and approximately 0, 300 or 1 900 mg candelilla wax/kg b.w. per day for a period of 12-13 months (Hodge, 1973). The number of deaths in the 5 % dose group was reported by the authors to exceed those in the lower or control groups, but the cause of death was not defined. The authors concluded that there was no evidence of a carcinogenic effect of candelilla wax in this study (JECFA, 1993c).

JECFA concluded that the long term study of Harrisson (1953) in rats described above under 'Subacute, subchronic and chronic toxicity studies' also did not provide any evidence of carcinogenicity.

The CONTAM Panel noted that the main components of candelilla wax (e.g. straight-chain hydrocarbons (C29-C33 n-alkanes) together with esters of acids and alcohols with even-numbered carbon chains (C28-C34) (JECFA, 2006)) do not give rise to concern with respect to carcinogenicity.

Developmental and reproductive toxicity

As reported by JECFA (1993c), in a very limited short-term study three male and three female rats were fed a diet containing a 1:1 mixture of styrene-butadiene polymer and candelilla wax at concentrations of 0, 680 or 3 420 mg/kg b.w. per day for 5 months prior to mating (Harrisson, 1949). The authors reported that two of the three females from each dose group conceived and produced normal litters.

3.12.2.4. Allergenicity

No information has been found regarding sensitizing capacity, adjuvanticity or irritancy of candelilla wax. The CONTAM Panel considers that it is unlikely that candelilla wax after dilution when used as a previous cargo will be of concern with regard to sensitization, adjuvanticity or irritancy.

3.12.3. Conclusions

JECFA concluded that dietary exposures to candelilla wax of less than 650 mg/person per day (approximately 10 mg/kg b.w. per day), the intake calculated by JECFA from a conservative exposure estimate based on the indicated uses of candelilla wax as a food additive, do not raise concern about safety. The EFSA ANS Panel noted that the available toxicity studies consistently reported no adverse effects associated with intake of the main components constituting candelilla wax and that the exposure estimates allowed to conclude that candelilla wax, within the currently authorised uses as

food additive, would not be of safety concern. The CONTAM Panel agreed with this position, and concluded that given the likely limited absorption of candelilla wax and the toxicological profile of its main component groups of chemicals, this wax will not pose any toxicological concern when used as a previous cargo. There is no evidence that it is genotoxic and there is no allergenic potential of concern. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated.

The CONTAM Panel therefore concludes that candelilla wax meets the criteria for acceptability as a previous cargo.

According to the information provided to EFSA, the CONTAM Panel noted that candelilla wax does not appear to be transported as a previous cargo.

3.13. WHITE MINERAL OILS (CAS No 8042-47-5)

White mineral oil (CAS 8042-47-5) is defined by ECHA³¹ as ‘a highly refined petroleum mineral oil consisting of a complex combination of hydrocarbons obtained from the intensive treatment of a petroleum fraction with sulfuric acid and oleum, or by hydrogenation, or by a combination of hydrogenation and acid treatment. Additional washing and treating steps may be included in the processing operation. It consists of saturated hydrocarbons having carbon numbers predominantly in the range of C15 through C50’. Often white mineral oils are deparaffinated (removal of n-alkanes) to ensure liquid properties.

White mineral oils are used mainly in the food industry (e.g. as release agents and for dedusting of grain and rice) and for pharmaceuticals.

Most edible oils contain mineral oil hydrocarbons from the environment, harvesting and/or processing. Concentrations around 10-50 mg/kg are common, but some edible oils also regularly contain above 100 mg/kg mineral oil (such as olive pomace oil, grapeseed oil or cottonseed oil; Wagner et al., 2001; Moret et al., 2003, Biedermann et al., 2009; EFSA 2012b). Mostly the mineral oils also contain aromatic components.

3.13.1. Previous evaluations

The SCF evaluated white mineral oils as previous cargo in 1996 and considered that oils with a carbon number of not less than 25, an average molecular mass not less than 480 and a viscosity not less than 8.5 centistokes at 100 °C were provisionally acceptable pending submission of further data on chronic toxicity/carcinogenicity (SCF, 1997a). The SCF considered that there were insufficient data to establish the safety of other mineral oils. In the 2003 SCF evaluation of acceptable previous cargoes, the Committee reconsidered white mineral oils and concluded that the information available was inadequate. The SCF therefore maintained their opinion as provisionally acceptable previous cargo (SCF, 2003).

Mineral oil hydrocarbons have been evaluated on a number of occasions for their safety for use as food additives by the SCF, EFSA and JECFA.

An ADI of 0-20 mg/kg b.w. was established by JECFA (1995) for high viscosity (> 11 mm²/s at 100 °C) mineral oils (synonyms: liquid paraffin; liquid petrolatum; food grade mineral oil; white mineral oil). In 2002 JECFA established an ADI of 0-10 mg/kg b.w. for medium- and low-viscosity, class I (8.5-11 mm²/s at 100 °C) mineral oils (synonyms: liquid paraffin; liquid petrolatum; food grade mineral oil; white mineral oil) (JECFA, 2002). A temporary group ADI of 0-0.01 mg/kg b.w. was established by JECFA (2002) for medium- and low-viscosity, class II (7.0-8.5 mm²/s at 100 °C) and

³¹ European Chemicals Agency (ECHA), online. White mineral oil. CAS 8042-47-5. Available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9ea08dfc-55a5-3699-e044-00144f67d031/DISS-9ea08dfc-55a5-3699-e044-00144f67d031_DISS-9ea08dfc-55a5-3699-e044-00144f67d031.html (consultation date: 2012-01-23).

class III (3.0-7.0 mm²/s at 100 °C) mineral oils, but this was withdrawn in 2012, due to the absence of suitable information to enable an ADI to be established or confirmed (JECFA, 2012).

The EFSA ANS Panel evaluated the safety of high viscosity white mineral oils (CAS Registry Number 8042-47-5, chain lengths C22-C60, average molecular weight: > 500 g/mol, viscosity at 100 °C $\geq$ 11 mm²/s, carbon number > 25 at 5 % distillation point) when used as food additives and established an ADI of 12 mg/kg b.w. based on a NOAEL of 1 200 mg/kg b.w. per day in a chronic (12 months) study in Fischer 344 rats (highest dose tested) (EFSA, 2009c).

The CONTAM Panel has provided a scientific opinion on Mineral Oil Hydrocarbons in Food, concluding that revision of the existing ADIs for some food grade mineral oil saturated hydrocarbons is warranted on the basis of new toxicological information (EFSA, 2012b). EFSA (2012b) noted that established ADIs are based on toxicological studies with poorly characterised products with regard to chemical composition. The CONTAM Panel concluded that for high-viscosity mineral oils and medium- and low viscosity class I mineral oils, the ADIs established by SCF, FAO/WHO and EFSA were based on toxicological studies in which no effects were observed at any tested dose. For those grades, it was concluded that although based on products with poor chemical characterisation, the existing ADIs were of low priority for revision. The upper bounds of the ADIs were all $\geq$ 4 mg/kg b.w.

White mineral oils, paraffinic, derived from petroleum-based hydrocarbon feedstocks, are approved for use as a food contact additive in plastics under Commission Regulation (EU) No 10/2011,²⁴ with no restrictions other than the generic overall migration limit of 60 mg/kg food.

For white mineral oil used as an active substance in pesticides, EFSA concluded that ‘In line with the low toxicity of paraffin oils (of high purity), no ADI, AOEL³² or ARfD³³ would be proposed, nor considered necessary, and no risk assessment for operators, workers and bystanders would be required’ (EFSA, 2008b, c).

3.13.2. Current evaluation

3.13.2.1. Expected impurities

White mineral oils are not expected to contain impurities of concern.

3.13.2.2. Reactivity and reaction products

White mineral oils do not react with edible fats and oils.

3.13.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Gastrointestinal absorption of white mineral oils is dependent on the physical properties and molecular composition of the oil, absorption decreasing with increasing carbon number and extent of side chain branching (EFSA, 2012b). Given the carbon numbers of high viscosity and medium and low viscosity (P70) white mineral oils, of > 28 and > 25 respectively, absorption will be limited. Any mineral oil absorbed is deposited mainly in the liver, mesenteric lymph nodes, spleen and perirenal fat, as shown by the results of toxicokinetic and toxicological studies (EFSA, 2012b). Deposited mineral oils will be slowly metabolised in the liver to the corresponding fatty alcohols and then fatty acids, with some metabolism also occurring in the small intestine, and will enter normal biochemical pathways (EFSA, 2012b).

³² Acceptable operator exposure level.

³³ Acute reference dose.

Acute toxicity

Mineral oils (high viscosity) and mineral oils (medium and low viscosity) have low acute toxicity, with LD₅₀ values > 5 000 mg/kg b.w.³¹(EFSA, 2012b). They are non-irritant or slightly irritant to skin and eyes.³¹

Subacute, subchronic and chronic toxicity studies

As reported by SCF (1995), JECFA (2002) and EFSA (2009c, 2012b), repeated ingestion of mineral oils results in accumulation of the oils in various tissues, both in humans and in experimental animals, in a dose- and time-dependent manner, depending on the molecular composition of the particular oils, the carbon number and the extent of side chain branching (EFSA, 2012b). This accumulation results in an inflammatory response, characterised by focal histiocytosis, particularly in the mesenteric lymph nodes, and granulomas or microgranulomas in the liver and mesenteric lymph nodes. Increases in relative organ weights of liver, lymph nodes, spleen and kidneys also occur, together with haematological changes indicative of a chronic inflammatory reaction and biochemical changes associated with mild hepatic damage. It has been demonstrated in a number of studies that the Fischer 344 rat is markedly more susceptible to the development of these changes than Sprague Dawley rats or other strains. The CONTAM Panel in their evaluation of mineral oil hydrocarbons considered that ‘the presence of microgranulomas/histiocytosis in MLN³⁴ is a non specific, adaptative change of low toxicological concern’ (EFSA, 2012b). The CONTAM Panel concluded however that the liver microgranulomas induced by mineral oils in Fischer 344 rats could be potentially relevant to humans and were the critical effect for risk assessment of mineral oils. The NOAEL for the critical effect in studies showing these effects was at least 100-fold greater than that for histiocytosis in the mesenteric lymph nodes. The lowest NOAEL identified for development of liver microgranulomas following administration of mineral hydrocarbons was 19 mg/kg b.w. per day, in a 90-day study in Fischer rats (Smith et al., 1996). This was however for mineral waxes, whereas in general the NOAEL for class II and II mineral oils was one order of magnitude greater.

These changes were not seen in toxicological studies with high viscosity mineral oils (e.g. P100) and medium and low viscosity class I mineral oils (e.g. P70(H)) and P100(H). For the high viscosity P100(H) mineral oils, the ADI of 20 mg/kg b.w. established by JECFA (2002) was based on a no-observed-effect level (NOEL) of 2 000 mg/kg b.w. per day, the highest dose tested in a 90 day study in Fischer rats. For the class I intermediate and low viscosity P70 mineral oils, the ADI of 10 mg/kg b.w. per day was based on an increased incidence of pigmented macrophages in male rats at a dose level of 2 000 mg/kg b.w per day, an effect considered of doubtful biological significance. A temporary group ADI of 0-0.01 mg/kg b.w. was established by JECFA (2002) for class II and III mineral oils, based on the occurrence of histiocytosis of mesenteric lymph nodes in 90-day studies in rats, with a NOAEL of 2 mg/kg b.w. per day and an uncertainty factor of 200. This effect is now considered to be of no toxicological relevance (EFSA, 2012b), and JECFA withdrew the ADIs of these classes in 2012, due to the absence of suitable information to enable an ADI to be established or confirmed (EFSA, 2012b).

Genotoxicity

The expert reviews carried out by the SCF, JECFA and EFSA have all concluded that refined high viscosity mineral oils (e.g. P100) and medium and low viscosity mineral oils with a very low content of aromatic compounds are not genotoxic. Negative results were obtained in bacterial mutagenicity tests without or with metabolic activation (Granella and Clonfero, 1991; Mackerer et al., 2003). Highly refined base oil was negative in a mouse lymphoma assay, with and without metabolic activation.³¹ As reported by EFSA (2009c), a series of five paraffinic base stocks and two naphthenic base stocks were tested in the rat bone marrow cytogenetic assay (CONCAWE, 1984, as cited in

³⁴ Mesenteric lymph nodes.

EFSA, 2009c). Negative findings in these base stock oils, all of which are less refined than white mineral oils, supports the lack of genotoxicity in refined white mineral oils.

Carcinogenicity

Refined high viscosity mineral oils (e.g. P100) and medium and low viscosity class I (e.g. P70) mineral oils (with a very low content of aromatics) have not shown any effects of toxicological significance, including carcinogenicity, in chronic toxicity/carcinogenicity studies. In the pivotal carcinogenicity study for these mineral oils, rats were administered P70H and P100H mineral oils at doses of 0, 60, 120, 240, or 1 200 mg/kg b.w. per day via the diet. No carcinogenic potential or chronic toxicity was observed (Exxon Biomedical Sciences, 2001, as cited in JECFA, 2002). EFSA has also concluded that, in chronic toxicity/carcinogenicity studies conducted with high viscosity and medium viscosity white oils, no carcinogenic effects were observed in any of the studies in F344 rats or in skin painting studies in mice (EFSA, 2009c, 2012b).

Developmental and reproductive toxicity

Refined high viscosity mineral oils (e.g. P100) and medium and low viscosity (e.g. P70) mineral oils are not considered to show developmental or reproductive toxicity, although there are limited data following administration by the oral route (EFSA, 2012b). As reported by EFSA (2012b), in a developmental toxicity study, a sample of white mineral oil (no detail available) was administered to female SD rats (20 rats/group) at dose levels of 0 or 5 000 mg/kg b.w. per day from days 6 through 19 of gestation. No maternal or fetal toxicity were observed³¹. As also reported by EFSA (2012b), a light paraffinic distillate extract with a low viscosity and carbon numbers predominantly in the range of C15 to C30 was administered by gavage at a dose level of 1000 mg/kg per day in a screening reproductive and development toxicity study to rats.³¹ There were no treatment-related effects on pup body weights, sex ratios, live litter sizes, viability indices, and general physical conditions and no treatment-related effects were observed on the parental animals. Studies by other routes of administration support the lack of developmental or reproductive toxicity of white mineral oils (EFSA, 2012b).

3.13.2.4. Allergenicity

In a skin sensitization study in guinea pigs undiluted highly refined base oil was tested, and found not to be a sensitizer (Anonymous, 1987g, as cited in ECHA, online). White mineral oil was found not to be sensitizing in the guinea pig maximization test (Magnusson and Kligman, 1969; Exxon Corporation, 1994, as cited in ECB, 2000b).

White mineral oil appears to be a non-irritant or only slightly irritating for the skin (Hoekstra and Phillips, 1963; Anonymous, 1987c, as cited in ECHA, online; Exxon Corporation, 1994, as cited in ECB, 2000b). However, the use of mineral oils of unspecified purity in cutting fluid has been associated with dermatitis in metal workers (Pryce et al., 1989). Emulsions of purified mineral oil with allergens have been much used as immune adjuvants in particular in animals (White, 1963) and the question has been raised whether mineral oil exposure may under some circumstances promote autoimmunity (Whitehouse, 2012).

Considering the high dilution factor, the CONTAM Panel considers that white mineral oils are not of concern with regard to sensitization, irritancy or adjuvanticity when used as a previous cargo.

3.13.3. Conclusions

The SCF, JECFA and/or EFSA have established ADIs of ≥ 4 mg/kg b.w. for high viscosity and medium- and low-viscosity, class I mineral oils. Whilst the CONTAM Panel considers that these may need to be revised as they were based on products with poor chemical characterisation, this is not a high priority given their toxicological profile, and there would be no toxicological concern from their use as previous cargoes. There are no ADIs for class II and III mineral oils. However, the lowest

relevant NOAEL available is 19 mg/kg b.w. per day from a 90-day study in Fischer rats. This was for mineral waxes, whereas in general the NOAEL for class II and III mineral oils was one order of magnitude greater. White mineral oils are not genotoxic and they would not be expected to be of concern for allergenicity when used as previous cargoes. They will not give rise to any reaction products with fats and oils of toxicological concern. The only potential impurities of toxicological concern are PAHs, which are controlled to low levels in these mineral oils.

The CONTAM Panel notes that some aliphatic hydrocarbons bioaccumulate in the body, such as branched and cyclic species in the mass range of 16-35 carbon atoms. However, since exposure to mineral oil hydrocarbons via contamination of edible fats and oils from previous cargoes occurs only rarely and mostly at levels lower than those observed in edible oils, it will contribute little to overall exposure. Further, humans are exposed to mineral oil hydrocarbons from numerous sources.

The CONTAM Panel therefore concludes that white mineral oils meet the criteria for acceptability as a previous cargo.

3.14. GLYCERINE (glycerol; glycerin) (CAS No 56-81-5)

The CONTAM Panel noted that the term glycerine is rarely used to refer to this substance. It is more normally referred to as glycerol (glycerin is the German name of the substance), and this is the preferred name. The IUPAC name is propane-1,2,3-triol.

Glycerol is a viscous liquid, miscible with water. It is the backbone of triglycerides (fats and oils). It is obtained from fats and oils, either from saponification or from transesterification with methanol (biodiesel; today the main source). It is also produced from propene via allyl chloride, dichloropropanol and epichlorohydrine. Synthetic glycerol is used only when the highest purity is required, as in pharmaceuticals and some food grade products.

Glycerol is used to produce e.g. glycerol trinitrate (nitroglycerol, dynamite), alkyd resins, polyurethanes, hydraulic fluids, antifreeze, cosmetics, humectants (e.g. in foods) and for the production of various food components, such as emulsifiers.

3.14.1. Previous evaluations

The SCF evaluated glycerol as a previous cargo in 1996 and considered it as acceptable (SCF, 1997a). This conclusion was based on the fact that glycerol was a food additive, E 422, and component of food, with an ADI not specified (SCF, 1981). In the 2003 SCF evaluation of acceptable previous cargoes, glycerol was not further considered as it was already considered acceptable (SCF, 2003).

JECFA evaluated glycerol as a food additive and established an ADI 'not specified' at its 20th meeting (JECFA, 1976). JECFA evaluated glycerol again in 2001 as a flavouring substance and maintained the ADI 'not specified' (JECFA, 2001).

The SCF evaluated glycerol as a food additive and as an extraction solvent for food, and agreed with the JECFA 1976 evaluation, that an ADI need not be specified (SCF, 1981).

The CONTAM Panel has evaluated crude glycerol derived from biodiesel production and concluded that such glycerol up to a level of 15 % in the diet of ruminants and up to 10 % in the diet of monogastric animals had no adverse effects on animal health (EFSA, 2010). Glycerol is considered as a feed material under Commission Regulation (EU) 892/2010,³⁵ with no maximum levels assigned.

Glycerol has been evaluated under the OECD SIDS programme on HPV chemicals (OECD, 2002). Overall, it was concluded that it was of low priority for further work.

³⁵ Commission Regulation (EU) No 892/2010 of 8 October 2010 on the status of certain products with regard to feed additives within the scope of Regulation (EC) No 1831/2003 of the European Parliament and of the Council, OJ L 266, 08.10.2010, p.8.

Glycerol is approved for use as a food contact additive in plastics under Commission Regulation (EU) No 10/2011,²⁴ with no restrictions other than the generic overall migration limit of 60 mg/kg food.

3.14.2. Current evaluation

3.14.2.1. Expected impurities

Crude glycerol from transesterification will contain alkali and methanol as the main impurities. When prepared by saponification it will contain alkali and free fatty acids as impurities. These impurities will not be of any concern when glycerol is used as a previous cargo.

Crude glycerol obtained by chemical synthesis could contain some unreacted allyl chloride, dichloropropanol and epichlorohydrin. Epichlorohydrin is fairly reactive in the presence of acid, but it is uncertain whether it reacts with components of edible fats and oils. However, as synthetic glycerol is only used after substantial purification, these impurities are not expected to be of concern when glycerol is used as a previous cargo.

3.14.2.2. Reactivity and reaction products

Glycerol may react with fats and oils through transesterification, but this is not expected to yield products of concern.

3.14.2.3. Toxicological profile

Absorption, distribution, metabolism and excretion

Glycerol occurs endogenously in the body as a result of hydrolysis of glycerol esters in the intestine, followed by absorption from the intestinal mucosa (JECFA, 2001). Following metabolism to glycerol-3-phosphate in the liver and to a minor extent in the kidney, intestine and other tissues, it is oxidized in several steps to pyruvic acid. Glycerol also combines with free fatty acids in the liver to form triglycerides. There is generally no excretion of free glycerol in the urine, due to complete metabolic conversion to other products (JECFA, 2001; OECD, 2002).

Acute toxicity

As reported by OECD (2002), glycerol is of low acute oral toxicity, with LD₅₀s > 20 000 mg/kg b.w. in rats, mice and rabbits. At doses approaching the LD₅₀, signs of toxicity include tremors, convulsions, CNS depression and hyperaemia of the gastrointestinal tract (OECD, 2002). Glycerol is slightly irritant to skin and eyes (OECD, 2002).

Subacute, subchronic and chronic toxicity studies

Rats were administered glycerol in the diet at levels of up to 60 %, providing intakes of 0, 1 000, 3 000, 6 000, 10 000, 15 000, 20 000, 30 000, 40 000, 500 00 or 60 000 mg/kg b.w. per day for 20 weeks (Guerrant and Whitlock, 1947, as cited in JECFA, 2001). Treatment-related changes were restricted to reduced body-weight gain in rats receiving > 40 000 mg/kg b.w. per day and hydropic and fatty degeneration of hepatocytes in rats receiving > 10 000 mg/kg b.w. per day. The NOAEL in this study was identified as 6 % glycerol in the diet, equivalent to 6 000 mg/kg b.w. per day (Guerrant and Whitlock, 1947, as cited in JECFA, 2001).

JECFA in 1976 reported the results of a long-term study in which rats were given glycerol in the diet at a concentration of 0, 5, 10 or 20 % (equivalent to 0, 2 500, 5 000, or 10 000 mg/kg b.w. per day) for 2 years, also including an interim kill at 12 months. No treatment-related effects were reported on body weight or on histological examination of major organs. The only treatment-related change was an increase in relative kidney weights at 10 000 mg/kg b.w. per day, unaccompanied by histopathological changes. The author of the study identified a NOEL of 10 000 mg/kg b.w. per day, the highest dose tested (Atlas Chemical Co., 1969, as cited in JECFA, 1976).

In another 2-year rat study reported by both JECFA (2001) and OECD (2002) male rats received 2 000, 4 000 or 8 000 mg/kg b.w. per day glycerol in the diet and females received 2 500, 5 000 and 10 000 mg/kg b.w. per day. No treatment-related effects were reported in this study other than a slight increase in food consumption in males receiving 4 000 or 8 000 mg/kg b.w. per day glycerol. The author of the report concluded that the NOAEL was greater than 10 000 mg/kg b.w. (Hine et al., 1953, as cited in JECFA, 2001 and OECD, 2002).

Genotoxicity

As reported by both JECFA (2001) and OECD (2002), glycerol did not induce mutations in a bacterial mutagenicity study, with and without metabolic activation and was negative in a mammalian cell gene mutation test (HGPRT). Glycerol did not induce chromosomal aberrations or sister chromatid exchanges in CHO cells (JECFA, 2001; OECD, 2002). In a bone marrow chromosome aberration test with rats, glycerol did not induce a statistically significant increase in chromosomal aberrations compared to controls (Varilyak and Kozachuk, 1985). OECD noted however that no reliable conclusions could be drawn from this study due to the limited details available, the small number of animals used and the absence of a positive control (OECD, 2002). Overall, glycerol is considered to possess no genotoxic potential.

Carcinogenicity

There was no evidence of carcinogenicity in two 2-year oral toxicity studies in the rat, reported under 'chronic toxicity' above. Data from tumour promotion studies in male mice indicated that oral administration of glycerol for up to 20 weeks had a weak promotion effect on the incidence of lung tumour formation (Nagahara, 1987; Inayama, 1986, as cited in OECD, 2002). Overall, these data do not indicate that glycerol has carcinogenic potential.

Developmental and reproductive toxicity

As reported by OECD (2002), a two generation study was conducted with glycerol administered by gavage as a 20 % solution in water (providing 2 000 mg/kg b.w. per day). No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility or reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of either the F1 or F2 generation. The NOAEL was therefore identified as 2 000 mg/kg b.w., the highest dose tested (Wegener, 1953, as cited in OECD, 2002).

In an oral gavage study, rats, mice and rabbits were administered glycerol at doses of 1 310, 1 280 and 1 180 mg/kg b.w. during part of the gestation period. No maternal toxicity or teratogenic effects were seen at the highest dose levels tested (N.T.I.S., 1974, as cited in OECD, 2002).

A study of fertility in 64 male workers involved in glycerol manufacture reported no significant effects on sperm quality parameters (Venable et al., 1980). The CONTAM Panel noted that although the workers were involved in glycerol manufacture, exposure to epichlorhydrin, allyl chloride and 1,3-dichloropropene was the specific focus of the study.

3.14.2.4. Allergenicity

Glycerol has low irritancy and is considered to be a weak sensitiser based on human data (El-Nagdy et al., 1973; Preston and Finch, 2003; Fairhurst and Wilkinson, 2007; Tamagawa-Mineoka et al., 2007). A guinea pig study on glycerol found no capacity for sensitization (Hine et al., 1953, as cited in JECFA, 2001 and OECD, 2002). No information has been found regarding adjuvanticity. The CONTAM Panel considers that the available data indicate that glycerol when used as a previous cargo would be of no concern as an allergen or adjuvant.

3.14.3. Conclusions

The CONTAM Panel notes that the name glycerine is rarely used to refer to this substance. It is more normally referred to as glycerol, which is the preferred name. The IUPAC name is propane-1,2,3-triol. The CONTAM Panel therefore recommends that the entry for the substance in the Annex to Commission Directive 96/3/EC⁵ is amended to ‘Glycerol (glycerine; glycerin; propane-1,2,3-triol) (CAS No 56-81-5)’.

Both JECFA and the SCF have established an ADI not specified for glycerol, which the CONTAM Panel considers appropriate. Glycerol is not genotoxic and there are no concerns regarding its allergenicity when it is used as a previous cargo. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological relevance.

The CONTAM Panel therefore concludes that glycerol meets the criteria for acceptability as a previous cargo.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Substances transported in bulk as previous cargoes are often rather crude and usually no specific information is available on the impurities present. Hence, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) often had to determine which impurities might be present primarily from assessment of the source or starting material and method of preparation of the substance to be transported. Chemicals transported as previous cargoes may vary in composition, depending on their method of preparation, details for which were obtained, in part, from information obtained from FOSFA. The CONTAM Panel based its evaluations on worst case assumptions on these aspects.³⁶
- The CONTAM Panel considered that it should be possible to measure the relevant components of a previous cargo in the edible oil or fat transported later. However, it is unrealistic to expect that corresponding analytical methods are immediately available, since fats and oils are not routinely analysed for the presence of most previous cargoes. It is sufficient that the development of a corresponding method is feasible with standard techniques.³⁶
- **Sodium silicate (water glass) solution (CAS No 1344-09-8).** Toxicological effects of sodium silicate following acute and repeat dosing are mostly due to high alkalinity. However, following ingestion it will be diluted and buffered by the neutralising capacity of the gastrointestinal tract. Thus the CONTAM Panel considered that the levels that would occur following oral ingestion of fats and oils transported subsequent to sodium silicate as a previous cargo would not give rise to any toxicological concern. Although there are no carcinogenicity studies available sodium silicate did not show any genotoxic activity in a variety of *in vitro* and *in vivo* assays thus indicating no genotoxic potential. Available data give no indication that sodium silicate is an allergen or an adjuvant at concentrations expected from its use as a previous cargo. Exposure to sodium silicates can be irritating or corrosive to the skin, however the potential levels arising in fats and oils following its transport as previous cargo would be of no concern. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern. The CONTAM Panel therefore concludes that sodium silicate solution meets the criteria for acceptability as a previous cargo for edible fats and oils.

³⁶ The addition of this bullet point aims to clarify the evaluation performed by the CONTAM Panel in relation to the impurities and the analytical methods. The clarification applies also to the previous two opinions by the CONTAM Panel on this topic (Part I of III (EFSA, 2011) and Part II of III (EFSA, 2012a)).

- Iso-octanol (isooctyl alcohol) (CAS no 26952-21-6), iso-nonanol (isononyl alcohol) (CAS no 27458-94-2) and iso-decanol (isodecyl alcohol) (CAS no 25339-17-7).** Iso-octanol, iso-nonanol and iso-decanol were classified by the former Scientific Committee on Food (SCF) in its last re-evaluation in 2003 as provisionally acceptable as previous cargoes because the information available was considered inadequate or limited. The CONTAM Panel used a ‘read across’ from the assessment profile of oxo-alcohols C9-C13 category (OECD, 2006) to fill data gaps on the toxicological profile of iso-octanol, iso-nonanol and iso-decanol. They are of a low order of toxicity following acute and repeated exposures upon oral, dermal or inhalational exposure. The lack of effects found in the limited studies available suggests that they are not genotoxic. They are not allergenic and there are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern. The CONTAM Panel therefore concludes that iso-decanol, iso-nonanol or iso-octanol meet the criteria for acceptability as previous cargoes for edible fats and oils.
- 1,3-Propanediol (1,3-Propylene glycol; trimethylene glycol) (CAS No 504-63-2).** 1,3-Propanediol is of low systemic toxicity when administered by the oral route. There are no carcinogenicity data but the available evidence indicates that 1,3-propanediol does not have any genotoxic potential. 1,3-Propanediol is not an allergen. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological concern. Therefore, the CONTAM Panel concludes that 1,3-propanediol meets the criteria for acceptability as a previous cargo for edible fats and oils.
- Isobutyl acetate (2-methylpropyl acetate) (CAS No 110-19-0).** The toxicity of acetate, one of the major metabolites of isobutyl acetate, was addressed in a previous Opinion of the CONTAM Panel (EFSA, 2012a), when it was considered that there would be no health concerns following the maximum potential carryover into edible fats and oils when it is transported as a previous cargo. In its evaluation of previous cargoes in 1996, the SCF concluded that isobutanol, the other major metabolite, was ‘not acceptable’, because ‘limited toxicological data indicates a suspicion of carcinogenic concerns.’ In a re-evaluation in 2003, the SCF maintained its previous opinion that this substance was not acceptable as a previous cargo because the Committee was aware of a number of issues that still needed clarification. The CONTAM Panel considered isobutanol as a previous cargo for edible fats and oils in 2009, and concluded that it was acceptable, based on the low level of toxicity observed in a more recent chronic study, as well as its volatility and ease of tank cleaning (EFSA, 2009b). Although the toxicological database for isobutyl acetate is limited, available data on this substance and on its hydrolysis products, acetic acid and isobutanol, suggest that isobutyl acetate is of relatively low systemic toxicity. It is not genotoxic or allergenic. There are no reaction products or impurities expected to be of toxicological concern. The CONTAM Panel therefore concludes that isobutyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.
- Sec-butyl acetate (CAS No 105-46-4).** There are no data on repeated-dose toxicity of sec-butyl acetate. It is a Cramer class I substance, with a threshold of toxicological concern of 1 800 µg/person per day. It is rapidly metabolised to acetate and sec-butanol, which can be further metabolised to methyl ethyl ketone (MEK). The CONTAM Panel has previously evaluated acetic acid and MEK for their suitability as previous cargoes for edible fats and oils and concluded that they meet the criteria for acceptability (EFSA, 2012a). sec-Butanol is not genotoxic. The information available on sec-butanol does not indicate any toxicological concern at the exposure levels that might occur from the transport of sec-butyl acetate as a previous cargo to edible fats and oils. sec-Butyl acetate is not allergenic. There are no reaction products or impurities of toxicological concern. The CONTAM Panel therefore concludes that sec-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

- **Tert-butyl acetate (CAS No 540-88-5).** The toxicological database on tert-butyl acetate is somewhat limited. The available data on tert-butyl acetate and on acetate and tert-butanol, its major metabolites, do not give rise to concerns regarding systemic toxicity, developmental toxicity or genotoxicity. Any carcinogenic risk would likely be from a non-genotoxic mode of action and would not be of concern at the levels of exposure that might occur from the use of tert-butyl acetate as a previous cargo for edible fats and oils. tert-Butyl acetate is not allergenic. There are no reaction products or impurities of toxicological concern. The CONTAM Panel therefore concludes that tert-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.
- **n-Butyl acetate (CAS No 123-86-4).** The SCF established a temporary acceptable daily intake (ADI) of 0-6 mg/kg for n-butyl acetate, on the basis of limited data. The toxicological database has several data gaps (no repeat dose studies by the oral route, no studies of chronic toxicity or carcinogenicity). However, there were sufficient data on its major metabolites, acetate and n-butanol, for the CONTAM Panel to conclude previously that these are not of concern, when used as previous cargoes. n-Butyl acetate is not genotoxic. The CONTAM Panel considers that the available information on the acute effects of n-butyl acetate and on its subchronic, reproductive and developmental toxicity following exposure by the inhalation route, together with information on its major metabolites, was sufficient to conclude that the risk from short-term exposure to n-butyl acetate when used as a previous cargo would not give rise to any toxicological concern. There are no concerns regarding the allergenicity of n-butyl acetate. There are no reaction products or impurities of toxicological concern. The CONTAM Panel therefore concludes that n-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.
- **Propylene tetramer (CAS No 6842-15-5).** Although specific studies on propylene tetramer itself are somewhat limited, data are available on many of its main components and mixtures of these. In general, the toxicological profile of alkenes depends on carbon length, and is similar for those with similar carbon length. The CONTAM Panel considers that propylene tetramer would not be of toxicological concern at the levels that would occur when used as a previous cargo for edible fats and oils. Although there are no studies of carcinogenicity, the CONTAM Panel concludes that in the absence of genotoxicity or of pathological changes in subchronic studies indicative of a potential carcinogenic hazard, there was no concern for possible carcinogenicity from the use of propylene tetramer as a previous cargo. Propylene tetramer is not allergenic. There are no reaction products or impurities of toxicological concern. The CONTAM Panel therefore concludes that propylene tetramer meets the criteria for acceptability as a previous cargo for edible fats and oils.
- **Montan wax (CAS No 8002-53-7).** No ADI or tolerable daily intake (TDI) has been established for montan wax by the SCF, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the European Food Safety Authority (EFSA). Data recently provided to the European Chemicals Agency (ECHA) indicate that montan wax is not mutagenic in a bacterial mutagenicity test, and the CONTAM Panel considers that it is not likely to be a significant sensitizer, adjuvant or irritant. In a subchronic toxicity study in rats, haematological changes and hepatotoxicity were observed at the lowest dose tested, of approximately 260 mg/kg body weight (b.w.) per day, and hence no no-observed-adverse-effect level (NOAEL) could be identified. There are no data on chronic toxicity or carcinogenicity. Montan wax is an ill-defined material for which it cannot be excluded that it contains components of concern. The CONTAM Panel therefore concludes that, given the deficiencies in the available data on montan wax, it does not meet the criteria for acceptability as a previous cargo.
- **Paraffin wax (food grade) (CAS No 8002-74-2 / 63231-60-7).** Paraffin wax may contain aromatic hydrocarbons, some of which are genotoxic carcinogens. Hence, the CONTAM Panel concluded that this entry to the Annex should be restricted to paraffin waxes that have

been treated to remove aromatic hydrocarbons and which otherwise meet relevant standards to be considered as ‘food grade’.

An ADI of 0-20 mg/kg b.w. has been established by both JECFA and the SCF for high molecular mass food-grade microcrystalline wax, with specifications as laid down according to Commission Directive 2008/84/EC and JECFA (2008). The CONTAM Panel (EFSA, 2012b) noted that this ADI was established from toxicological studies in which no effects were observed at any tested dose. Food grade paraffin wax is not genotoxic or allergenic. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated. The CONTAM Panel therefore concludes that paraffin wax (food grade, CAS No. 8002-74-2 / 63231-60-7) meets the criteria for acceptability as a previous cargo.

- **Carnauba wax (Brazil wax) (CAS No 8015-86-9).** JECFA have established an ADI of 0-7 mg/kg b.w. for carnauba wax, while the SCF concluded that its use as a glazing agent up to a maximum use level of 200 mg/kg of food was acceptable. The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) noted in its re-evaluation of carnauba wax as a food additive carried out in 2012, that available toxicity studies consistently reported no adverse effects associated with carnauba wax intake, and that the exposure estimates to carnauba wax allowed the conclusions that, within the currently authorised as a food additive, it would not be of safety concern. The CONTAM Panel considered, based on the outcome of these expert evaluations, the likely limited absorption of carnauba wax and the toxicological profile of its main component groups of chemicals, that this wax will not pose any toxicological concern when used as a previous cargo, based on normal assumptions regarding worst case carryover. There is no evidence that it is genotoxic and there is evidence that the allergenic potential is very low and therefore of no concern in the context of previous cargoes. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated. The CONTAM Panel noted however, the insolubility of carnauba wax in water, its high melting point (82 to 86 °C) and the fact that heating of ships’ tanks is normally to a maximum of 80 °C. The CONTAM Panel therefore has concerns regarding the feasibility of tank cleaning following transport of carnauba wax as a previous cargo, such that carryover may exceed the worst case normally assumed. The CONTAM Panel concludes that carnauba wax does not meet the criteria for acceptability as a previous cargo because of doubts concerning the efficiency of tank cleaning following transport of carnauba wax as a previous cargo.
- **Candelilla Wax (CAS No 8006-44-8).** JECFA concluded that dietary exposures to candelilla wax of less than 650 mg/person per day (approximately 10 mg/kg b.w. per day), the intake calculated by JECFA from a conservative exposure estimate based on the indicated uses of candelilla wax as a food additive, do not raise concern about safety. The ANS Panel noted in its re-evaluation of candelilla wax as a food additive carried out in 2012, that the available toxicity studies consistently reported no adverse effects associated with intake of the main components constituting candelilla wax and that the exposure estimates allowed the conclusion that, within the currently authorised uses as a food additive, it would not be of safety concern. The CONTAM Panel agreed with this position, and concluded that given the likely limited absorption of candelilla wax and the toxicological profile of its main component groups of chemicals, this wax will not pose any toxicological concern when used as a previous cargo. There is no evidence that it is genotoxic and there is no allergenic potential of concern. It will not give rise to any reaction products with fats and oils of toxicological concern. No impurities of toxicological concern are known or anticipated. The CONTAM Panel therefore concludes that candelilla wax meets the criteria for acceptability as a previous cargo.
- **White mineral Oils (CAS No 8042-47-5).** The SCF, JECFA and/or EFSA have established ADIs of ≥ 4 mg/kg b.w. for high viscosity and medium- and low-viscosity, class I mineral oils. Whilst the CONTAM Panel considers that these may need to be revised as they were

based on products with poor chemical characterisation, this is not a high priority given their toxicological profile, and there would be no toxicological concern from their use as previous cargoes. There are no ADIs for class II and III mineral oils. However, the lowest relevant NOAEL available is 19 mg/kg b.w. per day from a 90-day study in Fischer rats. This was for mineral waxes, whereas in general the NOAEL for class II and III mineral oils was one order of magnitude greater. White mineral oils are not genotoxic and they would not be expected to be of concern for allergenicity when used as previous cargoes. They will not give rise to any reaction products with fats and oils of toxicological concern. The only potential impurities of toxicological concern are polycyclic aromatic hydrocarbons (PAHs), which are controlled to very low levels in these mineral oils. The CONTAM Panel notes that some aliphatic hydrocarbons bioaccumulate in the body, such as branched and cyclic species in the mass range of 16-35 carbon atoms. However, since exposure to mineral oil hydrocarbons via contamination of edible fats and oils from previous cargoes occurs only rarely and mostly at levels lower than those observed in edible oils, it will contribute little to overall exposure. The CONTAM Panel therefore concludes that white mineral oils meet the criteria for acceptability as a previous cargo

- **Glycerol (Glycerine; glycerine; propane-1,2,3-triol) (CAS No 56-81-5).** Both JECFA and the SCF have established an ADI not specified for glycerol, which the CONTAM Panel considers appropriate. Glycerol is not genotoxic and there are no concerns regarding its allergenicity when it is used as a previous cargo. There are no reactions of concern with edible fats and oils, nor are any anticipated impurities likely to be present at levels of toxicological relevance. The CONTAM Panel therefore concludes that glycerol meets the criteria for acceptability as a previous cargo.

RECOMMENDATIONS

The CONTAM Panel recommends that a number of amendments be made to the entries of the substances in the Annex to Commission Directive 96/3/EC.⁵

- The entry for ‘Sodium silicate (water glass) (CAS No 1344-09-8)’ be amended to ‘**Sodium silicate (water glass) solution (CAS No 1344-09-8)**’ to reflect contemporary shipping practices.
- The entry for ‘1,3-Propylene glycol (trimethylene glycol; 1,3-propanediol) (CAS No 504-63-2)’ be amended to ‘**1,3-Propanediol (1,3-propylene glycol; trimethylene glycol (CAS No 504-63-2)**’ to reflect accepted chemical nomenclature.
- The entry for ‘iso-Butyl acetate (CAS No 110-19-0)’ be amended to ‘**Isobutyl acetate (2-methylpropyl acetate) (CAS No 110-19-0)**’ to reflect accepted chemical nomenclature.
- The entry for ‘Paraffin wax (CAS No 8002-74-2 / 63231-60-7)’ be amended to ‘**Paraffin wax (food grade) (CAS No 8002-74-2 / 63231-60-7)**’ since other grades may contain aromatic hydrocarbons which could pose an unacceptable risk if such waxes were to be transported as previous cargoes to edible fats and oils.
- The CONTAM Panel recommends that the entry for ‘Glycerine (glycerol; glycerin) (CAS No 56-81-5)’ be amended to ‘**Glycerol (glycerine; glycerin; propane-1,2,3-triol) (CAS No 56-81-5)**’ to reflect accepted chemical nomenclature.

The entries in the Annex to Commission Directive 96/3/EC for the substances evaluated in this Opinion as previous cargoes for edible fats and oils are listed in Table 4, amended as recommended by the CONTAM Panel.

Table 4: Substances in the list to Annex to Commission Directive 96/3/EC⁵ listed as acceptable previous cargoes for edible fats and oils with amendments recommended by the CONTAM Panel (entries in bold).

Substance (synonyms)	CAS Number
Sodium silicate (water glass) solution	1344-09-8
iso-Octanol (isooctyl alcohol)	26952-21-6
iso-Nonanol (isononyl alcohol)	27458-94-2
iso-Decanol (isodecyl alcohol)	25339-17-7
1,3-Propanediol (1,3-propylene glycol; trimethylene glycol)	504-63-2
Isobutyl acetate (2-methylpropyl acetate)	110-19-0
sec-Butyl acetate	105-46-4
tert-Butyl acetate	540-88-5
n-Butyl acetate	123-86-4
Propylene tetramer	6842-15-5
Paraffin wax (food grade)	8002-74-2 / 63231-60-7
Candelilla wax	8006-44-8
White mineral oils	8042-47-5
Glycerol (Glycerine; glycerine; propane-1,2,3-triol)	56-81-5

The CONTAM Panel recommends that when new substances are to be evaluated as previous cargoes for the bulk transport by sea of edible fats and oils, the interested party should provide information adequate for EFSA to carry out a scientific evaluation, including information on:

- the identity and specification (including impurities) of the substances to be evaluated,
- their chemical composition,
- the form in which they are transported (e.g. in solution),
- the chemical reactivity with fats and oils,
- published data on toxicological studies (including acute and chronic toxicity studies, reproductive and developmental toxicity studies, carcinogenicity and genotoxicity studies) and allergenicity and adjuvanticity.
- similar information on the toxicity of any significant impurities and reaction products with fats and oils.
- where an impurity (or reaction product) is not considered significant, an explanation for this conclusion.

Information should also be supplied on ease of tank cleaning, including any need for heating of the transfer equipment.

DOCUMENTATION PROVIDED TO EFSA

1. FOSFA International. Response from FOSFA International following a meeting the EFSA Working Group on Previous Cargoes, 3 April 2012. Submitted to EFSA on 18 April 2012.

WAXES

Question: the WG requested information on the current transport of the different waxes listed as acceptable previous cargoes for edible fats and oils.

With regard to the various waxes, the IBC Code does not differentiate but simply contains an entry “Waxes”, which is pollution Category Z and ship type 3 (lowest safety requirement ship). My contacts understand that in most cases where wax is carried, it is petroleum wax. They have never heard of anyone carrying beeswax, candelilla wax or carnauba wax, but they will check with the trade association membership to ensure they are not carried.

(Follow-up reply submitted by FOSFA to EFSA on 30.05.2012). I have made further enquiries into the carrying of waxes in bulk by sea. As far as I can determine, these waxes are not carried in bulk. There is not enough shipment to justify bulk cargoes. I believe that they are not carried in bulk by sea. Also, these products do not appear in the IBC Code, and products which do not appear in the code, and are not covered by a tripartite agreement (an agreement between the exporting country, the importing country and the flag state of the ship carrying the cargo) cannot be carried in bulk by sea.

PROPYLENE TETRAMER

Question: the WG requested information on the identity of the material that is carried as previous cargo for edible fats and oils.

Propylene Tetramer is a branched olefin produced by the polymerization of propylene. It is also commonly referred to as dodecene. Propylene Tetramer is used in the production of dodecylphenol, tridecyl alcohol, branched dodecylbenzene sulfonic acid, and dodecylsuccinic anhydride which in turn are used to produce plasticizers, surfactants, lube oil additives, emulsifiers for herbicides, and corrosion inhibitors in alkyd and epoxy coatings.

The material which is shipped seems to be a blend of isomers of which about 65% are 1-propene. Another MSDS lists a detailed breakdown of the isomers which are present:

Components	CAS Registration Number	Weight %
Trimethylheptene Isomers	102943-77-1	0.5 to 2
Tetramethylheptene Isomers	103982-56-5	3 to 6
Trimethyloctene Isomers	103985-01-9	3 to 6
Tetramethyloctene Isomers	105902-19-0	20 to 25
Trimethylnonene Isomers	54410-98-9	40 to 50
Dimethyldecene Isomers	55170-80-4	20 to 25
Tetramethylnonene Isomers	55771-41-0	0.5 to 2

I have not been able to get much information about the material which is shipped apart from a Material Safety Data Sheet (MSDS) which is rather vague on the various components. Thus, I have attached the detailed MSDS from TEXAS PETROCHEMICALS LP which gives much more information about the product.

2. FOSFA International. Response from FOSFA International. Submitted to EFSA on 16 July 2012.

1,3-PROPYLENE GLYCOL

Question: the WG requested information on the grade(s) of 1,3-propylene glycol most commonly transported.

The vast majority of propylene glycol carried by the chemical tanker fleet is 1,2-propylene glycol. The 1,3-propylene glycol which is carried is typified by the attached MSDS from Du Pont (1). This has a composition of > 99.7 % purity although many manufacturers quote 99% minimum, and occasionally 98 % min. It has been available in commercial quantities in the past, but is not an important product now. It was obtained as a by-product in the production of glycerol by either saponification or fermentation of animal fats, but is also produced from acrylaldehyde.

ISO-DECANOL, ISO-NONANOL, ISO-OCTANOL

Question: the WG requested information on the composition of the iso-decanols, iso-nonanols and iso-octanols as traded.

The iso-decanol, iso-nonanol, iso-octanol products which are traded are mixtures of isomers and are mainly produced by Oxo-process from olefins by addition of CO and hydrogen. They are generally described as, for example, 'ALCOHOLS, C9–C11 -ISO, C10-RICH', or 'C9-RICH'. The compositions are typified by the MSDS for Exxal 10 (2) and Neodol 9 (3). The purity is difficult to define. The multinational manufacturers such as Exxon have 99.0 % minimum purity, but some just state "rich", meaning that this is the majority chemical present. I believe that both types are carried in bulk by sea. Octanol Technical Grade (4) is sometimes carried and is described as 99.5 % minimum 2 ethyl-hexanol isomers.

PARAFFIN WAX - EDIBLE GRADE

Question: It was noted that this entry appears in the FOSFA list of acceptable previous cargoes as 'Paraffin wax - edible grade'. The WG requested information on the specifications understood by FOSFA for 'edible grade'? (The same applies for the entry White Mineral Oils).

Paraffin wax - edible grade is a highly refined paraffin wax. When a manufacturer states that their product is edible, FOSFA understands that it complies with all the relevant food ingredient legislation of the country in which it is to be used.

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ABBREVIATIONS

ACGIH	American Conference of Industrial Hygienists
ADH	Alcohol dehydrogenase
ADI	Acceptable daily intake
ALDH	Aldehyde dehydrogenases
ALT	Alanine aminotransferase
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
AST	Aspartate aminotransferase
b.w.	Body weight
CAC	Codex Alimentarius Commission
CCFO	Codex Committee for Fats and Oils
CHO	Chinese hamster ovary
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CNS	Central nervous system
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association of the US
FOSFA	Federation of Oils, Seeds and Fats Associations
GD	Gestation day
GMP	Good manufacturing practice
GRAS	Generally Recognized As Safe
HPV	High Production Volume
IUCLID	International Uniform Chemical Information Database
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LLNA	Local lymph node assay
LMPW	Low melting point paraffin wax
LMWPW	Lower melting point paraffin waxes
LOAEL	Lowest-observed-adverse-effect level
MCHC	Mean corpuscular hemoglobin concentration
MEK	Methyl ethyl ketone
MSDS	Material Safety Data Sheet
MTBE	Methyl tertiary butyl ether
NOAEC	No-observed-adverse-effect concentration
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PCE	Polychromatic erythrocytes
PND	Postnatal day
PTT	Polytrimethylene terephthalate
SCF	Scientific Committee on Food
SD	Sprague-Dawley (rats)
SIDS	Screening Information Data Set
TDI	Tolerable daily intake
TTC	Threshold of toxicological concern
WHO	World Health Organization

SCIENTIFIC OPINION

Scientific Opinion on the safety evaluation of the active substances iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite for use in food contact materials¹

**EFSA Panel on Food Contact Materials, Enzymes,
Flavourings and Processing Aids (CEF)^{2,3}**

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids deals with the safety evaluation of iron based oxygen absorber systems comprising iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite, incorporated in sachets, patches and cards. Iron, the main active ingredient reacts with oxygen to form iron hydroxide and iron oxide, thereby removing oxygen from the primary packaging. Only activated carbon has not been evaluated as such, but it meets the specifications for activated charcoal which is authorised as additive for plastic materials and articles in contact with foods. All other ingredients of the oxygen absorber formulations have been evaluated and approved for use as additives in plastic food contact materials and/or as food additives and/or food supplements or feed additives. The active system being based on solid ingredients and not intended for direct contact with liquid food or food with an external liquid surface, migration through the gas phase was screened for 9 representative active systems. No volatiles derived from the active mixtures were detected. Therefore the CEF Panel concluded that the substances do not raise a safety concern when used in oxygen absorbers in sachets, patches or cards, placed in the headspace of the packaging or when used in direct contact with food, excluding liquid food or foods that have an external aqueous liquid phase on the surface such as sliced fruits and fresh meat.

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¹ On request from the Food Standards Agency, UK, Question No EFSA-Q-2010-01489, adopted on 19 March 2013.

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KEY WORDS

Food contact materials; active and intelligent materials; oxygen absorbers; iron; safety assessment; evaluation.

SUMMARY

According to the Commission Regulation (EC) No 450/2009⁴ of the Commission of European Communities of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, substances responsible for the active or intelligent function need first to be evaluated by EFSA before their inclusion into a positive Community list. The procedure of the evaluation and the tasks of EFSA are described in the Regulation (EC) No 1935/2004⁵ of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

In the context of this evaluation procedure, following a request from the Food Standards Agency, UK, the Panel on Food Contact Materials, Enzymes and Processing Aids (CEF) was asked to deliver an opinion on the safety of mixtures comprising active iron (CAS No 7439-89-6, FCM Substance No 983), sodium chloride (CAS No 7647-14-5, FCM Substance No 983); water (CAS No 7732-18-5, FCM Substance No 515), silica gel (CAS No 7631-86-9, FCM Substance No 504), activated carbon (CAS No 7440-440/ 64365-11-3, FCM Substance No 984), monosodium glutamate (CAS No 142-47-2, FCM Substance No 1023), potassium acid tartrate (CAS No 868-14-4, FCM Substance No 1018), powdered cellulose (CAS No 9004-34-6, FCM Substance No 553), malic acid (CAS No 6915-15-7, FCM Substance No 499), chabazite (CAS No 12251-32-0, FCM Substance No 1019), hydroxypropyl cellulose (CAS No 9004-64-2, FCM Substance No 559), potassium carbonate (CAS No 584-08-7, FCM Substance No 21), sodium thiosulfate (CAS No 7772-98-7, FCM Substance No 519), propylene glycol (CAS No 57-55-6, FCM Substance No 109), glycerin (CAS No 56-81-5, FCM Substance No 103), polyethyleneglycol sorbitan monooleate (CAS No 9005-65-6, FCM Substance No 569), sodium propionate (CAS No 137-40-6, FCM Substance No 1020) and clinoptilolite (CAS No 12173-10-3, FCM Substance No 1021), for use as iron based oxygen absorbers in sachets, patches or cards. The dossier was submitted on behalf of Multisorb Technologies, USA.

Iron, the main active ingredient reacts with oxygen to form iron hydroxide and iron oxide, thereby removing oxygen from the primary packaging.

Only activated carbon was not evaluated as such, but it meets the specifications for activated charcoal which is authorised as additive for plastic materials and articles in contact with foods. All other ingredients of the oxygen absorber formulations have been evaluated and approved for use as additives in plastic food contact materials and/or as food additives and/or as food supplements or feed additives.

As the active system is based on solid ingredients, only migration through the gas phase is expected. Therefore, the applicant screened by headspace gas chromatography coupled to mass spectrometry the volatiles produced by 9 representatives active systems. No volatiles derived from the active mixtures were detected.

The CEF Panel concluded that the substances: iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite, do not raise a safety concern when used in oxygen absorbers in sachets, patches or cards, placed in the headspace of the packaging or when used in direct contact with food, excluding liquid food or foods that have an external aqueous liquid phase on the surface such as sliced fruits and fresh meat.

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Directive 95/45/EC with exception of ash content which can be up to 10 % (w/w).

⁴ Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, OJ L 135.30.05.2009, p.3-11.

⁵ Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, OJ L338.13.11.2004, p.4-17.

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BACKGROUND AS PROVIDED BY THE LEGISLATION

Regulation (EC) No 450/2009 of the Commission of European Communities is a specific measure that lays down specific rules for active and intelligent materials and articles intended for contact with foodstuffs in addition to the general requirements established in Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food. Active materials and articles are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.

The substance(s) responsible for the active and/or intelligent function of the material should be included in a positive list by the Commission following a safety evaluation by the EFSA according to the procedure described in the abovementioned regulations.

According to this procedure the industry submits applications to the Member States competent Authorities which transmit the applications to EFSA for evaluation. The application is supported by a technical dossier submitted by the industry following the EFSA guidelines on “submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food” (EFSA, 2009).

In this context, EFSA received an application from the Food Standards Agency, UK , requesting the evaluation of mixtures comprising iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite, in sachets, patches and cards, for use as oxygen absorber.

TERMS OF REFERENCE AS PROVIDED BY THE APPLICANT

The EFSA is required to carry out an assessment on the risks originating from the migration into food of the iron , sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite, used in oxygen absorbing systems in food contact materials, and deliver a scientific opinion, according to the Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food.

The opinion of EFSA will be considered by the Commission for adoption of a Community list of authorised substances where according to the Regulation (EC) No 450/2009 there will be specified:

- (a) the identity of the substance(s);
- (b) the function of the substance(s);
- (c) the reference number;
- (d) if necessary, the conditions of use of the substance(s) or component;
- (e) if necessary, restrictions and/or specifications of use of the substance(s);
- (f) if necessary, conditions of use of the material or article to which the substance or component is added or into which it is incorporated.

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the Food Standards Agency, UK, to evaluate the safety of various iron based oxygen absorber systems for food packaging. These absorbers are composed of iron (CAS No 7439-89-6, FCM Substance No 983), sodium chloride (CAS No 7647-14-5, FCM Substance No 983); water (CAS No 7732-18-5, FCM Substance No 515), silica gel (CAS No 7631-86-9, FCM Substance No 504), activated carbon (CAS No 7440-440/ 64365-11-3, FCM Substance No 984), monosodium glutamate (CAS No 142-47-2, FCM Substance No 1023), potassium acid tartrate (CAS No 868-14-4, FCM Substance No), powdered cellulose (CAS No 9004-34-6, FCM Substance No 553), malic acid (CAS No 6915-15-7, FCM Substance No 499), chabazite (CAS No 12251-32-0, FCM Substance No), hydroxypropyl cellulose (CAS No 9004-64-2, FCM Substance No 559), potassium carbonate (CAS No 584-08-7, FCM Substance No 21), sodium thiosulfate (CAS No 7772-98-7, FCM Substance No 519), propylene glycol (CAS No 57-55-6, FCM Substance No 109), glycerin (CAS No 56-81-5, FCM Substance No 103), polyethyleneglycol sorbitan monooleate (CAS No 9005-65-6, FCM Substance No 569), sodium propionate (CAS No 137-40-6, FCM Substance No) and clinoptilolite (CAS No 12173-10-3, FCM Substance No 1021). The request has been registered in the EFSA's register of questions under the number EFSA-Q-2010-01489. The dossier was submitted on behalf of Multisorb Technologies, USA.

2. General information

According to the applicant, the oxygen absorbers are prepared by mixing together iron, sodium chloride, water and depending on the intended use some of the following substances: silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite. These substances are used in different mixtures formulated according to the target oxygen absorption capacity and the food contact applications. The mixtures are placed into sachets, patches or cards.

Sachets, patches and cards are intended to be used for dry foods applications (such as tea/coffee, bakery products, dried fruits, vegetables, spices, flour, pasta) and fat containing foods (such as processed, cured and smoked meats, cheese, nuts and bakery products containing fat, such as cakes and cookies). They are not intended for direct contact with liquid food or food with an external liquid surface. The range of contact conditions is from chilled up to ambient temperature for long time storage.

Sachets, patches, cards are constituted by multilayer materials including plastic films and/or paper and adhesive in their structures. Layers are either perforated plastic or non woven substrates in order to allow gas exchanges with the atmosphere surrounding the product.

After filling, sachets, patches are heat sealed. For cards both layers between which the active powder is deposited, are stuck together with an adhesive. All applied technologies are used to prevent the physical release to food of the active mixtures under foreseeable condition of uses.

Mixtures based on the above listed chemicals have not been evaluated by the SCF or EFSA in the past. However, the substances constituting the oxygen absorber systems (iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polysorbate 80, sodium propionate are authorised either for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) or as food additives (Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the

European Parliament and of the Council by establishing a Union list of food additives⁶) or food supplements (sodium chloride) (Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements⁷), as follows:

- Iron powder is authorised as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with a specific restriction of 48 mg iron/kg food based on a Provisional Maximum TDI (PMTDI) of 0.8 mg/kg bw set by JECFA/WHO (1983) and agreed by the SCF (1990) (FCM Substance No 983).
- Sodium chloride is an authorised food supplement (Regulation EC No 1170/2009) with no specific restriction.
- Water is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restrictions. The water specifications must be in compliance with Directive 98/83/EC⁸ (FCM Substance No 515).
- Silica gel (silicon dioxide) is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with the restriction ‘For synthetic amorphous silicon dioxide: primary particles of 1 – 100 nm which are aggregated to a size of 0.1 – 1 µm which may form agglomerates within the size distribution of 0.3 µm to the mm size’ (FCM Substance No 504). It is also listed in Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008⁹ of the European Parliament and of the Council by establishing a Union list of Food Additives, with a specific maximum level of 10g/kg or higher, depending on the foodstuffs (E 551).
- Activated carbon should meet the requirements for activated charcoal, which is authorised as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with the following restriction: “Only for use in PET at maximum 10 mg/kg of polymer (evaluated by EFSA in 2004¹⁰). Same purity requirements as for Vegetable Carbon (E 153) set out by Commission Directive 95/45/EC¹¹ with exception of ash content which can be up to 10 % (w/w)” (FCM Substance No 984).
- Monosodium glutamate is listed in the Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives (E 621), with a specific maximum level of 10g/kg, individually or in combination, expressed as glutamic acid.

⁶ Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives, OJ L295, p.1-177

⁷ Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements, OJ L 314, 01.12.2009, p.36-42.

⁸ Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, OJ L 330, 5.12.1998, p.32.

⁹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L354, p.16-33

¹⁰ EFSA (European Food Safety Authority), 2004, Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Iron. The EFSA Journal 2004, 125, 1-34.

¹¹ Commission directive 95/45/EC, of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs, OJ L 226, 22.9.1995, p. 1.

- Potassium acid tartrate is listed in the Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives, (E 336), with no specific restriction.
- Cellulose (powdered) is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restrictions (FCM Substance No 553).
- Malic acid (E 296) is a food ingredient and an authorised food additive, listed in the Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives, with a specific maximum level of 3 g/kg or higher, depending on the foodstuffs. It is also authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with the restriction “in case of use as a monomer only to be used as a co-monomer in aliphatic polyesters up to maximum level of 1 % on a molar basis” (FCM Substance No 499).
- Chabazite (calcium aluminium silicate) (E 556) is listed in the Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives, with no specific restriction.
- Hydroxypropyl cellulose is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 559).
- Potassium carbonate is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 21 – carbonic acid, salts).
- Sodium thiosulfate is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with a restriction of 7 mg/kg expressed as SO₂ (FCM Substance No 519).
- Propylene glycol is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 109).
- Glycerin is authorised as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 103).
- Polyethyleneglycol sorbitan monooleate (polysorbate 80) is authorized as additive or monomer for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 569).
- Sodium propionate is listed in the Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives, with no specific restriction.
- Clinoptilolite is a natural zeolite comprising a microporous arrangement of silica and alumina tetrahedra. It has the complex formula: (Na,K,Ca)₂₋₃Al₃(Al, 2Si)₁₃O₃₆·12(H₂O). According to an Opinion of the FEEDAP Panel (EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2013), clinoptilolite is considered safe as a feed additive up to 10 000 mg clinoptilolite/kg complete feed for all animal species.

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the EFSA guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food (EFSA, 2009).

Non-toxicity data:

- Data on identity
- Data on physical and chemical properties
- Data on manufacturing process
- Data on function, intended use and authorisation
- Data on overall migration studies
- Data on identification and migration studies of volatiles

Toxicity data:

- None

4. Evaluation

4.1. Non-toxicological data

The main active ingredient of each commercial oxygen absorber is iron which reacts with oxygen to form iron hydroxide and iron oxide, thereby removing oxygen from the primary packaging.

Depending on the intended uses, the oxygen absorber mixture may contain in addition to iron powder, water and sodium chloride, some of the following substances added to provide a suitable reaction medium depending on their own properties: silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite.

Activated carbon, powdered cellulose, chabazite, silica gel and clinoptilolite are used as water activity modifiers. Hydroxypropyl cellulose, glycerine, propylene glycol are used as binders. Monosodium glutamate, potassium acid tartrate, potassium carbonate, sodium thiosulfate and malic acid act as buffers to keep the pH at the optimum range to promote the iron oxidation. Sodium chloride and water play the role of electrolyte to facilitate the reaction. Polyethyleneglycol sorbitan monooleate is used to decrease the surface tensions and to permit a good dispersion of chemical ingredients. Sodium propionate acts as antimicrobial agent.

Overall migration was measured by direct contact (total immersion or one side contact) with food simulants. However, these experiments were not considered relevant due to the design of sachets, cards or patches which are constituted by perforated and/or non-woven materials and because direct contact with liquid food or food with an external liquid surface is not intended by the applicant. Therefore those data were not taken into account.

As the active system is based on solid ingredients, only migration through the gas phase is expected. Therefore, the applicant screened by headspace gas chromatography coupled to mass spectrometry (HS-GCMS) the volatiles produced by 9 representatives active systems, placed for 30 minutes at 100 °C in sealed glass vessels. No volatiles derived from the active mixtures were detected.

4.2. Toxicological data

All ingredients of the oxygen absorber formulations have been evaluated and approved for use as additives in plastic food contact materials, as food additives or food supplements. Activated carbon was not evaluated as such, but it meets the specifications for activated charcoal, which is authorised as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) i.e. same purity requirements as for Vegetable Carbon (E 153) set out by Commission Directive 95/45/EC with the exception of ash content which may be up to 10 % (w7w). All these ingredients are expected to be stable in normal storage and handling conditions. Thus no toxicity studies are required.

As regards clinoptilolite, the Panel noted that due to the insolubility of the substance no appropriate genotoxicity *in vitro* testing is feasible and only inconclusive information on the occurrence of chromosomal aberrations *in vivo* in mouse are available. However, 'read across' from studies on other zeolites (notably zeolite A or silver zeolite A; EFSA, 2005) does not give rise to concern for genotoxicity. Moreover, the Panel noted that according to an Opinion of the FEEDAP Panel (EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2013) clinoptilolite is not absorbed in target animals and is considered safe as a feed additive up to 10 000 mg clinoptilolite/kg complete feed for all animal species.

Based on the above information and considering that no migration into food is expected in the intended conditions of use, the Panel concluded that the composition of the oxygen absorbers would not give rise to safety concerns.

CONCLUSIONS

The CEF Panel concluded that the substances: iron, sodium chloride, water, silica gel, activated carbon, monosodium glutamate, potassium acid tartrate, powdered cellulose, malic acid, chabazite, hydroxypropyl cellulose, potassium carbonate, sodium thiosulfate, propylene glycol, glycerin, polyethyleneglycol sorbitan monooleate, sodium propionate and clinoptilolite, do not raise a safety concern when used in oxygen absorbers in sachets, patches or cards, placed in the headspace of the packaging or when used in direct contact with food, excluding liquid food or foods that have an external aqueous liquid phase on the surface such as sliced fruits and fresh meat.

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Directive 95/45/EC with exception of ash content which can be up to 10 % (w/w).

DOCUMENTATION PROVIDED TO EFSA

1. Dossier referenced: 10A11J124I. November 2010. Submitted on behalf of the applicant, Multisorb Technologies, USA.

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GLOSSARY AND ABBREVIATIONS

EC	European Commission
FCM	Food Contact Materials
FEEDAP	Additives and Products or Substances used in Animal Feed
JECFA/WHO	The Joint FAO/WHO Committee on Food Additives
LMWF	Low molecular weight fraction
LOAEL	Low observed adverse effect level
Mn	Number average molecular weight
Mw	Weight average molecular weight
NDA	Dietetic Products, Nutrition and Allergies
PET	Poly(ethylene terephthalate)
PMTDI	Provisional Maximum Tolerable Daily Intake
REF No	Reference Number
SCF	Scientific committee on food
SML	Specific Migration Limit
w/w	Weight by weight
Overall migration:	The sum of the amounts of volatile and non volatile substances, except water, released from a food contact material or article into food or food stimulant
Specific migration:	The amount of a specific substance released from a food contact material or article into food or food stimulant

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Re-evaluation of glycerol (E 422) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS),
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Abstract

The ANS Panel provides a scientific opinion re-evaluating the safety of glycerol (E 422) used as a food additive. In 1981, the Scientific Committee on Food (SCF) endorsed the conclusion from the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976 of 'acceptable daily intake (ADI) for man not specified'. The Panel concluded that glycerol has low acute toxicity and that local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies was likely due to hygroscopic and osmotic effects of glycerol. Glycerol did not raise concern with respect to genotoxicity and was of no concern with regard to carcinogenicity. Reproductive and prenatal developmental studies were limited to conclude on reproductive toxicity but no dose-related adverse effects were reported. None of the animal studies available identified an adverse effect for glycerol. The Panel conservatively estimated the lowest oral dose of glycerol required for therapeutic effect to be 125 mg/kg bw per hour and noted that infants and toddlers can be exposed to that dose by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel concluded that there is no need for a numerical ADI and no safety concern regarding the use of glycerol (E 422) as a food additive at the refined exposure assessment for the reported uses. The Panel also concluded that the manufacturing process of glycerol should not allow the production of a food additive, which contains genotoxic and carcinogenic residuals at a level which would result in a margin of exposure below 10,000. The Panel recommended modification of the EU specifications for E 422. The Panel also recommended that more information on uses and use levels and analytical data should be made available to the Panel.

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Keywords: glycerol, glycerine, 1,2,3-propanetriol, trihydroxypropane, E 422, CAS Registry Number 56-81-5, food additive

Requestor: European Commission

Question number: EFSA-Q-2011-00519

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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of glycerol (E 422) when used as a food additive.

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that came available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Glycerol (E 422) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

Glycerol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976. Based on the available toxicological studies and the fact that glycerol occurs naturally in fats and other substances consumed via food, JECFA allocated to glycerol an acceptable daily intake (ADI) 'not specified'.

The Scientific Committee for Food (SCF) evaluated glycerol as a food additive in 1981 and endorsed the conclusion from JECFA in 1976 of 'ADI for man not specified'. However, a rationale for the conclusion was not provided.

A request to use glycerol without restriction (*quantum satis* level) as a sweetener in concentrated juices especially intended for young children was rejected by the SCF in 1999.

Glycerol has also been reviewed by TemaNord in 2002. It was concluded that no new data were available indicating a need for a re-evaluation of glycerol. However, it was further concluded that the present permitted *quantum satis* use may lead to the conclusion that all levels are acceptable. In addition, it was concluded that it should be ensured that glycerol is not used as a sugar replacement.

The Panel noted that in addition to the information provided by industry on the manufacturing process, glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern.

Glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract, it is then distributed into the total body water space and primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as carbon dioxide, with only minor amounts excreted via urine or faeces. The Panel noted that glycerol concentrations in blood higher than 92 mg/L would result in renal elimination of glycerol in rats and humans.

The Panel considered that glycerol has a low acute toxicity.

Short-term or subchronic studies were not performed according to current test guidelines. In a subchronic toxicity study (in drinking water) in rats, the effects reported were observed with doses in the range of the oral median lethal dose (LD₅₀) values for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg body weight (bw) per day, the lowest dose tested), and dogs (100% glycerol at 5,600 mg/kg bw per day) was likely due to the hygroscopic and osmotic effects of large bolus doses of glycerol administered by gavage.

Glycerol did not show any genotoxic activity in a variety of *in vitro* assays. A lack of valid *in vivo* genotoxicity data was not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in tumour incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.

The reports of the two and multigeneration reproductive toxicity studies have limitations but no adverse effects were reported. Prenatal developmental toxicity studies were also limited but showed

no dose-related developmental and maternal effects up to the highest dose tested (1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits, respectively).

The Panel considered that none of the animal studies available identified an adverse effect for glycerol.

The Panel considered that the therapeutic oral use of glycerol at 1,000–1,500 mg/kg bw given as bolus in patients with glaucoma triggered an increase in plasma osmolality and dehydration and resulted in some patients in side effects such as headache, nausea and vomiting in some individuals. Given the dose- and time period-range reported in a study, the Panel calculated that the minimum dose of glycerol required to induce a therapeutic reduction in intracranial pressures was within the range of 125–333 mg/kg bw per hour. The Panel considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

The Panel considered that the exposure estimates in all exposure scenarios resulted in overestimates of the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008. Although use levels and analytical data were only available for 28 out of the total of 68 food categories in which glycerol (E 422) is authorised for use according to Annex II, the Panel considered that this did not result in an underestimation of the exposure because:

- glycerol (E 422) belongs to Group I food additives, and therefore almost all the food categories for which glycerol (E 422) is authorised correspond to the general Group I food additives authorisation. Given the information on the uses provided by food industry and information from the Mintel's Global New Products Database (GNPD), it may be assumed that glycerol is not used in a number of these food categories.
- approximately 94% of the food products labelled with glycerol (E 422) in the Mintel GNPD belonged to food subcategories that were considered in the exposure assessment.

Furthermore, the Panel noted that the additional exposure to glycerol via natural sources, including wine and honey (the sources for which analytical data were available) did not significantly change the exposure to glycerol (E 422). The highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use is consumption of a beverage. Therefore, the Panel calculated the volume of flavoured drinks required to be consumed in order to exceed the acute bolus exposure (125 mg/kg bw per hour) calculated by the Panel to be the minimum dose required to have a therapeutic effect. When considering the available data, the Panel noted that infants and toddlers can be exposed to more than 125 mg glycerol/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink.

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels received from industry and Member States in 28 food categories out of 68 food categories in which glycerol (E 422) is authorised;
- the highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario;
- glycerol (E 422) as a food additive is identical to a compound which is a normal constituent in the body (an endogenous compound) and is a regular component of the diet;
- sufficient toxicity data were available;
- the toxicological studies in animals did not provide any indication for adverse effects, including at the highest dose tested in a chronic toxicity study (10,000 mg/kg bw per day);

the Panel concluded that there is no need for a numerical ADI for glycerol (E 422).

The Panel concluded that there is no safety concern regarding the use of glycerol (E 422) as a food additive according to Annex II and III (Part 1, 2, 3, 4 and 5) for the general population at the refined exposure assessment for the reported uses of glycerol as a food additive.

However, the Panel identified that there remain uncertainties over the lack of identification and quantification of residuals, especially those that are genotoxic and carcinogenic. The Panel noted that

these residuals are mostly present when chemical synthesis is used to produce glycerol. The Panel concluded that the manufacturing process for glycerol should not allow the production of a food additive, which contains these residuals at a level, which would result in a margin of exposure (MOE) below 10,000.

The Panel concluded that if 3-monochloropropane-1,2-diol (3-MCPD) is present at its maximum authorised level of 0.1 mg 3-MCPD/kg glycerol, the maximum exposure to 3-MCPD was below the tolerable daily intake (TDI) of 0.8 µg/kg bw per day, and therefore exposure via glycerol (E 422) alone was of no concern.

The Panel could not calculate exposures to other genotoxic impurities or contaminants that may be present in glycerol (E 422) as a result of the manufacturing process, e.g. glycidol, due to the lack of data on their concentrations in the food additive.

The Panel concluded that infants and toddlers could be exposed to more than 125 mg/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel further concluded that the acute bolus exposure to glycerol (E 422) by its use as a food additive should stay below doses at which pharmacological or side effects could occur.

The Panel recommended that:

- given that during the manufacturing processes of glycerol, genotoxic impurities – e.g. glycidol, epichlorohydrin – could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- given that during the manufacturing processes of glycerol, other potential impurities of toxicological concern (e.g. dichlorohydrin) could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- more data should be generated to decrease uncertainty arising from the presence in the food additive (E 422) of compounds of toxicological concern (e.g. acrolein, 3-MCPD or 3-MCPD ester), which can be produced under some food processing conditions (e.g. use of glycerol (E 422) in parallel with lactic acid bacteria; use of glycerol (E 422) in food containing significant amounts of sodium chloride (more than 5%) and treated at temperatures above 160°C, etc.);
- a numerical limit for acrolein should be included in the EU specifications for glycerol (E 422);
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for glycerol (E 422) should be revised in order to ensure that glycerol (E 422) as a food additive will not be a significant source of exposure to those toxic elements in food;
- more information on uses and use levels and analytical data should be made available to the Panel in order to perform an adequate exposure assessment, in particular in the case of estimate acute exposure, more data on flavoured drinks is needed.

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1. Introduction

The present opinion deals with the re-evaluation of glycerol (E 422) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008¹ of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under Regulation (EU) No 257/2010². This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee for Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU³ of 2001. The report 'Food additives in Europe 2000'⁴ submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.2. Interpretation of the terms of references

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

1.3. Information on existing authorisations and evaluations

Glycerol (E 422) is authorised as a food additive in the EU in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.⁵

¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 1–26.

² Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

³ COM(2001) 542 final.

⁴ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

⁵ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

Glycerol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976 (JECFA, 1976). Based on the available toxicological studies and the fact that glycerol occurs naturally in fats and other substances consumed via food, JECFA allocated to glycerol an acceptable daily intake (ADI) 'not specified'.

The Scientific Committee for Food (SCF) evaluated glycerol as a food additive in 1981 and endorsed the conclusion from JECFA (1976) of 'ADI for man not specified' (SCF, 1981). However, a rationale for the conclusion was not provided. The SCF Opinion cited the JECFA evaluation (JECFA, 1976) but no further studies were described.

A request to use glycerol without restriction (*quantum satis* (QS) level) as a sweetener in concentrated juices especially intended for young children was rejected by the SCF in 1999 (SCF, 1999).

Glycerol has also been reviewed by TemaNord (2002). It was concluded that no new data were available indicating a need for a re-evaluation of glycerol. However, it was further concluded that the present permitted QS use may lead to the conclusion that all levels are acceptable. In addition, it was concluded that it should be ensured that glycerol is not used as a sugar replacement.

Glycerol has been registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation 1907/2006 (ECHA, online).

Glycerol is permitted in cosmetic products in the EU (European Commission database-CosIng⁶).

2. Data and methodologies

2.1. Data

The Panel was not provided with a newly submitted dossier. EFSA launched public calls for data^{7,8,9} to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to January 2017. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however, not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database¹⁰) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of glycerol (E 422) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of glycerol (E 422) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg body weight (bw) per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as 'equivalent'. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day

⁶ Available online: <http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple>

⁷ Call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Published: 9 June 2010. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/ans100609>

⁸ Call for food additives usages level and/or concentration data in food and beverages intended to human consumption. Published: 27 March 2013. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/130327>

⁹ Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: <http://www.efsa.europa.eu/en/dataclosed/call/140324.htm>

¹⁰ Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to glycerol (E 422) from its use as a food additive, as well as due to natural occurrence, was estimated by combining food consumption data from the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.4.1). Uncertainties in the exposure assessment were identified and discussed. Acute dietary exposure to glycerol (E 422) per meal was estimated as the exposure via one food category (food categories as defined in legislation) consumed in one meal (acute exposure per food category).

In the context of this re-evaluation, the Panel followed the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010 (EFSA ANS Panel, 2014).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

Glycerol (E 422) is a polyalcohol containing three hydroxyl (–OH) groups with the molecular formula $C_3H_8O_3$. It has a molecular weight of 92.1 g/mol. The CAS Registry Number is 56-81-5. The EINECS number is 200-289-5. Its chemical name is 1,2,3-propanetriol. The structural formula is presented in Figure 1.

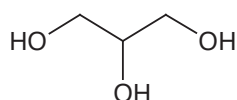


Figure 1: Structural formula of glycerol (E 422)

Physicochemical properties of glycerol are shown in Table 1 (OECD, 2002).

Table 1: Physicochemical properties of glycerol (OECD, 2002)

Physicochemical properties of glycerol	
Melting point (°C)	18
Boiling point (°C, 1,013 hPa)	290
Vapour pressure (hPa)	0.000106 at 25°C (calculated) and 0.0033 at 50°C (measured)
Solubility in water	Miscible
Relative density (20°C)	1.26
Log $P_{o/w}$	–1.76
Dissociation constant	7×10^{-15}
Flash point (°C)	160
Autoflammability (°C)	393
Dynamic viscosity (mPa s; 20°C)	1,410
Surface tension (mN/m; 20°C)	63.4

Glycerol is described as a clear, colourless, hygroscopic syrupy liquid, with not more than a slight characteristic odour, which is neither harsh nor disagreeable (Commission Regulation (EU) No 231/2012). The substance is miscible in water and ethanol and immiscible in ether (JECFA, 2006).

Glycerol is hygroscopic and absorbs water from air. When contact with strong oxidising agents, such as chromium trioxide, potassium chlorate or potassium permanganate, it may be explosive (Merck Index, 1996).

The most common synonyms are 2-propanol, 1,3-dihydroxy-; 1,2,3-propanetriol; 1,2,3-trihydroxypropane, glyceryl alcohol, glycerin or glycerine (SciFinder¹¹, software).

¹¹ SciFinder® the choice for chemistry researchTM.

3.1.2. Specifications

The specifications for glycerol (E 422) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006) are listed in Table 2.

Table 2: Specifications for glycerol (E 422) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Assay	Content not less than 98% of glycerol on an anhydrous basis	Not less than 99% on an anhydrous basis
Description	Clear, colourless, hygroscopic syrupy liquid, with not more than a slight characteristic odour, which is neither harsh nor disagreeable	Clear, colourless, hygroscopic, syrupy liquid, having a not more than a slight characteristic odour, which is neither harsh nor disagreeable
Identification		
Acrolein formation on heating	Heat a few drops of the sample in a test tube with about 0.5 g of potassium bisulfate. The characteristic pungent vapours of acrolein are evolved	–
Specific gravity (25/25 °C)	Not less than 1.257	–
Refractive index $[n]_D^{20}$	Between 1.471 and 1.474	–
Solubility	–	Miscible with water and with ethanol; immiscible with ether
Test for glycerol	–	Passes test
Purity		
Water	Not more than 5% (Karl Fischer method)	Not more than 5% (Karl Fischer method)
Sulfated ash	Not more than 0.01% determined at $800 \pm 25^\circ\text{C}$	Not more than 0.01%
Butanetriols	Not more than 0.2%	Not more than 0.2%
Acrolein, glucose and ammonium compounds	Heat a mixture of 5 mL of glycerol and 5 mL of potassium hydroxide solution (1 in 10) at 60°C for 5 min. It neither becomes yellow nor emits an odour of ammonia	–
Fatty acids and esters	Not more than 0.1% calculated as butyric acid	Not more than 30 mg/kg
Chlorinated compounds	Not more than 30 mg/kg (as chlorine)	Not more than 30 mg/kg (as chloride ion)
3-monochloropropane-1,2-diol (3-MCPD))	Not more than 0.1 mg/kg	
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Chlorides	–	Not more than 10 mg/kg
Colour	–	The colour of the sample, when viewed downward against a white surface in a 50-mL Nessler tube, is not darker than the colour of a standard made by diluting 0.4 mL of ferric chloride TSC with water to 50 mL and similarly viewed in a Nessler tube of approximately the same diameter and colour as that containing the sample

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Readily carbonisable substances	–	Rinse a glass-stoppered, 25-mL cylinder with sulfuric acid TS, and allow to drain for 10 min. Add 5 mL of the sample and 5 mL of sulfuric acid TS, shake vigorously for 1 min, and allow to stand for 1 h. The mixture is no darker than <i>Matching Fluid H</i>

The Panel noted that EU specification for glycerol content is not less than 98% on an anhydrous basis whereas JECFA specifies content not less than 99%.

The Panel noted that in the EU specifications for glycerol (E 422) there is no specific numerical limit for acrolein and that acrolein is classified as a substance of acute toxicity 2 when swallowed, according to Regulation (EC) No 1272/2008¹² (Index No. 605-008-00-3).

The Panel noted that, while the EU specifications have a limit for chlorinated compounds, the identities of specific chlorinated compounds are not indicated.

The Panel noted that, according to the EU specifications for glycerol (E 422), impurities of the toxic elements lead, mercury, arsenic and cadmium are accepted up to a concentration of 2, 1, 3 and 1 mg/kg, respectively. Contamination at those levels would have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010a, 2012).

The Panel also noted that a limit of 0.1 mg/kg for 3-monochloropropane-1,2-diol (3-MCPD), a compound of toxicological concern, is defined in the EU specifications for glycerol (E 422). A tolerable daily intake (TDI) of 0.8 µg/kg bw per day for 3-MCPD has been derived by the EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel, 2016)¹³.

3.1.3. Manufacturing process

3.1.3.1. Glycerol produced from fats and oils

According to Christoph et al. (2006), glycerol is mainly produced from fats and oils by high pressure splitting. It is obtained by feeding water and fat in counter current into a splitting column at 2–6 MPa and 220–260°C. This glycerol is marketed as 88% saponification- or hydrolysis-crude glycerol (Christoph et al., 2006).

The production of glycerol after saponification (treatment with caustic alkali or alkali carbonates) of neutral fats and oils is only partially applied. The final product is obtained from crude glycerol by purification via distillation or ion exchange chromatography (Christoph et al., 2006).

According to CEFIC (2013a [Documentation provided to EFSA n. 3]), 'vegetal' glycerol can be produced from vegetable oils after a treatment at a temperature higher than 100°C at low pressure, followed by distillation and further purification. The Panel noted that refined oils and fats may be contaminated with glycidol (EFSA CONTAM Panel, 2016). Glycidol is characterised as probably carcinogenic to humans 2A (IARC, 2000; BfR, 2009) and as a carcinogenic and genotoxic compound by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2016).

3.1.3.2. Glycerol produced by chemical synthesis

Glycerol can be produced from propene (Christoph et al., 2006). Three pathways are known: (i) via the conversion of propene to acrolein and allyl alcohol; (ii) via the conversion of propene to propylene oxide and allyl alcohol; and (iii) via the conversion of propene to allyl chloride and epichlorohydrin.

In the pathway via acrolein, propene is oxidised to acrolein, which is then reduced to allyl alcohol. Allyl alcohol is then epoxidised with hydrogen peroxide to glycidol, which is further hydrolysed to glycerol.

¹² Regulation (EC) No 1972/2008 of the European Parliament and the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

¹³ The Panel is aware that the EFSA CONTAM Panel agreed at its 82nd plenary meeting to reopen its Scientific Opinion on the risks for human health related to the presence of 3- and 2-MCPD, and their fatty acid esters, and glycidyl fatty acid esters (EFSA-Q-2014-00535) to address the identified scientific divergence with the JECFA opinion. EFSA CONTAM Panel minutes available online: http://www.efsa.europa.eu/sites/default/files/event/170124-m_0.pdf

In the pathway via propylene oxide, propene is epoxidised to propylene oxide, which is then isomerised to allyl alcohol. In a second step, epoxidation is carried out to form glycidol, which is hydrolysed to glycerol.

In the pathway via allyl chloride, propene is oxidised with hypochlorite to dichlorohydrin, which, in the presence of calcium hydroxide or sodium hydroxide, is converted to epichlorohydrin. Epichlorohydrin is then hydrolysed to glycerol at 80–200°C with an aqueous solution of sodium hydroxide or sodium carbonate.

The Panel noted that in the synthesis pathway of glycerol via either acrolein or via propylene oxide, glycidol is produced as an intermediate, which may be present in the final product.

The Panel also noted that in the synthesis pathway of glycerol via allyl chloride, epichlorohydrin is formed as an intermediate, which may be present in the final product. Epichlorohydrin is classified as carcinogen 2A according to the International Agency for Research on Cancer (IARC, 1999).

The Panel further noted that there are no limits in the specifications for other starting or intermediate chemicals used in the synthesis of glycerol, including glycidol, propene, allyl alcohol, propylene oxide, allyl chloride and dichlorohydrin, and no numerical limit in the specifications for acrolein.

3.1.3.3. Glycerol produced by microbial fermentation

The Panel noted that processes are described for glycerol production via microbial fermentation using either yeasts (*Saccharomyces cerevisiae* or osmotolerant yeasts), bacteria (*Lactobacillus lycopersici* and *Bacillus subtilis*) or algae (*Dunaliella tertiolecta* and *Dunaliella bardawil*) (Wang et al., 2001). However, the Panel has no indication if these processes are used on an industrial level.

Schier et al. (2009) presented a literature survey of potential contaminants/intermediates resulting from the manufacturing or purification processes of glycerol. The authors indicated that when, e.g. glycerol is produced via hydrogenolysis of carbohydrates, ethylene glycol can be present as a contaminant. It is also indicated that when glycerol is produced via microbial fermentation, dioxane and aniline can be present as contaminants. All these contaminants are substances of toxicological concern.

Overall, the Panel noted that in addition to the information provided by CEFIC (2013), glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern. This should be considered in the EU specifications for glycerol (E 422). The Panel considered that the manufacturing process for glycerol (E 422) should not allow the production of a food additive which contains residuals of genotoxic or/and carcinogenic concern at a level which would result in a margin of exposure (MOE) below 10,000.

3.1.4. Methods of analysis in food

Different methods for the analysis of glycerol are available in the scientific literature.

A gas chromatography (GC) method for the determination of alcohols, phenols, glycols (including glycerol) and sugars has been published. Acetylation followed by GC/flame ionisation detection (FID) analysis is used (Wachowiak and Connors, 1979). No information is given on recovery rates or limit of detection (LOD).

Glycerol may be measured in wine vinegar, wine, and vinegar directly without any sample preparation by high-performance liquid chromatography (HPLC) using an anion exclusion column (Dewaele et al., 1991). There are two AOAC Official Methods (AOAC, 2000) for the qualitative and quantitative analysis of glycerol in eggs. Various procedures for the determination of glycerol in wine are based on enzymatic and analytical techniques, such as spectrophotometry, fluorimetry, amperometry, chemiluminescence and potentiometry, are cited by Fernandes et al. (2004), who have proposed an enzymatic method coupled with a colorimetric measurement which can be applied on flow during wine manufacturing. The Panel noted that glycerol (E 422) as a food additive is not authorised for those uses, but in wine it may be present as a result of the activity of the yeast.

Nuclear magnetic resonance (NMR) spectroscopy has been employed by Scano et al. (2012) to determine glycerol content in processed mullet (*Mugil cephalus*) roes but no information is given related to the LOD or recovery rates. According to de Souza et al. (2013), the glycerol content was analysed in various beverages: sugar cane liquor, grape juices, grape soda, cola soda and wine and three enzymatic kits combined with amperometric and/or colorimetric analysis were tested. The Panel noted that out of those samples tested glycerol (E 422) as a food additive is authorised only in sugar cane liquor and in soda drinks. Recovery rates were in accordance with internationally accepted performance criteria (Bravenboer et al., 1995) and the LOD was 1 µmol/L equal to 92.1 µg/L.

Amperometric polyenzymatic biosensors based on gold planar or nanocomposite electrode containing multiwalled carbon nanotubes for the determination of glycerol have been developed (Monosik et al., 2012). The biosensors were tested in wine samples only. Ion chromatography (IC) coupled with isotope ratio mass spectrometry (IRMS) using a liquid interface/chemical oxidation of organic matter has been used to quantify solutions containing glycerol in mixtures with carbohydrates and organic acids but the only real sample tested was red wine (Guyon et al., 2013). The Panel noted that glycerol (E 422) as a food additive is not authorised in wine.

Straadt et al. (2014) studied the levels of metabolites – including glycerol – in fresh porcine meat by using NMR spectroscopy. The Panel noted that the use of glycerol (E 422) as a food additive is not authorised in fresh meat.

An HPLC method for the analysis of glycerol in an alcoholic beverage (spirit) coming from ginger is described by Leonel et al. (2015). No information is given concerning performance criteria of the method.

A method for glycerol quantification in sugarcane spirits (cachaças) has been reported. The method is based on sample derivatisation using benzoyl chloride followed by solid-phase extraction and HPLC with diode array detection (Garcia et al., 2015). The reported LOD was 0.25 mg/L.

3.1.5. Stability of the substance and reaction and fate in food

Glycerol is highly stable under ordinary conditions of storage and use, remaining free from objectionable colour, odour or taste with the passage of time. Glycerol solutions subject to heat should not be processed or stored in iron- or copper-containing vessels since iron and copper salts will catalyse the oxidation of glycerol under such conditions (SDA, 1990).

According to industry, glycerol is stable at normal storage and use temperatures. However, temperatures above 54°C can result in decomposition. Decomposition products can include, but are not limited to, acrolein. Moisture and strong oxidisers should be avoided (Dow, online).

According to Leonel et al. (2015), lactic acid bacteria can metabolise glycerol to produce acrolein. Lactic acid bacteria can be used in manufacturing of yogurt, cheese, cultured butter, sour cream (with fat content above 20%), sausage, cucumber pickles, olives and sauerkraut; if glycerol (E 422) is added to those food items, acrolein may be formed. In the absence of data on the amount of acrolein formed, the Panel considered that more data should be generated to decrease uncertainty and allow for a risk assessment.

According to the EFSA CONTAM (2016) opinion, 'In model food heating systems containing water, sodium chloride and glycerol or lipid precursors 3-MCPD production increases with increasing temperature once above 160°C, and with NaCl concentration up to 10% with acylglycerol precursors but at about 5% NaCl with glycerol. The optimum water content is 15–20% for 3-MCPD' and 'Baked goods are the major source of 3- and 2-MCPD and the formation of these contaminants in model bakery systems has been studied in some detail in model systems (Hamlet et al., 2003, 2004a,b). Free glycerol produced by the action of yeast enzymes is the major precursor and the formation reactions of 3- and 2-MCPD follow zero-order kinetics. The levels of 3-MCPD and 2-MCPD formed increase exponentially with temperature up to the maximum (about 220°C) used in baking'. Therefore, the Panel considered that glycerol (E 422) should not be added in food containing significant amounts of salt (more than 5%) treated at temperatures above 160°C, as 3-MCPD or 3-MCPD esters can be formed.

3.2. Authorised uses and use levels

Maximum levels of glycerol (E 422) have been defined in Annex II to Regulation (EC) No 1333/2008¹⁴ on food additives, as amended. In this opinion, these levels are named maximum permitted levels (MPLs).

Glycerol (E 422) is included in the Group I of food additives authorised at QS. Overall, glycerol (E 422) is an authorised food additive in the EU at QS in 68 food categories.

Table 3 summarises foods that are permitted to contain glycerol (E 422) at QS as set by Annex II to Regulation (EC) No 1333/2008.

¹⁴ Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

Table 3: Foods in which glycerol (E 422) is authorised according to the Annex II to Regulation (EC) No 1333/2008

Food Category number	Food category name	E-Number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		QS
01.4	Flavoured fermented milk products including heat-treated products	Group I		QS
01.6.3	Other creams	Group I		QS
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	QS
01.7.5	Processed cheese	Group I		QS
01.7.6	Cheese products (excluding products falling in category 16)	Group I		QS
01.8	Dairy analogues including beverage whiteners	Group I		QS
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		QS
02.3	Vegetable oil pan spray	Group I		QS
03	Edible ices	Group I		QS
04.2.1	Dried fruit and vegetables	Group I		QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		QS
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		QS
04.2.5.4	Nut butters and nut spreads	Group I		QS
04.2.6	Processed potato products	Group I		QS
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	E 422		QS
05.2	Other confectionery including breath refreshing microsweeteners	Group I		QS
05.3	Chewing gum	Group I		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		QS
06.2.2	Starches	Group I		QS
06.3	Breakfast cereals	Group I		QS
06.4.2	Dry pasta	Group I	Only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS
06.4.4	Potato Gnocchi	Group I	Except fresh refrigerated potato gnocchi	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		QS
06.5	Noodles	Group I		QS
06.6	Batters	Group I		QS
06.7	Pre-cooked or processed cereals	Group I		QS
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	QS
07.2	Fine bakery wares	Group I		QS

Food Category number	Food category name	E-Number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
08.3.1	Non-heat-treated meat products	Group I		QS
08.3.2	Heat-treated meat products	Group I	Except <i>foie gras</i> , <i>foie gras entier</i> , <i>blocs de foie gras</i> , <i>Libamáj</i> , <i>libamáj egészben</i> , <i>libamáj tömbben</i>	QS
08.3.3	Casings and coatings and decorations for meat	Group I		QS
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		QS
09.3	Fish roe	Group I	Only processed fish roe	QS
10.2	Processed eggs and egg products	Group I		QS
11.2	Other sugars and syrups	Group I		QS
11.4.1	Table Top Sweeteners in liquid form	E 422		QS
12.1.2	Salt substitutes	Group I		QS
12.2.2	Seasonings and condiments	Group I		QS
12.3	Vinegars	Group I		QS
12.4	Mustard	Group I		QS
12.5	Soups and broth	Group I		QS
12.6	Sauces	Group I		QS
12.7	Salads and savoury based sandwich spreads	Group I		QS
12.8	Yeast and yeast products	Group I		QS
12.9	Protein products excluding products covered in category 1.8	Group I		QS
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group I		QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	QS
14.1.4	Flavoured drinks	Group I		QS
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	QS
14.2.3	Cider and perry	Group I		QS
14.2.4	Fruit wine and made wine	Group I		QS
14.2.5	Mead	Group I		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	QS
14.2.7.1	Aromatised wines	Group I		QS
14.2.7.2	Aromatised wine-based drinks	Group I		QS
14.2.7.3	Aromatised wine-product cocktails	Group I		QS

Food Category number	Food category name	E-Number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		QS
15.2	Processed nuts	Group I		QS
16	Desserts excluding products covered in category 1, 3 and 4	Group I		QS
17.1 ^(a)	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		QS
17.2 ^(a)	Food supplements supplied in a liquid form	Group I		QS
17.3 ^(a)	Food supplements supplied in a syrup-type or chewable form	Group I		QS
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	Group I		QS

MPL: maximum permitted level; QS: *quantum satis*.

(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, Part 1 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a carrier in all food additives at QS.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a food additive other than a carrier in all food additives at QS.

In addition, according to Annex III, Part 3 of Regulation (EC) No 1333/2008, glycerol (E 422) is authorised as a food additive including as a carrier in food enzymes with a maximum level in enzyme preparation and in final food (beverages or not) at QS.

According to Annex III, Part 4 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a food additive including carriers in all food flavourings at QS.

Finally, according to Annex III, Part 5, Section A, of Regulation (EC) No 1333/2008, glycerol (E 422) is authorised as a food additive in all nutrients at QS.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of glycerol (E 422)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS, as is the case for glycerol (E 422) (Table 3).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued public calls^{15,16} for occurrence data (usage level and/or concentration data) on glycerol (E 422). In response to these calls, both types of data were submitted to EFSA by industry and Member States, respectively.

¹⁵ <http://www.efsa.europa.eu/sites/default/files/consultation/ans15062010%2C0.pdf>

¹⁶ <http://www.efsa.europa.eu/sites/default/files/consultation/130327%2C0.pdf>

3.3.1.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels ($n = 91$) of glycerol (E 422) in foods for 20 out of the 68 food categories in which glycerol (E 422) is authorised.

These data were provided by FoodDrinkEurope (FDE), the International Chewing Gum Association (ICGA), the Association of the European Self-Medication Industry (AESPG), Cutisin, Nutricia and Riemser Arzneimittel AG. Data were mainly reported for edible ices (FCS 03).

The Panel noted that the use levels of glycerol (E 422) reported by the food industry were high in some foods ($> 50,000$ mg/kg in dietary supplements, chewing gums and fine bakery wares) and varied largely between food categories: from < 0.01 mg/kg in breakfast cereals to 100,000 mg/kg in chewing gums.

Appendix A provides data on the use levels of glycerol (E 422) in foods as reported by industry.

3.3.1.2. Summarised data on concentration levels in food submitted by Member States

In total, 7,745 analytical results were reported to EFSA by one country, Germany. These data were mainly for wine and other products defined by the Council Regulation (EC) No 1234/2007¹⁷ (FCS 14.2.2). Foods were sampled between 2000 and 2013, and all of them were analysed in the year of collection.

Almost 98% of the analytical results on glycerol (E 422) were quantified. In 72 samples, the results were not quantified ($<$ limit of quantification (LOQ)) and in 89 samples, the results were reported as not detected ($<$ LOD).

Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, but all samples were derived from accredited laboratories.

In order to include only data over the last 10-year period, analytical results sampled before 2004 ($n = 522$) were excluded from further analyses.

In total, 1,133 analytical results reported for glycerol (E 422) in foods were taken into account by the Panel in the exposure assessment considering only direct addition of glycerol (E 422) to food according to Annex II of Regulation (EC) No 1333/2008. These data covered 14 out of 68 food categories in which glycerol (E 422) is authorised as a food additive according to Annex II to Regulation No 1333/2008.

The Panel noted that the remaining 6,090 analytical results were reported in food categories in which glycerol (E 422) is not authorised for direct addition according to Annex II of Regulation (EC) No 1333/2008, including: 'Honey as defined in Directive 2001/110/EC¹⁸' (FCS 11.3) and 'wine and other products defined by Regulation (EC) No 1234/2007 and alcohol-free counterparts' (FCS 14.2.2). It should be noted that glycerol (E 422) was quantified in almost all samples of these two food categories. The authorised use of glycerol (E 422) according to Annex III to Regulation (EC) No 1333/2008 (Parts 1, 2, 3, 4 and 5, Section A) may have resulted in carry-over and its detection in the wine food category. Natural fermentation could also result in the presence of glycerol in wine and other alcoholic beverages (VCF, 1963-2016), as well as in honey (Huidobro et al., 1993). Therefore, the analytical results in these food categories (honey (FCS 11.3), wine (FCS 14.2.2), other alcoholic beverages (FCS 14.2.3, 14.2.4, 14.2.5, 14.2.7.1, 14.2.7.2, 14.2.7.3, 14.2.8)) were not considered in the exposure assessment of glycerol (E 422) as a food additive according to Annex II, but included in the overall estimation of the exposure to glycerol (E 422) according to Annex II, via carry-over (Annex III) and natural sources.

Appendix B shows the analytical results of glycerol (E 422) in foods as reported by Member States.

When both concentration levels, i.e. reported use and analytical levels, were available (only for a few food categories), they were comparable (Appendices A and B).

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 800,000 are or have been available on the EU food market. Mintel started covering

¹⁷ Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation). OJ L 299, 16.11.2007, p. 1–149.

¹⁸ Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ L 10, 12.1.2002, p. 1–6.

EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.¹⁹

For the purpose of this Scientific Opinion, the Mintel GNPD²⁰ was used for checking the labelling of products containing glycerol (E 422) within the European's food products as the Mintel GNPD shows the compulsory ingredient information presented on food product labels.

According to the Mintel GNPD, glycerol (E 422) was labelled on more than 10,000 food products between 2011 and 2016, with an increasing number of food items during the last 3 years.

Appendix C presents the percentage of the food products labelled with glycerol (E 422) between 2011 and 2016, out of the total number of food products per food subcategory according to the Mintel GNPD food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently²¹ added in the Comprehensive database were also taken into account in this assessment.¹⁰

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 4).

Table 4: Population groups considered for the exposure estimates of glycerol (E 422)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 month of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 month up to and including 35 month of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK
Children ^(a)	From 36 month up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly ^(a)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

¹⁹ Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.

²⁰ <http://www.gnpd.com/sinatra/home/> accessed on 26/10/2016.

²¹ Available online: <http://www.efsa.europa.eu/en/press/news/150428.htm>

Consumption records in the EFSA Comprehensive Database were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

3.3.3.2. Food categories considered for the exposure assessment of glycerol (E 422)

The food categories in which the use of glycerol (E 422) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for 10 food categories (Appendix D) and may have resulted in an underestimation of the exposure. The food categories which were not taken into account for this reason are described below (in ascending order of the FCS codes):

- 01.6.3 Other creams
- 02.3 Vegetable oil pan spray
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4
- 06.6 Batters
- 06.7 Pre-cooked or processed cereals
- 08.3.3 Casings and coatings and decorations for meat
- 12.1.2 Salt substitutes
- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars
- 14.2.4 Fruit wine and made wine
- 14.2.5 Mead

For the following food categories, the restrictions/exceptions which apply to the use of glycerol (E 422) could not be taken into account, and therefore, the whole food category was considered in the exposure assessment. This applies to 2 food categories and may have resulted in an overestimation of the exposure.

- 07.1 Bread and rolls, except products in 07.1.1 and 07.1.2
- 08.3.2 Heat-treated meat products, except *foie gras*, *foie gras entier*, *blocs de foie gras*, *Libamáj*, *libamáj egészben*, *libamáj tömbben*

In the EFSA Comprehensive database, no information is provided on the type of food supplements consumed by infants and young children. In the exposure assessment, even if this food category refers in the EU regulation to foods supplements excluding infants and young children, it was in this opinion therefore assumed that the food supplements consumed in these population groups were the same as those consumed in the older population groups for which concentration data were supplied, resulting in an overestimation of the exposure to glycerol (E 422) in these two population groups.

Furthermore, 30 food categories were not taken into account because no concentration data (reported use levels or analytical data) were provided to EFSA. For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied.

Overall, 28 food categories out of 68 were included in the present exposure assessment to glycerol (E 422) (Appendix D).

3.4. Exposure estimate

3.4.1. Exposure to glycerol (E 422) from its use as a food additive

The Panel estimated chronic exposure to glycerol (E 422) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to glycerol (E 422) was calculated by multiplying glycerol (E 422) concentrations for each food category (Appendix D) with their respective consumption amount per kilogram body weight for each individual in the EFSA Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with

only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The exposure was estimated for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 4). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not estimated.

It should be noted that, in two dietary surveys from Finland, namely DIPP_2001_2009 and NWSSP07-08, the consumption of grain-based products including bread and fine bakery products was coded at the level of their ingredients (flour), which resulted in a very low exposure to glycerol in all Finnish populations compared with the other studies. Therefore, these two studies were excluded from the assessment.

Exposure assessment to glycerol (E 422) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) the reported use levels or analytical data (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below. Furthermore, considering that

- glycerol (E 422) is authorised in alcoholic beverages (several categories under FCS 14.2) but no use data were submitted by industry for this food category, and
- the presence of glycerol in alcoholic beverages can also be due to natural fermentation and carry-over,

the analytical data of glycerol in alcoholic beverages submitted to EFSA were considered as resulting from natural fermentation and carry-over and not from usage by food industry. The presence of glycerol in honey was considered to be solely derived from fermentation. Therefore, for glycerol (E 422), dietary exposure according to the *refined exposure assessment scenario* was assessed using two sets of concentration data:

- 1) Reported use levels and analytical data considering food categories for which direct addition of glycerol (E 422) is authorised according to Annex II to Regulation No 1333/2008, excluding alcoholic beverages (FCS 14.2) (data set 1).
- 2) Reported use levels and analytical data considering food categories for which direct addition of glycerol (E 422) is authorised according to Annex II to Regulation No 1333/2008, and analytical data for food categories which may contain glycerol due to carry-over (Annex III) and from natural sources (i.e. honey, wines and other alcoholic beverages) for which data were available (data set 2). Results using this data set are presented in Appendices F and G.

The exposure scenarios can consider only food categories for which concentration data were available to the Panel.

3.4.1.1. Maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As glycerol (E 422) is authorised according to QS in all food categories, a *maximum level exposure assessment scenario* was performed based on the maximum reported use levels provided by industry or high level of analytical data provided by MSs, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014), whichever was highest or available. The same food categories as included in data set 1 were considered for this scenario.

The Panel considers the exposure estimates derived following this scenario as the most conservative for the food categories taken into account, since it is assumed that the consumer will be continuously (over a long period of time) exposed to glycerol (E 422) in foods belonging to these food categories at maximum reported use levels/high level of analytical data.

3.4.1.2. Refined exposure assessment scenario

The *refined exposure assessment scenario* is based on use levels reported by industry and analytical results reported by Member States. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix D summarises the concentration levels of glycerol (E 422) used in the refined exposure assessment scenario. For the two concentration data sets, the Panel calculated two refined exposure

estimates based on different model populations. In these two refined exposure assessment scenarios, the concentration levels considered were extracted from the whole data available to EFSA (i.e. reported use levels and analytical results):

- The *brand-loyal consumer scenario*: It was assumed that a consumer is exposed long-term to glycerol (E 422) present at the maximum reported use/analytical level for one food category. This exposure estimate is calculated as follows:
 - Combining food consumption with the maximum of the reported use levels or the 95th percentile of the analytical results, whichever was highest or available, for the main contributing food category at the individual level
 - Using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for the remaining food categories
- The *non-brand-loyal consumer scenario*: It was assumed that a consumer is exposed long-term to glycerol (E 422) present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for all food categories.

If both usage and analytical data were available for the same food category, the most reliable value under consideration was used. For glycerol (E 422), the Panel considered due to a low sample size of the analytical data, that the reported use levels were more reliable. However, the Panel also noted that the levels were comparable for both sources in the respective food categories.

To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010) was used. In the present opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (medium bound (MB)). Subsequently, per food category, the mean or median, whichever was highest, MB concentration was calculated.

3.4.1.3. Dietary exposure to glycerol (E 422)

Table 5 summarises the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008 in six population groups (Table 4) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix E.

Table 5: Summary of exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks– 11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	10–138	158–583	239–480	128–326	68–185	76–138
95th percentile	269–457	484–938	489–910	283–629	161–404	168–268
Refined estimated exposure assessment scenario (data set 1)						
Brand-loyal scenario						
Mean	9–126	138–453	199–369	101–248	57–144	65–119
95th percentile	223–435	400–796	420–786	217–500	136–320	136–265
Non-brand-loyal scenario						
Mean	6–100	89–308	112–240	74–162	50–100	53–77
95th percentile	153–266	261–418	222–460	149–298	110–197	98–147

In the *maximum level exposure assessment scenario*, mean exposure to glycerol (E 422) ranged from 10 mg/kg bw per day in infants to 583 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 161 mg/kg bw per day in adults to 938 mg/kg bw per day in toddlers.

In the *refined brand-loyal exposure scenario* considering data set 1, the mean exposure to glycerol (E 422) ranged from 9 mg/kg bw per day in infants to 453 mg/kg bw per day in toddlers, and the 95th percentile from 136 mg/kg bw per day in adults and the elderly to 796 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, the mean exposure to glycerol (E 422) ranged from 6 mg/kg bw per day in infants to 308 mg/kg bw per day in toddlers, and the 95th percentile from 98 mg/kg bw per day in the elderly to 460 mg/kg bw per day in children.

The Panel noted that the exposure to glycerol (E 422) using data set 2 (Appendix F), both *brand-loyal* and *non-brand-loyal scenarios*, did not significantly change the exposure to glycerol (E 422) using data set 1 (Table 5).

The Panel also noted that only one, rather high, use level (30,000 mg/kg) was reported by industry for food category 07.1 'bread and rolls' (Appendix A) (no analytical data were reported for this food category). This level was for a niche product ('chapattis'), i.e. a not commonly eaten food item. This use level was applied to the whole food category, which will result in a significant increase in the exposure estimates (between 1.7 and up to 19 times more depending on the population groups at the mean for the *non-brand-loyal scenario*), especially since the consumption of food category 07.1 'bread and rolls' is fairly high in many surveys.

3.4.1.4. Main food categories contributing to exposure to glycerol (E 422)

Maximum level exposure assessment scenario (Table 6)

Table 6: Main food categories contributing to the exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) ^(a)					
03	Edible ices	–	14.7–14.7 (1)	5.6–13.2 (3)	5.1–5.1 (1)	–	–
05.2	Other confectionery including breath refreshing microsweets	–	–	34.6–34.6 (1)	5.9–39.6 (2)	8.3–8.3 (1)	–
07.1	Bread and rolls	27.7–85.1 (5)	9.4–68.7 (10)	17.7–51 (17)	22–44.8 (16)	27.1–64.1 (17)	31–74 (14)
07.2	Fine bakery wares	7.9–46.4 (4)	7.1–50.2 (10)	5.1–58.4 (17)	19.8–49.1 (15)	6.5–39.5 (17)	9.3–44 (14)
08.3	Meat products	7.2–7.2 (1)	–	–	–	–	–
12.6	Sauces	5.2–31.4 (4)	6.5–14.3 (7)	5.4–14 (12)	7.4–14.4 (10)	9–17.5 (10)	6.5–16.5 (10)
14.1.4	Flavoured drinks	10.2–26.9 (2)	7.1–35.7 (8)	5.7–36.4 (18)	11.8–38.3 (17)	6.3–30.6 (17)	6.5–15.6 (8)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	38.5–73.8 (2)	28.7–28.7 (1)	10.2–10.2 (1)	–	5.6–8.2 (3)	8–9.8 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Refined exposure assessment scenario using data set 1 (Tables 7 and 8)

Table 7: Main food categories contributing to the exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) using *the brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) ^(a)					
03	Edible ices	–	14.0 (1)	8.8 (1)	–	–	–
05.2	Other confectionery including breath refreshing microsweets	–	–	41.2 (1)	45.9 (1)	8.2 (1)	–
07.1	Bread and rolls	30.4–93.0 (5)	11.0–84.7 (10)	22.6–66.7 (17)	29.1–55.7 (16)	33.9–73.9 (17)	37.3–86.4 (14)
07.2	Fine bakery wares	19.4–42.8 (3)	14.1–51.5 (9)	16.9–62.2 (16)	14.8–53.6 (15)	5.5–39.2 (16)	10.7–43.4 (12)
08.3	Meat products	7.7 (1)	5.6 (1)	–	–	–	–
12.6	Sauces	14.4–30.9 (3)	5.9–11.7 (3)	5.0–11.6 (7)	5.2–10.6 (9)	6.8–14.9 (9)	5.8–13.2 (7)
14.1.4	Flavoured drinks	8.4–29.4 (2)	6.5–40.3 (7)	7.8–40.8 (16)	8.6–43.2 (17)	5.1–32.7 (16)	6.5–10.5 (7)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	36.2–76.1 (2)	27.6 (1)	9.2 (1)	–	5.3–10.1 (3)	6.0–9.7 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Table 8: Main food categories contributing to exposure to glycerol (E 422) from its use as food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2)) (data set 1) using *the non-brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) ^(a)					
05.2	Other confectionery including breath refreshing microsweets	–	–	36.8 (1)	41.1 (1)	5.9 (1)	–
07.1	Bread and rolls	47.9–94.7 (5)	23.3–86.3 (10)	6.3–74.9 (18)	45.6–74.6 (16)	51.3–83.6 (17)	60.2–89.6 (14)
07.2	Fine bakery wares	8.7–16.7 (3)	8.9–20.3 (9)	8.4–29.9 (16)	7.5–23.8 (15)	8.5–15.8 (14)	7–18.4 (11)
08.3	Meat products	16.9 (1)	12.0 (1)	5.0 (1)	–	–	–
12.6	Sauces	13.5–22.5 (3)	6.1–10.3 (5)	5.0–15.0 (10)	6.6–10.6 (10)	6.6–13.7 (10)	5.4–13.2 (9)
14.1.4	Flavoured drinks	6.6–17.6 (2)	5.4–26.6 (7)	5.7–28.9 (17)	7.8–33.5 (17)	6.4–22.1 (15)	5.7–8 (7)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	26.0–65.2 (2)	27.8 (1)	10.3 (1)	–	6.1 (1)	5.2–6.5 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

3.4.1.5. Uncertainty analysis

Uncertainties in the exposure assessment of glycerol (E 422) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the sources of uncertainties have been summarised and evaluated in Table 9.

Table 9: Qualitative evaluation of influence of uncertainties on the dietary exposure estimates of glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 (using data set 1)

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database:	
Uncertainties to which foods the levels refer to	+/-
Levels considered applicable to all foods within the food category	+/-
Food categories included in the maximum level exposure and refined exposure assessment scenarios:	
Exclusion of food categories due to missing FoodEx linkage (n = 10)	-
Concentration data not available for 30 food categories	-
Restrictions/exceptions not taken into account (e.g. bread and rolls)	+
Food supplements consumed by infants and young children	+
Reported use levels:	
Data not available from industry for 48 food categories	-
Use level of a niche product used for the whole food category bread and rolls	+/-
Analytical data:	
Not representative of foods on the EU market (coming from only one MS)	+/-
Food categories in which the presence of glycerol comes from natural sources	+
Treatment of left-censored data: use of MB scenario	+/-
Maximum level exposure assessment scenario:	
Food categories which may contain glycerol (E 422) due to carry-over not considered	-
Exposure calculations based on the maximum use levels (reported use from industries or analytical data)	+
Uncertainty on the actual use of glycerol (E 422) in food categories authorised for the Group I food additives	+
Refined exposure assessment scenarios:	
Food categories which may contain glycerol (E 422) due to carry-over was only considered for wine	-
Exposure calculations based on the maximum or mean levels (reported use from industries or analytical data)	+/-
Uncertainty on the actual use of glycerol (E 422) in food categories authorised for the Group I food additives	+
Uncertainty in possible national differences in use levels of food categories	+/-
Uncertainty in possible fermentation in other food products (fruit juice)	-

MB: middle bound.

(a):+, uncertainty with potential to cause overestimation of exposure; -, uncertainty with potential to cause underestimation of exposure.

Glycerol (E 422) is authorised as a Group I food additive in 66 food categories and has a specific authorised use in two food categories (Table 3). Since the majority of food categories correspond to the general Group I food additives authorisation, glycerol may not necessarily be used in some of these food categories. This may explain why reported use levels and analytical data of glycerol (E 422) were only available for 28 food categories, including one food category for which glycerol (E 422) has a specific use. The Panel calculated, based on the information in the Mintel GNPD, that out of the

foods authorised to contain glycerol (E 422) according to Annex II to Regulation (EC) No 1333/2008, 18–50% of the amount of food consumed per population group was reported to potentially contain glycerol (E 422) as a food additive. Based on this, the Panel noted that the information from the Mintel GNPD supported the observation that due to its Group I authorisation, glycerol may be used in less food categories than it is authorised.

Furthermore, the Panel noted that information from the Mintel GNPD (Appendix C) indicated that 54 out of 76 food subcategories, categorised according to the Mintel GNPD nomenclature, in which glycerol (E 422) was labelled were included in the current exposure assessment. These 54 food subcategories represented approximately 94% of the food products labelled with glycerol (E 422) in the database. In the remaining 22 food subcategories, in which glycerol (E 422) was labelled but which were not included in the exposure assessment, glycerol (E 422) was authorised in 18 food subcategories.

Given these observations, the Panel considered overall that the uncertainties identified would, in general, result in an overestimation of the exposure to glycerol (E 422) from its use as a food additive according to Annex II in both the maximum level and refined exposure scenario.

The Panel noted that food categories which may contain glycerol (E 422) due to carry-over (Annex III, Part 1, 2, 3, 4 and 5) were only partly considered (via wine and other alcoholic beverages) in the current exposure assessment using data set 2, and that the exposure to glycerol via natural sources included only wine, other alcoholic beverages and honey. Taking these sources also into consideration did, however, not significantly change the exposure to glycerol (E 422) (Appendix F).

3.4.2. Exposure to 3-MCPD from the use of glycerol (E 422) as a food additive

According to the EU specifications for glycerol (E 422), 3-MCPD may be present at its maximum level of 0.1 mg/kg glycerol. On this basis, the Panel calculated the exposure to 3-MCPD from the use of glycerol (E 422) as a food additive in six population groups according to the different exposure scenarios (Section 3.4.1) (Table 10).

Table 10: Summary of exposure to 3-MCPD from the use of glycerol (E 422) as a food additive in the *maximum level exposure assessment scenario* and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in ng/kg bw per day)

	Infants (12 weeks– 11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	1–14	16–58	24–48	13–33	7–19	8–14
95th percentile	27–46	48–94	49–91	28–63	16–41	17–27
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	1–13	14–45	20–37	10–25	6–14	6–12
95th percentile	22–44	40–80	42–79	22–50	14–32	14–26
Non-brand-loyal scenario						
Mean	0.6–10	9–31	11–24	7–16	5–10	5–8
95th percentile	15–27	26–42	22–46	15–30	11–20	10–15

In the *maximum level exposure assessment scenario*, the mean exposure to 3-MCPD ranged from 1 ng/kg bw per day in infants to 58 ng/kg bw per day in toddlers. The 95th percentile of exposure ranged from 16 ng/kg bw per day in adults to 94 ng/kg bw per day in toddlers. In the *refined brand-loyal exposure scenario*, mean exposure to 3-MCPD ranged from 1 ng/kg bw per day in infants to 45 ng/kg bw per day in toddlers, and the 95th percentile from 14 ng/kg bw per day in adults and the elderly to 80 ng/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to 3-MCPD ranged from 0.6 ng/kg bw per day in infants to 31 ng/kg bw per day in toddlers, and the 95th percentile from 10 ng/kg bw per day in the elderly to 46 ng/kg bw per day in children.

The Panel could not calculate exposure to any genotoxic impurities or other contaminants that may be present in glycerol (E 422), e.g. glycidol, due to the lack of data on their concentrations in the food additive.

3.4.3. Estimated acute exposure to glycerol (E 422)

The Panel noted that during therapeutic use of glycerol, some effects (nausea, headache and/or vomiting) were reported. Using a conservative approach (see Section 3.5.7), the Panel calculated that these effects may be observed after oral intake of a bolus dose of 125 mg glycerol/kg bw per hour.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use is consumption of a beverage.

Therefore, the Panel calculated the volume of flavoured drinks that is required to be consumed in order to exceed the acute bolus exposure at which effects were reported during therapeutic use.

The calculation was done using either the highest value (12,700 mg/L) or the mean (rounded up to 5,000 mg/L) of the reported analytical data for glycerol in flavoured drinks and the mean body weight of each population, and considered the 95th percentile (Table 11).

Table 11: Volume (mL) required to be consumed to exceed the acute bolus exposure of 125 mg/kg bw per hour

Concentration of glycerol (E 422) in flavoured drinks (mg/L)	Volume (mL)				
	Infants	Toddlers	Children	Adolescents	Adults
12,700	70	130	230	500	700
5,000	175	300	600	1,300	1,800

From the results on Table 11, the Panel noted that for infants and toddlers, consumption of less than the volume of a can (330 mL) is sufficient to exceed the dose of 125 mg glycerol/kg bw per hour.

However, the Panel is aware that the approach used to assess the acute exposure has limitations. Thus, the Panel noted that the uncertainty about the exposure calculation is extremely high as:

- the calculation was done based on only 5 analytical data;
- there was a wide variation among the 5 values available (2 < LOD and the 3 others being 3,200, 7,700 and 12,700 mg/L).

In addition, acute dietary exposure to glycerol (E 422) per meal was estimated as the exposure via one food category (food categories as defined in legislation) consumed in one meal (acute exposure per food category). Calculations were based on food consumption data (EFSA Comprehensive Database) and using data set 1 (Section 3.4.1). Acute exposure per food category was estimated for each individual by multiplying the consumed amount of each food category per meal by its high occurrence level. Appendix H lists the mean and 95th percentile of the acute exposure per food category, meal and population group.

The Panel is aware that the approach used to assess the acute exposure has its limitations. Acute exposure is any possible exposure to a compound during a single meal or day. This exposure can be high (e.g. if someone consumes much of a food or the food contains a high level), but can also be lower (e.g. if the consumer consumes a food with a low occurrence level). The probability of being exposed to high levels depends on the amounts consumed and the chance that a consumer selects a food with a high occurrence level (EFSA PPR Panel, 2012). In the acute exposure calculations performed for glycerol (E 422), the Panel assumed that the probability of selecting a food with high levels of glycerol was equal to 1 for one food category, ignoring the presence of concentrations lower than this high value. This was done due to the limited occurrence data available for the exposure assessment and limitations in the models available to assess acute exposure. It is therefore estimated that acute exposure estimates reported here are likely overestimates of the exposure. The acute exposure estimates per food category should therefore be interpreted as upper 'worst case' acute exposure estimates via these food categories.

The acute exposure assessment was furthermore hampered by the same limitations regarding the analytical data (only provided by one country) and usage levels (very limited, except for edible ice) as the chronic exposure assessment. Considering that concentration data were not made available for all

authorised food categories, these categories could also not be considered in the acute exposure assessment.

3.4.4. Exposure via the regular diet

The Panel noted that glycerol is liberated from normal lipid constituents (e.g. triglycerides) but considered that this source of exposure to glycerol is not relevant to the exposure assessment since the glycerol is re-esterified at or soon after absorption (EFSA NDA Panel, 2010), and does not become systemically available as glycerol.

3.4.5. Exposure via other sources

Exposure to glycerol due to the following uses was not considered in this opinion.

3.4.5.1. Glycerol as an ingredient in food for sports people

The use of glycerol in beverages for athletes to enhance and maintain hydration status and to improve endurance exercise performance is described (Van Rosendal et al., 2010; Savoie et al., 2016). Glycerol when used as a component of a hyperhydration strategy is prohibited by the World Anti-Doping Agency (WADA) (Martindale, online; Savoie et al., 2016; WADA, 2016).

3.4.5.2. Pharmaceutical use

Glycerol is used in pharmaceutical products as an active ingredient as well as an excipient, e.g. solvent, plasticiser or lubricant (EMA, 2003; Martindale, online). When glycerol is used as an excipient in medicinal products for oral administration and the glycerol intake is equal or above 10 g per single dose of the medicinal product, the information 'May cause headache, stomach upset and diarrhea' is required for the package leaflet (EMA, 2003). As an active ingredient, glycerol is used as an osmotic dehydrating agent. It is given orally, e.g. for the reduction in intra-ocular pressure before and after ophthalmic surgery and as an adjunct in the management of acute glaucoma or to reduce intracranial pressure (Martindale, online) (see Section 3.5.7).

3.5. Biological and Toxicological studies

The Panel noted that not all the studies performed with glycerol have used glycerol concurring with the EU specifications for the food additive E 422.

3.5.1. Absorption, distribution, metabolism and excretion

3.5.1.1. Absorption

In animals

Höber and Höber (1937) examined the absorption of unlabelled glycerol from the ligated intestine in anaesthetised rats (no further details provided). After washing, the intestinal lumen was filled with 4 mL 100 mM glycerol solution. After an exposure period of 25 min, the lumen was emptied by rinsing with Ringer's solution and residual glycerol in the washing fluid was determined analytically. The absorption varied between 70 and 89%. Similar experiments with ligated rat stomach (Herting et al., 1956) revealed lower absorption (10–35%) after an exposure period of 6 h.

Rapid absorption of radiolabelled glycerol in rats after oral administration was demonstrated by Gidez and Karnovsky (1954). Experiments with ^{14}C -labelled glycerol were performed in 24 h fasted male Wistar rats (number of rats employed was not reported). Rats were administered 60 or 112 mg/rat by either gavage or intravenous (i.v.) injection and exhaled $^{14}\text{CO}_2$ was measured every 15 min for 60 min and then every 20–30 min for up to 240 min. No significant differences were observed between the two routes of administration in terms of exhaled $^{14}\text{CO}_2$. Peak levels of $^{14}\text{CO}_2$ were reached 50–80 min after administration, suggesting rapid absorption and metabolism after oral administration.

The mean endogenous glycerol level in whole blood of untreated rats ($n = 200$) was reported to be 0.254 mM (23 mg/L) (Ackerman et al., 1975).

Kato et al. (2005) examined the absorption of glycerol using a closed loop of the rat small intestine *in situ*. The absorption of glycerol was saturable and was inhibited by Na^+ -free conditions, suggesting the involvement of a Na^+ -dependent carrier-mediated transport system. Similar results were presented by Yuasa et al. (2003).

In humans

In a clinical study performed by McCurdy et al. (1966; see also section 3.2.7), five volunteers (no further details) consumed a 50% solution of unlabelled glycerol in water at a dose level of 1,000–1,270 mg/kg bw after overnight dehydration and administration of pitressin tannate (to further inhibit diuresis). The mean pretreatment glycerol concentration in serum was 0.51 mM (47 mg/L). The serum glycerol levels were measured every 20 min after ingestion for a total of 140 min. Twenty minutes after ingestion, the mean serum concentration increased to 7.7 mM (710 mg/L) and reached a maximum of 16.2 mM (1,490 mg/L) 80 min after the treatment. Thereafter, the serum level declined to 13.3 mM at 140 min. Glycerol appeared in urine and the amount accounted for 7.5–13.9% of the ingested dose (urine collected for 2.5 h after ingestion, from 6 volunteers). Although no conclusions can be drawn on the total absorption, the authors of this study stated that the data indicated rapid absorption of glycerol after ingestion.

Pelkonen et al. (1967) determined the glycerol serum level in 10 volunteers (pretreatment level 0.1 mM or 9.2 mg/L) every 15 min for 60 min after oral treatment with 70 mg glycerol/kg bw (as 5% aqueous solution of unlabelled glycerol). The maximum serum concentration of glycerol occurred 15 min after ingestion, 0.4 mM (37 mg/L). The serum level declined thereafter to pretreatment levels by 1 h.

3.5.1.2. Distribution

In a study in rats, it was shown that there was a net synthesis of blood glucose and liver glycogen as a result of ^{14}C -glycerol administration (88–89 mg/rat; intraperitoneal (i.p.)). Fifteen minutes after administration, 70% of newly formed blood glucose was derived directly from glycerol and 30 min after treatment this value increased to 100%. After 30 minutes, 39% of the newly formed liver glycogen was derived from the administered ^{14}C -glycerol. This value decreased during the following 1–6 h after application and fell to 15% at 6 h (Gidez and Karnovsky, 1954).

In further experiments, the incorporation of ^{14}C -glycerol into liver lipids was also reported (Gidez and Karnovsky, 1954). At a dose of 1 mM ^{14}C -labelled glycerol (92 mg/rats; i.p.), most radioactivity was found in liver lipids (5.9% of applied dose) 6 h after application with only minor amounts found in other organs (e.g. 0.26% in the brain and 0.16% in the kidney).

3.5.1.3. Metabolism

Glycerol occurs naturally in fats (e.g. triglycerides, phospholipids) and is also an endogenous metabolite (Tao et al., 1983). After ingestion of triglyceride, hydrolysis in the gastrointestinal tract yields glycerol and fatty acids which are then absorbed by the mucosa. Glycerol is also released by adipose tissue lipolysis; the catabolic process leading to the breakdown of triglycerides stored in fat cells. Glycerol may then be metabolised through a number of pathways (Lin, 1977; Brisson et al., 2001).

Glycerol may be phosphorylated by glycerol kinase to glycerol phosphate followed by oxidation to dihydroxyacetone phosphate via the enzyme glycerol phosphate dehydrogenase (Tao et al., 1983; Brisson et al., 2001). Dihydroxyacetone phosphate can participate in glycolysis and be further oxidised to carbon dioxide (oxidised as an energy substrate) or gluconeogenesis after formation of the intermediate glyceraldehyde 3-phosphate (Tao et al., 1983; Brisson et al., 2001). Exogenous glycerol also participates in lipogenesis (e.g. formation of triglycerides). When rats were i.p. injected with radiolabelled glycerol, radioactivity was found in lipids (Gidez and Karnovsky, 1954). With respect to glycerol utilisation, the liver accounts for up to at least 75% of the total body glycerol-utilising capacity and the kidney accounts for up to 25% (Lin, 1977; Tao et al., 1983). At physiological serum concentrations up to 1 mM glycerol (92 mg/L), uptake into hepatocytes follows first order kinetics (Tao et al., 1983).

Gidez and Karnovsky (1954) examined the relationship between the amount of glycerol oxidised compared to the amount administered. At dose levels above 30 mg/rat (about 150 mg/kg bw), an approximately constant proportion of the administered glycerol appeared to be oxidised and exhaled as $^{14}\text{CO}_2$. At lower concentrations, the proportion converted to CO_2 was higher.

3.5.1.4. Excretion

Rats

In male Sprague–Dawley rats ($n = 4$ per group) most of the absorbed glycerol was metabolised and exhaled as CO_2 (Michael and Coots, 1971). Within 51 h after gavage of 14–20 mg/kg bw ^{14}C -labelled glycerol, 73.3% of recovered radioactivity was exhaled as $^{14}\text{CO}_2$, 5.2% excreted in urine and 2% in faeces or gastrointestinal contents and 19.5% in the carcass.

At glycerol concentrations in the blood up to 1 mM, glycerol excretion in the urine was reported to be undetectable. At higher glycerol levels, a renal excretion of glycerol occurs (Ackerman et al., 1975; Lin, 1977). In male SIV rats, higher glycerol levels in blood were induced by i.v. infusion (Ackerman et al. (1975). Excretion of glycerol via the urine occurred but the interindividual variance of this effect was substantial. At a steady state blood concentration of 1.9 mM (infusion of glycerol at a rate of 500 mg/kg bw per hour; $n = 20$), an average and standard error of the mean of $3.7 \pm 3.5\%$, respectively, of the applied glycerol was eliminated in urine. The infusion of 700 mg/kg bw per hour ($n = 4$) resulted in a range of steady state blood levels between 2 and 40 mM, and the range of renal excretion of glycerol varied between 3.5% and 23% without a clear relationship to the blood level.

Humans

The excretion of glycerol via urine was reported to occur in humans at high dose levels (McCurdy et al., 1966). In six volunteers (no further details), glycerol appeared in urine and accounted for 7.5–13.9% of applied dose (urine collected for 2.5 h after ingestion) after ingestion of 1,000–1,270 mg/kg bw unlabelled glycerol (no further data).

The disappearance of serum glycerol in humans was analysed in individuals that were injected i.v. with 2,400 mg unlabelled glycerol over a period of 2 min (Pelkonen et al., 1967). The serum levels of glycerol were determined over the subsequent 75–90 min. The removal rates of glycerol in four volunteers varied between 1.35 and 2.21 $\mu\text{mol/kg bw per minute}$ (mean 1.7 $\mu\text{mol/kg bw per minute}$). The half-life (at equilibration; $t_0 = 10$ min after injection) was 10.7–12.3 min (mean 11.7 min). A twofold increase in mean half-life and removal rate were measured in similar trials with 8 diabetics (Pelkonen et al., 1967).

It was shown in volunteers (9 lean and 13 obese subjects, no data about sex and age) that the removal of glycerol from the human blood after infusion varied in proportion to the glycerol plasma concentrations (Bortz et al., 1972). A basal turnover rate of approximately 100 $\mu\text{mol/minute}$ was reported in adult volunteers. At plasma concentrations up to 0.45 mM (41.4 mg/L), the turnover rate increased to 700 $\mu\text{mol/minute}$ in obese subjects, and to a maximum of 400 $\mu\text{mol/minute}$ in lean subjects (0.22 mM glycerol level in plasma). In an intravenous glycerol tolerance test, the turnover rate was calculated as 1.5 $\mu\text{mol/kg bw per minute}$.

Overall, the Panel considered that glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract; distributed into the total body water space and is primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as CO_2 , with only minor amounts excreted via urine or faeces. The serum concentration of free glycerol in humans has been reported to be normally between 0.46 and 18.5 mg/L (Lin, 1977; Tao et al., 1983). The Panel noted that at glycerol concentrations in blood up to 92 mg/L, no glycerol was detected in the urine but higher levels result in renal elimination of glycerol in rats (Ackerman et al., 1975) and humans (McCurdy et al., 1966).

3.5.2. Acute toxicity

The oral median lethal dose (LD_{50}) values for glycerol were reported to be 12,600–28,800 mg/kg bw (rat); 15,000–38,000 mg/kg bw (mouse); 7,750–11,500 mg/kg bw (guinea pig) and 17,600–27,000 mg/kg bw in the rabbit (BIBRA, 1993 [Documentation provided to EFSA n. 2]).

Diuresis was reported to occur in dogs 15 min after gavage of 2,500 g/kg bw (Johnson et al., 1933) and in rats after 'toxic dose levels' (no further details; Suzuki et al., 1977).

No irritation was apparently seen when undiluted glycerol was applied to the lining of the oral cavities of rats, rabbits and dogs (Informatics Inc, 1973, as referred to by BIBRA, 1993 [Documentation provided to EFSA n. 2]).

The Panel considered that glycerol has a low acute toxicity.

3.5.3. Short-term and subchronic toxicity

3.5.3.1. Rats

In a study by Staples et al. (1967), female rats (10 animals/group; strain not reported) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol by gavage (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day), control animals received 3 mL water by

gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 0.75 mL/kg bw (2,800 mg/kg bw per day) and higher, the authors reported dose-dependent local irritant effects including slight to severe hyperaemia, petechial haemorrhages and erosions but no systemic toxicity. Administration of 3,800 mg glycerol/kg bw (thrice daily) as either a 20, 40, 60, 80 or 100% aqueous solution (dose volume varied between 3 (for 100%) and 15 mL (for 20%)) resulted in local irritant effects at all dilutions but with severity reduced by dilution such that 1 animal in 10 showed slight effects in the 20% group.

Thirty adult male Wistar rats (6 animals/group) were treated by gavage with 0 (control/saline), 200, 400, 800 and 1,600 mg glycerol/kg bw per day (bidistilled glycerine, 99.85% glycerol) for 28 days (Lisenko et al., 2015). Glycerol did not induce any statistically significant change in average daily weight gain or water consumption or excretion levels. Glycerol supplementation did induce a significant decrease in the average daily feed intake levels. Supplementation with glycerol did not affect plasma triglyceride, glucose, total cholesterol, lipoprotein concentrations, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Plasma glucose and total cholesterol levels demonstrated a significant increase in values up to a dose of 800 mg/kg, followed by a decrease at 1,600 mg/kg bw per day. In terms of high-density lipoprotein levels, there was an increase up to a dose of 800 mg/kg bw per day, followed by a decrease at 1,600 mg/kg bw per day, but levels were still within the normal range for the species. For very low-density lipoprotein and low-density lipoprotein levels, there was a decrease with increasing glycerol doses, which remained within the standards. The liver enzymes AST and ALT showed a decrease trend up to the dose of 800 mg/kg bw per day, with an increase at 1,600 mg/kg bw per day. Glycerol supplementation did not result in any change in relative organ weights. Leucocyte levels differed between the groups, although all were within the normal range. The differential count for lymphocytes and neutrophils did not change between the experimental groups. No lesions were observed in any tissue (pancreas, kidney, liver) from animals receiving different levels of glycerol.

In a limited study by Fischer et al. (1949), male rats (20 animals/group; strain not reported) were treated for 44 days with 0, 100, 500, 1,000 or 2,000 mg glycerol/kg bw per day by gavage. The authors reported no clinically adverse signs at any dose; no effects on mortality or body weight gain. For the haematological parameters examined, there were no effects reported on haemoglobin, erythrocyte, leucocyte and lymphocyte counts and differential blood counts. Histopathological examination was restricted to liver, kidney and urinary bladder, and no effects were observed.

Adult male Wistar rats were subjected to 6 weeks of aerobic training (after an acclimatisation to swimming, the animals swam for 60 min daily, at a frequency of 5 times per week, with relative overload of 5% of their body mass) (Andrade et al., 2014). In the last 4 weeks, the animals were treated with saline, glucose or glycerol (800 mg/kg bw per day) by gavage (it is not stated how frequently animals were dosed). Treatment with glycerol did not induce statistically significant differences in body mass, water consumption, urinary production or relative weight of liver or kidney compared to negative control. Glycerol supplementation did induce a significant reduction in food consumption compared to negative control. In terms of blood biochemical parameters, no difference was observed in glycaemic level or lipoprotein concentrations in plasma compared to negative control. However, a statistically significant increase in both plasma triacylglyceride and total cholesterol levels was observed after supplementation with glycerol. Statistically significant increases in adipocyte diameter and area, as well as villous:crypt ratios of the duodenum and ileum were observed following glycerol supplementation. No differences were observed in adipocyte density or the jejunum villous: crypt ratio in glycerol-supplemented animals compared to negative control.

In a study by Anderson et al. (1950), female rats (5 animals/group; strain not reported) were treated for 26 weeks with 0 or 5% glycerol by volume in the drinking water (either derived from natural sources or synthetically synthesised), equivalent to 0 and 4,500 mg glycerol/kg bw per day). Haematology was performed monthly and was composed of erythrocyte and leucocyte counts, haemoglobin and differential blood counts. At 26 weeks, necropsy and histopathology of heart, lungs, liver, spleen, stomach, intestines, kidneys, thymus, thyroid and adrenals was performed, and no effects were observed by the authors except mineralisation in renal tubules. Glycerol treatment had no effects on haematological parameters. The Panel considered mineralisation in renal tubules to be due to the high dose of glycerol used in the study.

In a 3-month study by Löser et al. (1954), male and female rats (strain unspecified, 6 animals/group) were treated with glycerol as a 0, 1%, 2%, 5%, 10% or 20% solution in drinking water (equivalent to 0, 800, 1,600, 4,000, 8,100 and 16,200 mg/kg bw per day and 0, 900, 1,800, 4,500, 9,100 and 18,200 mg/kg bw per day for males and female, respectively). No effects were noted in the

animals receiving 1%, 2%, 55 or 10% glycerol. In the group receiving 20% glycerol, 2 animals out of 12 animals died. The remaining 18 exhibited some retardation in weight gain and development. However, they recovered while under treatment and had no abnormalities at the end of the study. The authors reported that some organs were investigated (liver, spleen, kidney, endocrine organs) and that no effects of glycerol were found. Identical results for glycerol were reported in a shorter version in a publication, by Bornmann (1954). The Panel considered it probable that this paper reported the same study.

3.5.3.2. Dogs

In a study by Staples et al. (1967), mongrel dogs (1–2 animals/group) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day) by gavage, control animals received 3 mL water by gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 5,600 mg/kg bw per day and higher, the authors reported dose-dependent local irritating effects including hyperaemia, petichial haemorrhages and erosions.

Overall, the Panel noted that no studies were performed according to current test guidelines. The Panel also noted that in a subchronic toxicity study (in drinking water) in rats (Löser et al., 1954) the effects reported were observed with doses in the range of the LD₅₀ for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg bw per day, the lowest dose tested (Staples et al., 1967)), and dogs (100% glycerol at 5,600 mg/kg bw per day (Staples et al., 1967)) was likely due to the hygroscopic and osmotic effects of large doses of glycerol administered by gavage.

3.5.4. Genotoxicity

3.5.4.1. *In vitro*

Numerous studies have been published for glycerol on reverse gene mutation in bacteria, chromosome aberrations and gene mutations in mammalian cells. A summary of available data is presented in Table 12.

Table 12: Summary of studies on genotoxicity of glycerol *in vitro*

Test system	Tested organisms	Tested concentrations vehicle ^(a)	Cytotoxic concentration	Results –MA	Results +MA	Reference
Gene mutation in bacteria						
Ames test	<i>Salmonella</i> Typhimurium TA100, TA1535, TA1537, TA98, TA1538; <i>Escherichia coli</i> WP2uvrA	0.005–5 mg/plate (7 dose levels) water	Not cytotoxic but tested up to 5 mg/plate	Negative	Negative	Shimizu et al. (1985)
Ames test	<i>S. Typhimurium</i> TA100, TA1535, TA1537, TA98	0.01–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Haworth et al. (1983)
Ames test	<i>S. Typhimurium</i> TA100, TA1535, TA1537, TA98, TA1538	0.001–10 mg/plate DMSO	Tested up to 10 mg/plate	Negative	Negative	Clark et al. (1979)
Ames test	<i>S. Typhimurium</i> TA100, TA1535, TA1537, TA98, TA1538	0.2–1 mg/plate n.d.	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
Ames test	<i>S. Typhimurium</i> TA102 and TA97	0.1–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Fujita et al. (1994)
Ames test	<i>S. Typhimurium</i> TA100, TA1535, TA1537, TA98, TA92, TA94	Up to 50 mg/plate BP	Tested up to 50 mg/plate	Negative	Negative	Ishidate et al. (1984)
Ames test	<i>S. Typhimurium</i> TA100	Up to 92 mg/plate n.d.	No data	Negative	Negative	Stolzenberg and Hine (1979)
Ames test	<i>S. Typhimurium</i> TA100	0.5 mg/plate n.d.	No data	Negative	n.d.	Yamaguchi (1982)

Test system	Tested organisms	Tested concentrations vehicle ^(a)	Cytotoxic concentration	Results –MA	Results +MA	Reference
Ames test	<i>S. Typhimurium</i> TA100	n.d. n.d.	No data	Negative	n.d.	Higashimoto et al. (1991)
Chromosomal aberrations						
Chromosome aberration test	Chinese hamster lung fibroblast cells	Up to 1 mg/mL (3 dose levels) PS	Not cytotoxic	Negative	n.d.	Ishidate et al. (1984)
Chromosome aberration test	Chinese hamster ovary (CHO) cells	0, 69, or 92 mg/mL water	Not cytotoxic ^(g)	Negative	n.d.	Galloway et al. (1987)
Chromosome aberration test	CHO cells	0.1–1 mg/mL (6 dose levels) water	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
Gene mutation in mammalian cells						
HGPRT gene mutation assay	CHO cells	0.1–1 mg/mL (6 dose levels) water	Not cytotoxic	Questionable	Negative	Doolittle et al. (1988)
DNA damage						
Unscheduled DNA synthesis	Isolated male rat hepatocytes	0.1–1 mg/mL (5 dose levels) water	n.d.	Negative	Not applicable	Doolittle et al. (1988)
Sister chromatid exchange assay	CHO cells	0.2–1 mg/mL (5 dose levels) water	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
rec assay	<i>Bacillus subtilis</i> H17 or M45	n.d. n.d.	n.d.	Positive	n.d.	Nonaka (1982)

DMSO: dimethylsulfoxide; CHO: Chinese hamster ovary; HGPRT: hypoxanthine-guanine phosphoribosyltransferase; MA: metabolic activation system; n.d.: no data available; PB: phosphate buffer; PS: physiological saline.

The most reliable data for the bacterial reverse mutation assay in *Salmonella* Typhimurium strains TA100, TA1535, TA1537, TA98, TA1538 and *Escherichia coli* WP2uvrA were presented by Shimizu et al. (1985). The study is comparable to current standards. Glycerol concentrations up to 5 mg/plate were tested, the maximal dose level recommended in OECD Guideline 471. Negative results were obtained with and without addition of S9 mix (prepared from livers of male rats pre-treated with polychlorinated biphenyl). Vehicle and positive controls were performed and showed the expected results. No mutagenic activity was detected in other studies (Clark et al., 1979; Stolzenberg and Hine, 1979; Yamaguchi, 1982; Haworth et al., 1983; Ishidate et al., 1984; Doolittle et al., 1988; Higashimoto et al., 1991; Fujita et al., 1994).

Ishidate et al. (1984) published a chromosome aberration test in Chinese hamster lung fibroblast cells (see Table 12). The cells were exposed to three different doses for 24 and 48 h without addition of a metabolic activation system. The maximum dose tested was 1 mg/mL. This concentration represented the highest non-cytotoxic dose level tested in preliminary tests (50% cell-growth inhibition). One hundred metaphases per concentration were analysed for polyploid cells and structural chromosomal aberrations (no further details). Chromosome and chromatid gaps were included in the evaluation of clastogenic effects. No data were presented about concurrent positive controls but clastogenic effects were reported in parallel experiments with other test substances. The exposure to glycerol resulted in no increase in polyploidy or chromosome aberration. The authors considered glycerol to be non-clastogenic and non-mutagenic in this test system, but concentrations up to the cytotoxic threshold were not tested. However, no cytotoxic effects, nor clastogenic activity, was reported by Galloway et al. (1987) at higher concentrations (92 mg/mL), a dose level 18 times greater than the maximum dose level recommended *in vitro* by OECD Guideline no. 473. In this study, no concurrent positive control was tested for verification of the results. The concentrations used by Doolittle et al. (1988, see Table 12) also gave negative results but did not induce cytotoxic effects nor reach the recommended maximum concentration. Overall, however, the available studies are sufficient

for evaluation of this endpoint and were considered by the Panel to be sufficiently robust to support the results reported, indicating no clastogenic activity of glycerol in *in vitro* studies.

Equivocal results were reported in a mammalian cell gene mutation assay in trials without addition of a metabolic activation system (Doolittle et al., 1988). An increase in the mutation frequency compared to negative (4.8-fold increase) and solvent control (12-fold increase) was found at a concentration of 0.8 mg/mL but no clear increase at the highest dose level of 1 mg/mL. The effect was not dose-dependent and therefore considered by the authors to be without biological relevance. The Panel noted that mutation frequencies in the concurrent untreated and solvent treated controls were unusually low. No independent second trial was performed. Negative results were obtained with addition of a metabolic activation system. Other studies on this endpoint were not available.

No genotoxic activity was reported in limited studies on DNA damage in mammalian cells (Doolittle et al., 1988). There were only limited details reported for the rec assay in *B. subtilis* (Nonaka, 1982).

3.5.4.2. *In vivo*

Barilyak and Kozachuk (1985) reported a bone marrow chromosome aberration assay and a dominant lethal assay, both performed in male rats. No other *in vivo* genotoxicity data were available for glycerol.

A single gavage dose of 1 g/kg bw glycerol ($n = 10$, controls $n = 11$) induced an increase in chromosomal aberrations ($2.2 \pm 0.6\%$ vs 0% in control; without gaps) and polyploidy cells ($3.2 \pm 0.8\%$ vs $0.5 \pm 0.2\%$ in control) (Barilyak and Kozachuk, 1985). The bone marrow was processed for cytogenetic analysis 50 h after treatment and at least 50 metaphases per animal were analysed. However, the Panel noted that incidence of chromosomal aberrations observed was low and fell within the range of values often observed in untreated or solvent control animals. Moreover, the Panel also noted methodological shortcomings (e.g. collection of bone marrow outside the recommended range of 12–36 h, no positive control; no aberrations in 1,000 control metaphases; no statistical analysis and one dose level only) and limited information (e.g. no data on toxic effects). On this basis, the Panel considered this study not reliable. In the same publication, a rat dominant lethal assay was documented. Male rats were exposed to 0, 10, 100 or 1,000 mg/kg bw glycerol 'at the stage of late spermatids' (2 weeks before fertilisation) and mated with untreated females ($n = 11$ –12 per dose). A dose-dependent and significant increase in post-implantation loss was reported with a statistically significant increase at ≥ 100 mg/kg bw. The Panel noted that the reported positive results were based on insufficient number of female animals to assess dominant lethal mutations and the number of treated male animals was not indicated. On this basis, the Panel considered this study not reliable.

Overall, glycerol did not show any genotoxic activity in different *in vitro* assays, which include negative results in the bacterial reverse mutation assay (Ames test), in chromosome aberration assays and in studies on DNA damage in mammalian cells. Questionable results obtained in a hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene mutation assay did not show a dose-response effect and were therefore judged of no biological significance. A lack of valid *in vivo* genotoxicity data is not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

3.5.5. Chronic toxicity and carcinogenicity

3.5.5.1. Mice

Witschi et al. (1989) investigated the effects on lung and liver tumour incidences in male and female CH3 mice (between 31 and 51 mice/group) after administration for 1 year of drinking water containing 0% or 5% glycerol (equivalent to 0 and 6,500 mg glycerol/kg bw per day for males and 0 and 8,300 mg/kg bw per day for female). Although the reporting of this study was limited, the Panel noted that lung and liver tumour incidences were not significantly elevated in male or female mice (no further parameters examined).

3.5.5.2. Rats

As reported by JECFA (1976), male and female Sprague-Dawley rats (9 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% natural or synthetic glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 50 weeks (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976). At necropsy, growths in the pituitary and histopathological effects were observed in the kidney in females

treated with either test compounds. The Panel noted limited methodological detail, effects only in females and data lacking any dose–response effects.

Hine et al. (1953) examined the effects of supplementing the diet with glycerol (natural and synthetic, no further details on test substances) for up to 2 years on Long-Evans male and female rats (between 10 and 14 animals per group) at 0, 2,500, 5,000 or 10,000 mg/kg bw per day. The Panel noted that the study has a number of limitations – all rats in the high-dose group were culled after 1 year, with no concurrent control group; no clinical chemistry; limited parameters in haematology and urinalysis and limited histopathology. Body weight and food consumption were measured weekly and body weight gain calculated at 4 week intervals. Clinical signs were recorded daily and careful inspections for health and activity were made once a week. Urinalysis and blood sampling for haematology were performed at 3, 6, 12, 18 and 24 month (n = 5 per dose, no data about sex). Urinalysis was limited to microscopical examination and determination of glucose and albumin concentrations. Haematology was limited to erythrocyte counts, leucocyte counts and haemoglobin levels. High-dose rats were sacrificed after 1 year and liver glycogen and lipids determined (no control values were available). For the other groups, including the control group, the study was terminated after 105 weeks. In all groups, complete necropsy was performed and weights for the liver, lung, kidney, heart and spleen determined. Histopathology was limited to the liver, spleen, adrenals, kidney, small intestine, reproductive organs and urinary bladder. Lipid stains in the liver were prepared for all groups sacrificed after 105 weeks. The results were similar for the groups treated with natural or synthetic glycerol. No data were presented on food consumption, clinical signs or mortality. No significant effects were found on the body weight gain (statistical analysis restricted to mean values at week 13, 52 and 105); data on absolute body weight were not given. No consistent treatment-related effects were found in haematology, urinalysis, evaluation of organ weights and pathology. Liver glycogen and lipids did not differ between the two high-dose groups, a comparison with the control group was not possible. Similarly, data on organ weights and pathological effects in the high-dose group (10,000 mg/kg bw per day) were compared with a control group sacrificed at 105 weeks, limiting the validity of the results in the high-dose group. No no observed adverse effect level (NOAEL) was identified by the authors of the study. Although this study had minor limitations (e.g. lacking concurrent control in the 1-year study group), the Panel noted that no adverse effects were reported in animals receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in the tumours incidences up to doses of 5,000 mg/kg bw per day for 2 years, the highest dose tested.

JECFA reported a study in rat (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976) in which male and female Sprague–Dawley rats (24 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 2 years. Animals were treated with natural glycerol (99.8% glycerol, similar caloric density for test and control diets) or synthetic glycerol (99.76% glycerol and 1,2,3-butanetriol and 1,2,4-butanetriol, 0.09%, similar caloric density for test and control diets). For both natural and synthetic glycerol, at all dose levels, glycerol treatment resulted in an increase in body weight gain (no further details). At the 10% dose level, there was an increase in relative heart weights (no data regarding other dose levels). At necropsy, the 20% glycerol group was examined for mortality, body weight, efficiency of caloric utilisation, water consumption, urinalysis (volume, specific gravity, sediment, acetone, albumin, sugar and oxalate), haematology (white blood cells and differential count, red blood cells, haematocrit, and haemoglobin) and clinical chemistry (blood glucose, urea, nitrogen, plasma cholesterol, serum alkaline phosphatase and transaminases, and bromosulfophthalein retention). In addition, organ weights were determined and complete histopathology performed (no further details). The only reported change noted was an increase in relative kidney weight at 20% glycerol dose level with both natural and synthetic glycerol. The Panel considered that the increase in relative heart weights did not show a dose–response relationship and that the increase in relative kidney weight at 20% glycerol dose level in both studies as adaptive since no histopathological changes were reported.

Overall, the Panel considered glycerol to be of no concern with regard to carcinogenicity.

3.5.5.3. Promoting and/or co-carcinogenic effects of glycerol

The Panel noted a series of studies, which had investigated potential modulating effects of glycerol administration on the development of cancer in animal models treated with an initiating agent (Inayama, 1986; Inayama et al., 1986; Nagahara, 1987; Witschi et al., 1989; Nagahara et al., 1990;

Yano et al., 1993, 1994; Itoh et al., 2002; Kanojia and Vaidya, 2006). The Panel considered these studies not relevant for the risk assessment of glycerol as a food additive.

3.5.6. Reproductive and developmental toxicity

3.5.6.1. Reproductive toxicity

In a study reported by Johnson et al. (1933), rats were kept on diets containing 61% starch and no glycerol (control), or 20% starch plus 41% glycerol (equivalent to 20,500 mg/kg bw per day), or no starch plus 61% glycerol (equivalent to 30,500 mg/kg bw per day), limited information, e.g. no data about number of animals per group. Reproduction was impaired by diets containing 41% glycerol and no pregnancies occurred in rats that were on diets containing 61% glycerol (undernourished conditions). However, this was an old study and the documentation of the results is not sufficient for evaluation of this endpoint.

In a study performed by Guerrant et al. (1947), groups of male and female rats (3 males and 6 females per group) received a synthetic diet containing 0 or 30% glycerol (equivalent to 15,000 mg/kg bw per day) and were carried through seven successive generations. Young rats from treated females weighed on average 20% less than controls. The Panel noted this study was limited: the number of animals per group was low and the diet changed during the generations.

Ten male and 10 female rats were orally gavaged daily either with glycerol (20% solution in water) at a dose level of 2,000 mg/kg bw per day or with distilled water (control) for 8 weeks before mating (Wegener, 1953). After mating, one subgroup of females ($n = 5$) received glycerol throughout pregnancy and up to parturition. The other subgroup ($n = 5$) was treated until weaning of the F_1 generation. Body weights of F_1 rats were measured every second day during the post-natal period from day 15–60. At post-natal day 100, F_1 rats were sacrificed except for 10/sex which were used to produce the F_2 -generation. The authors reported that there were no effects of glycerol on fertility of parental (P) rats, F_1 litter size or F_1 weight gain (however, data were not presented). In total, 12 F_1 rats died during post-natal day 1–50 in the treatment group and three rats in the control; this mortality was not further discussed and could not be judged by the Panel as the total number of pups/dam was not presented. The onset of oestrus cycle in F_1 females was comparable in both groups and histopathology of F_1 rats revealed no effects on testes, ovaries, thyroid glands, pituitary and adrenal glands (no other organs were examined). F_2 litter size was comparable to control. No further parameters were investigated. The Panel noted that the study has limitations due to some methodological weaknesses (e.g. low number of pregnant P rats, no F_1 pup weights, no data about external malformations or toxic effects in P generation) but there is no indication for adverse effects on reproductive performance of P rats at a dose of 2,000 mg/kg bw per day, the highest dose level tested. The Panel, therefore, considered that this study was limited as the number of animals per (sub) group was very low and the reporting was limited.

3.5.6.2. Developmental toxicity

Several developmental toxicity studies of glycerol were conducted in CD-1 mice, Wistar rats golden hamsters and Dutch-belted rabbits (FDRL, 1974a,b,c). Animals were administered different doses of glycerol (not specified) in water by gavage; the control groups were vehicle-treated (dose volume is not reported). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects.

Groups of 25 adult female albino CD-1 outbred mice were dosed by gavage from gestation day (GD) 6 to 15 with 0, 12.8, 59.4, 276 or 1,280 mg glycerol/kg bw per day (dose volume 10 mL/kg bw per day) (FDRL 1974a). At necropsy on GD 17, the surviving dams appeared to be completely normal and the number of implantations, and live fetuses was comparable to the control group. Doses up to 1,280 mg glycerol/kg bw per day had no effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions and average implant sites and also fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 25–29 virgin adult female Wistar rats were dosed by gavage from GD 6 and to 15 with 0, 13.1, 60.8, 282 or 1,310 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2, or 5 mL/kg bw per day)

(FDRL, 1974b). At necropsy on GD 20, doses up to 1,600 mg glycerol/kg bw per day appeared to be completely normal and had no effects on implantation or on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 15–20 virgin adult female Dutch-belted rabbits were treated by gavage once daily from GD 6 to 18 with doses of 0, 11.8, 54.8, 254.5 or 1,180 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2 or 5 mL/kg bw per day) (FDRL, 1974c). Only 10–14 rabbits became pregnant and were evaluated at termination. At necropsy on GD 29, the surviving dams appeared normal throughout the observation period and had normal fetuses. No effect was observed on the number of implantations. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

The Panel noted that the identically performed tests with glycerol in mice, rats and rabbits by the FDRL (1974a,b,c) were reported in a limited way but cover the organogenesis period of the reproductive cycle. These prenatal developmental toxicity studies showed no dose-related developmental and maternal effects up to the highest dose tested 1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits.

3.5.7. Other studies

3.5.7.1. Observations in humans

Glycerol has been used therapeutically by intravenously or oral administration to mobilise oedema and to induce osmotic diuresis. Clinical experience and data from clinical studies are found in the literature indicating that doses up to 60,000 mg intravenously, infused over 1–4 h, for a treatment duration of 1 week and in some studies, several weeks (Frank et al., 1981), and bolus oral doses (initial dose 1,500 mg/kg bw, followed by 500–700 mg/kg bw every 3 h) (Cantore et al., 1964) or 500–1,000 mg/kg bw (Wald and McLaurin, 1982).

The Panel considered that the therapeutic administration of glycerol by oral administration to patients reported to be suffering disease that could impact significantly on their physiological functionality was not relevant in the safety assessment of glycerol (E 422) as a food additive. However, glycerol has been prescribed for reduction in intraocular pressure in patients with glaucoma and may be prescribed prior to intraocular surgery (Bartlett, 1991). For this indication, glycerol was reported to be administered orally as a bolus dose of 1,000–1,500 mg/kg bw (as a 50% solution) with a daily total oral dose not higher than 120,000 mg (about 1,700 mg/kg bw) (Gilman, 1990). The Panel considered that the therapeutic administration of glycerol by oral administration to patients reported to be suffering ocular disease – such as glaucoma and administration of glycerol to control participants in clinical studies – were relevant for the safety assessment of glycerol (E 422) as a food additive.

The Panel noted that for oral therapies to patients with ocular disease or healthy controls, glycerol was administered as a bolus at 1,350 mg/kg bw (Buckell and Walsh, 1964); 1,500 mg/kg bw (Drance, 1964); 1,500 mg/kg bw (Consul and Kulsretha, 1965); 1,000 mg/kg bw (Kornblueth et al., 1967); 1,000–1,270 mg/kg bw (McCurdy et al., 1966); 1,350 mg/kg bw (Charan and Sarda, 1967); 1,200 mg/kg bw (Krupin et al., 1970) and 1,260 mg/kg bw (Friedman et al., 1980). The only side effects observed in these oral therapies to patients with ocular disease or healthy controls were either none or one or more of nausea, headache and/or vomiting.

Guidelines for glycerol use in hyperhydration and rehydration associated with exercise report 28 studies in which oral doses between 500 mg/kg bw and 1,500 mg/kg bw were given in the total of 238 subjects (Van Rosendal et al., 2010). Three studies reported side effects after rapidly administering the glycerol as a concentrated bolus followed by fluid ingestion. Four subjects in two of the trials were nauseous after glycerol ingestion. In another study, two subjects developed diarrhoea in the 24 h after the trial. In a further three studies, a low incidence of gastrointestinal distress (bloating) or light-headedness were reported.

Using the studies of Wald and McLaurin (1982), the Panel noted that patients were administered glycerol orally as bolus doses of 500–1,000 mg/kg bw every 3–4 h, dependent on the patients' intracranial pressures (ICPs). Specific individual dosages ranged from 4,000 mg to 70,000 mg (average 54,000 mg) and it was administered via a nasogastric tube as a 50% solution (by mixing a 100%

glycerol solution with an equal volume of either 5% dextrose in water or 5% dextrose in 0.4% normal saline, depending upon the systemic electrolyte status). If no change in ICP was achieved or a significant volume of solution was aspirated from the stomach, intravenous mannitol was administered and another trial of the drug was initiated 4–24 h later. For the six patients in which data were reported in this study, maximum ICP reduction and maximum serum glycerol concentration occurred around 60–90 min after oral bolus ingestion. In most cases, the serum glycerol concentration returned to pre-treatment levels around 3 h after oral administration.

Given the dose- and time period-range reported in this study, the Panel calculated that the dose of glycerol required to induce a therapeutic reduction in ICP was within the range of 125–333 mg/kg bw per hour.

The Panel therefore considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

Glycerol therapy in diabetics

Hyperosmolar non-ketotic coma occurred in diabetic patients after repeated use of oral and intravenous glycerol (e.g. Oakley and Ellis, 1976; Sear, 1976). According to Sear (1976), the non-ketotic hyperosmolar hyperglycaemic state usually occurs in cases of maturity onset diabetes or pre-diabetes and not in non-diabetic subjects.

3.6. Discussion

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

The EU specification for glycerol content is not less than 98% on an anhydrous basis, whereas JECFA specifies a content not less than 99%. The Panel noted that in the EU specifications for glycerol (E 422) there is no specific limit for acrolein and while the EU specifications has a limit for chlorinated compounds, the identities of specific chlorinated compounds are not identified. The Panel considered that the identification of possible chlorinated impurities would be needed for their evaluation of toxicological significance.

According to the EU specifications for glycerol (E 422), impurities of the toxic elements lead, mercury, arsenic and cadmium are accepted up to a concentration of 2, 1, 3 and 1 mg/kg, respectively. The Panel considered that contamination at those levels would have a significant impact on the exposure to these metals for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010a,b, 2012).

The Panel noted that glycidol may be present in glycerol from its manufacture from either refined oils and fats or when it is chemically synthesised. Glycidol is characterised as probably carcinogenic to humans 2A (IARC 2000) and as a carcinogenic and genotoxic compound by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2016). The Panel considered that its presence in glycerol (E 422) would raise a safety concern.

The Panel also noted that in the synthesis pathway of glycerol via allyl chloride, epichlorohydrin is formed as an intermediate, which may be present in the final product. Epichlorohydrin is classified as carcinogen 2A according to IARC (1999) and the Panel considered that its presence in glycerol (E 422) would raise a safety concern.

Furthermore, some chemically synthesised preparations of glycerol, starting or intermediate chemicals used in the synthesis, including propene, allyl alcohol, propylene oxide, allyl chloride and dichlorohydrin may be present and there are no maximum limits for these substances in the EU specifications for glycerol (E 422).

The Panel considered that lactic acid bacteria may be used in manufacturing of some foods (e.g. yogurt, cheese, cultured butter, sour cream (with fat content above 20%), sausage, cucumber pickles, olives and sauerkraut) and that if glycerol (E 422) is added to those food items, acrolein may be formed.

The Panel noted that in addition to the information provided by CEFIC (2013a [Documentation provided to EFSA n. 3]), glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants in glycerol (E 422), which are of toxicological concern. This should be considered in the EU specifications for glycerol (E 422). The Panel considered

that the manufacturing process for glycerol (E 422) should not allow the production of a food additive which contains residuals of genotoxic or/and carcinogenic concern at a level which would result in a MOE below 10,000.

Glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract; distributed into the total body water space and is primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as CO₂, with only minor amounts excreted via urine or faeces. The Panel noted that glycerol concentrations in blood higher than 92 mg/L would result in renal elimination of glycerol in rats (Ackerman et al., 1975) and humans (McCurdy et al., 1966).

Glycerol has a low acute toxicity.

Short-term or subchronic studies were not performed according to current test guidelines. In a subchronic toxicity study (in drinking water) in rats, the effects reported were observed with doses in the range of the LD₅₀ for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg bw per day, the lowest dose tested (Staples et al., 1967)), and dogs (100% glycerol at 5,600 mg/kg bw per day (Staples et al., 1967)) was likely due to the hygroscopic and osmotic effects of glycerol.

Glycerol did not show any genotoxic activity in different *in vitro* assays, which include negative results in the bacterial reverse mutation assay (Ames test), in chromosome aberration assays and in studies on DNA damage in mammalian cells. Questionable results obtained in a HGPRT gene mutation assay did not show a dose–response effect and were therefore judged of no biological significance. A lack of valid *in vivo* genotoxicity data was not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in the tumours incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.

The reports of the two- and multigeneration reproductive toxicity studies have limitations but no adverse effects were reported. Prenatal developmental toxicity studies were also limited but showed no dose-related developmental and maternal effects up to the highest dose tested (1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits, respectively).

The Panel considered that none of the animal studies available identified an adverse effect for glycerol.

The Panel considered that the therapeutic oral use of glycerol at 1,000–1,500 mg/kg bw given as bolus in patients with glaucoma triggered an increase in plasma osmolality and dehydration, and resulted in side effects such as headache, nausea and/or vomiting in some individuals. Given the dose- and time period-range reported in the Wald and McLaurin (1982) study, the Panel calculated that the dose of glycerol required to induce a therapeutic reduction in ICP was within the range of 125–333 mg/kg bw per hour. The Panel considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

The exposure estimates in all exposure scenarios resulted in overestimates of the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008 (Section 3.4.1). Although, use levels and analytical data were only available for 28 out of the total of 68 food categories in which glycerol (E 422) is authorised for use according to Annex II, the Panel considered that this did not result in an underestimation of the exposure because:

- glycerol (E 422) belongs to Group I food additives, and therefore almost all the food categories for which glycerol (E 422) is authorised correspond to the general Group I food additives authorisation (Table 3). Given the information on the uses provided by food industry and information from the Mintel GNPD, it may be assumed that glycerol (E 422) is not used in some of these food categories.

- approximately 94% of the food products labelled with glycerol (E 422) in the Mintel GNPD belonged to food subcategories that were considered in the exposure assessment (Appendix C).

Furthermore, the Panel noted that the additional exposure to glycerol via natural sources, including wine, other alcoholic beverages and honey (the sources for which analytical data were available) did not significantly change the exposure to glycerol (E 422) (Appendix F). The Panel further noted that the exposure to glycerol from its use according to Annex III (Parts 1, 2, 3, 4 and 5) was only partly considered in the exposure assessment (via wine and other alcoholic beverages). The highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was, however, estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario. The food categories contributing most to the mean exposure to glycerol (E 422) were bread and rolls and fine bakery wares for which brand-loyalty was not assumed relevant.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use, is consumption of a beverage. Therefore, the Panel calculated the volume of flavoured drinks required to be consumed in order to exceed the acute bolus exposure (125 mg/kg bw per hour), calculated by the Panel, to be the minimum dose required to have a therapeutic effect. When considering the available data, the Panel noted that infants and toddlers can be exposed to more than 125 mg glycerol/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink.

The Panel considered that the exposure to 3-MCPD coming from the use of glycerol (E 422) as a food additive at a maximum level of 0.1 mg 3-MCPD/kg glycerol, did not exceed the TDI of 0.8 µg/kg bw per day for 3-MCPD (EFSA CONTAM Panel, 2016) in any of the exposure assessment scenarios (maximum being up to 6% in the children population at the 95th percentile in the non-brand-loyal scenario; Section 3.4.3).

4. Conclusions

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels received from industry and Member States in 28 food categories out of 68 food categories in which glycerol (E 422) is authorised;
- the highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario;
- glycerol (E 422) as a food additive is identical to a compound which is a normal constituent in the body (an endogenous compound) and is a regular component of the diet;
- sufficient toxicity data were available;
- the toxicological studies in animals did not provide any indication for adverse effects, including at the highest dose tested in a chronic toxicity study (10,000 mg/kg bw per day);

the Panel concluded that there is no need for a numerical ADI for glycerol (E 422).

The Panel concluded that there is no safety concern regarding the use of glycerol (E 422) as a food additive according to Annex II and III (Part 1, 2, 3, 4 and 5) for the general population at the refined exposure assessment for the reported uses of glycerol as food additive.

However, the Panel identified that there remain uncertainties over the lack of identification and quantification of residuals especially those that are genotoxic and carcinogenic. The Panel noted that these residuals are mostly present when chemical synthesis is used to produce glycerol. The Panel concluded that the manufacturing process for glycerol should not allow the production of a food additive, which contains these residuals at a level which would result in a MOE below 10,000.

The Panel concluded that if 3-MCPD is present at its maximum authorised level of 0.1 mg 3-MCPD/kg glycerol, the maximum exposure to 3-MCPD was below the TDI of 0.8 µg/kg bw per day, and therefore exposure via glycerol (E 422) alone was of no concern.

The Panel could not calculate exposures to other genotoxic impurities or contaminants that may be present in glycerol (E 422) as a result of the manufacturing process, e.g. glycidol, due to the lack of data on their concentrations in the food additive.

The Panel concluded that infants and toddlers could be exposed to more than 125 mg/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel further concluded that the acute bolus exposure to glycerol (E 422) by its use as a food additive should stay below doses at which pharmacological or side effects could occur.

5. Recommendations

The Panel recommended that:

- given that during the manufacturing processes of glycerol, genotoxic impurities – e.g. glycidol, epichlorohydrin – could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- given that during the manufacturing processes of glycerol, other potential impurities of toxicological concern (e.g. dichlorohydrin) could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- more data should be generated to decrease uncertainty arising from the presence in the food additive (E 422) of compounds of toxicological concern (e.g. acrolein, 3-MCPD or 3-MCPD ester), which can be produced under some food processing conditions (e.g. use of glycerol (E 422) in parallel with lactic acid bacteria; use of glycerol (E 422) in food containing significant amounts of sodium chloride (more than 5%) and treated at temperatures above 160°C, etc.);
- a numerical limit for acrolein should be included in the EU specifications for glycerol (E 422);
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for glycerol (E 422) should be revised in order to ensure that glycerol (E 422) as a food additive will not be a significant source of exposure to those toxic elements in food;
- more information on uses and use levels and analytical data should be made available to the Panel in order to perform an adequate exposure assessment, in particular in the case of estimate acute exposure, more data on flavoured drinks is needed.

Documentation provided to EFSA

- 1) AESGP (Association of the European Self-Medication Industry), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
- 2) BIBRA, 1993. Toxicity profile: glycerol. Submitted by CEFIC, February 2013.
- 3) CEFIC, 2013a. Flow chart manufacturing process vegetal glycerine and complementary documents. Submitted by CEFIC, February 2013.
- 4) CEFIC, 2013b. SIDS Initial Assessment Report for SIAM 14 (OECD SIDS, 2002). Submitted by CEFIC, February 2013.
- 5) Cutisin, 2010. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Cutisin, sro Jilemnice, December 2010.
- 6) FDE (FoodDrinkEurope), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
- 7) ICGA (International Chewing Gum Association), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
- 8) Nutricia, 2011. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Nutricia, June 2011.
- 9) Pre-evaluation document prepared by Fraunhofer, March 2012.

- 10) Riemser Arzneimittel AG, 2010. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Riemser Arzneimittel AG, May 2010.

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Abbreviations

3-MCPD	3-monochloropropane-1,2-diol
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESPG	Association of the European Self-Medication Industry
ALT	alanine aminotransferase
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
AST	aspartate aminotransferase
BfR	Bundesinstitut für Risikobewert
bw	body weight
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
CONTAM	EFSA Panel on Contaminants in Food Chain
DMSO	dimethylsulfoxide
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
FCS	food categorisation system
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FID	flame ionisation detection
GC	gas chromatography
GD	gestation day
GNPD	Global New Products Database
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
IC	ion chromatography
ICGA	International Chewing Gum Association
ICP	intracranial pressure
i.p.	intraperitoneal
IRMS	isotope ratio mass spectrometry
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives

LD ₅₀	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
MB	medium bound
MPL	maximum permitted level
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PS	physiological saline
QS	<i>quantum satis</i>
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCF	Scientific Committee on Food
SDA	The Soap and Detergent Association
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
TDI	tolerable daily intake
VCF	Volatile Compounds in Food
WADA	World Anti-Doping Agency
WHO	World Health Organization

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of glycerol (E 422) provided by industry

Food category number	Food category name	MPL	Number of samples	Usage level mean		Information provided by	Comments
				Typical mean (range)	Mean max (range)		
01.6.3	Other creams	QS	3	17,181.8 (50–50,000)	20,515.2 (50–60,000)	FDE	
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS	1	2.5	2.5	FDE	
03	Edible ices	QS	16	187.5 (8–930)	929.8	FDE	Non-milk-based ice-cream
			36	2,312.7 (0.6–20016.3)	20,016.3	FDE	Milk-based ice-cream
05.2	Other confectionery including breath freshening microsweet	QS	3	11,945.7 (5,837–15,000)	21,945.7 (5,837–30,000)	FDE	
05.3	Chewing gum	QS	1	17,100	100,000	ICGA	Used in most of chewing gum recipes (from 50% up to 90% of recipes depending on producers). Highly representative
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS	3 (1 NP)	67,723.3 (50,870–77,300)	90,766.7 (77,300–100,000)	FDE	<i>Without taking into account NP</i>
06.3	Breakfast cereals	QS	1	0.0017	0.0017	FDE	
07.1	Bread and rolls – except products in 7.1.1 and 7.1.2	QS	1 NP	30,000	31,000	FDE	Refers to 'chapattis' bread
07.2	Fine bakery wares	QS	6 (2 NP)	21,631 (0–56,000)	30,464.3 (0–63,000)	FDE	
08.3.1	Non-heat-treated meat products	QS	1	1,500	1,500	FDE	

Food category number	Food category name	MPL	Number of samples	Usage level mean		Information provided by	Comments
				Typical mean (range)	Mean max (range)		
08.3.3	Casings and coatings and decorations for meat	QS	1	250	250	FDE	(20–30%) glycerol/dry solid of casing. Dry solid of casing approx 75%. Casing = (0.5–0.85%) by weight of foods (i.e. sausages)
			–	1.1	1.9	Cutisin ^(a)	
12.2.2	Seasonings and condiments	QS	1	48,800	48,800	FDE	
12.5	Soups and broth	QS	5	2.9 (0.8–10)	3 (0.8–10)	FDE	
12.6	Sauces	QS	5	20,013.1 (4–50,000)	20,015.9 (4–50,000)	FDE	
12.8	Yeast and yeast products	QS	1	43.5	43.6	FDE	
13.2	Dietary food for special medical purposes (FSMP) regulated according to Directive 1999/21/EC	QS	–	390.0	1,180.0	Nutricia ^(a)	Typical level = 0.66 g/L, Maximum level = 2 g/L. Glycerol is used as a carrier in a nutrient preparation where it is present at a level of 59%. Levels in final foods: typical = 660 mg/L x 59% = 390 mg/L; maximum: 2,000 mg/L x 59% = 1,180 mg/L
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS	1	34,967	34,967	FDE	
16	Desserts excluding products covered in category 1, 3 and 4	QS	1	6	6	FDE	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS	3	138,333.3 (2,000–330,000)	143,400 (2,200–330,000)	AESGP	
			3	4,682.8 (0.2–9,269.8)	9,269.8	Riemser Arzneimittel AG ^(a)	
17.3	Food supplements supplied in a syrup-type or chewable form	QS	1	85,000	94,000	AESGP	

MPL: maximum permitted level; QS: *quantum satis*; NP: Niche product.
(a): Call for data from 2010.

Appendix B – Summary of analytical results (mg/kg) of glycerol (E 422) provided by Members States

Food category number	Food category name	Restrictions	MPL	Number of samples	% LC	LOD		LOQ		All data – medium-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
04.2.1	Dried fruit and vegetables		QS	11	100	0.4	0.4	2.0	500.0	0.2	250.0	227.3	250.0	250.0
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC		QS	31	0					40.0	83.0	116.1	278.0	442.0
07.2	Fine bakery wares		QS	1	0					19900.0	19900.0	19900.0	19900.0	19900.0
11.3	Honey as defined in Directive 2001/110/EC		–	289	6	5.0	5.0	10.0	15.0	7.5	40.0	77.7	265.1	1080.0
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	1	0	0.4	0.4	2.0	2.0	390.0	390.0	390.0	390.0	390.0
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices		QS	167	31	0.4	500.0	1.0	2000.0	0.2	350.0	419.4	880.0	1470.0
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products		QS	3	100			500.0	500.0	250.0	250.0	250.0	250.0	250.0
14.1.4	Flavoured drinks		QS	5	40	500.0	500.0	1500.0	1500.0	250.0	3200.0	4820.0	12700.0	12700.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts		–	5,792	1	40.0	150.0	40.0	700.0	75.0	6575.0	6490.8	9300.0	61000.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	Only alcohol-free	–	9	0	40.0	150.0	40.0	150.0	1300.0	7200.0	5460.0	8200.0	8200.0
14.2.3	Cider and perry		QS	252	0	40.0	500.0	40.0	1500.0	1100.0	3000.0	3068.1	4580.0	11110.0
14.2.4	Fruit wine and made wine		QS	117	0	80.0	166.7	200.0	500.0	700.0	4300.0	4237.5	6600.0	8700.0
14.2.5	Mead		QS	72	0	80.0	166.7	200.0	500.0	3300.0	5895.0	6445.4	9200.0	14200.0
14.2.7.1	Aromatised wines		QS	64	2	40.0	150.0	40.0	700.0	35.0	4655.0	4835.5	7440.0	16800.0
14.2.7.2	Aromatised wine-based drinks		QS	259	0	40.0	150.0	40.0	150.0	1800.0	7400.0	9328.8	23200.0	48800.0
14.2.7.3	Aromatised wine-product cocktails		QS	72	0	40.0	150.0	40.0	150.0	1600.0	3900.0	6386.1	23600.0	34900.0

Food category number	Food category name	Restrictions	MPL	Number of samples	% LC	LOD		LOQ		All data – medium-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	78	0	40.0	166.7	40.0	700.0	900.0	3525.0	3889.5	6600.0	9600.0

% LC: % of left-censored data; p95: 95th percentile; LOD: limit of detection; LOQ: limit of quantification.

Appendix C – Number and percentage of food products labelled with glycerol (E 422) out of the total number of food products present in the Mintel GNPD per food subcategory between 2011 and 2016

Food subcategory ^(a)	Total number of products	Food products labelled with glycerol (E 422)	
		Number	%
Gum	1,423	942	66.2
Snack/cereal/energy bars	5,386	1,415	26.3
Cakes, pastries & sweet goods	13,966	3,498	25.0
Mixed assortments	332	63	19.0
Liquorice	819	103	12.6
Sticks, liquids & sprays	92	11	12.0
Meal kits	2,200	218	9.9
Sandwiches/wraps	2,765	197	7.1
Pastilles, gums, jellies & chews	4,018	239	5.9
Bread & bread products	10,721	612	5.7
Chocolate countlines	2,473	131	5.3
Lollipops	419	22	5.3
Sweet biscuits/cookies	18,562	909	4.9
Baking ingredients & mixes	9,565	387	4.0
Medicated confectionery	1,092	36	3.3
Non-individually wrapped chocolate pieces	5,531	178	3.2
Individually wrapped chocolate pieces	2,683	81	3.0
Other frozen desserts	1,690	48	2.8
Snack mixes	1,566	42	2.7
Chocolate tablets	8,871	212	2.4
Seasonal chocolate	5,765	130	2.3
Shelf-stable desserts	3,203	68	2.1
Boiled sweets	1,008	21	2.1
Toffees, caramels & nougat	1,990	39	2.0
Cold cereals	6,699	129	1.9
Dairy-based frozen products	8,342	151	1.8
Other natural sweeteners	723	12	1.7
Standard & power mints	898	13	1.4
Meat snacks	1,072	13	1.2
Marshmallows	522	6	1.1
Chilled desserts	6,588	68	1.0
Sports drinks	848	8	0.9
Dessert toppings	669	6	0.9
Other sugar confectionery	1,159	10	0.9
Water-based frozen desserts	1,282	10	0.8
Hot cereals	1,306	10	0.8
Meal replacements & other drinks	1,370	10	0.7
Flavoured alcoholic beverages	2,102	15	0.7
Caramel & cream spreads	310	2	0.6
Baby snacks	340	2	0.6
Vodka	620	3	0.5
Rice snacks	425	2	0.5
Hors d'oeuvres/canapes	4,262	20	0.5
Meat pastes & pates	3,183	12	0.4

Food subcategory ^(a)	Total number of products	Food products labelled with glycerol (E 422)	
		Number	%
Fruit snacks	3,759	14	0.4
Beverage concentrates	2,449	8	0.3
Pizzas	4,618	15	0.3
Energy drinks	1,772	5	0.3
Seasonings	10,041	26	0.3
Spoonable yogurt	10,427	26	0.2
Wheat & other grain-based snacks	2,076	5	0.2
Meat substitutes	2,540	6	0.2
Savoury biscuits/crackers	5,047	10	0.2
Prepared meals	11,608	20	0.2
Sucrose	1,172	2	0.2
Fish products	13,353	22	0.2
Chocolate spreads	1,223	2	0.2
Soft cheese desserts	1,610	2	0.1
Meat products	17,204	18	0.1
Other sauces & seasonings	984	1	0.1
Carbonated soft drinks	5,991	6	0.1
Pastry dishes	2,024	2	0.1
Wine	4,164	4	0.1
Malt & other hot beverages	1,072	1	0.1
Rice/nut/grain & seed based drinks	1,296	1	0.1
Poultry products	6,944	5	0.1
Fruit/flavoured still drinks	3,043	2	0.1
Liqueur	1,811	1	0.1
Table sauces	6,326	3	0.0
Processed cheese	2,229	1	0.0
Fruit	2,949	1	0.0
Soft cheese & semi-soft cheese	6,051	2	0.0
Pasta sauces	4,017	1	0.0
Confiture & fruit spreads	5,004	1	0.0
Pickled condiments	5,869	1	0.0
Beer	8,696	1	0.0
Total sample	461,729	10,319	2.2^(b)

(a): According to Mintel food categorisation.

(b): In total, around 2.2% of the foods available on the Mintel GNPD are labelled with glycerol (E 422) between 2011 and 2016.¹⁹

Appendix D – Concentration levels of glycerol (E 422) used in the refined and acute exposure scenarios (mg/kg or mL/kg as appropriate)

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level ^(b)	
01.3	Unflavoured fermented milk products, heat-treated after fermentation	QS	–	–	Not taken into account (no concentration data)
01.4	Flavoured fermented milk products including heat-treated products	QS	–	–	Not taken into account (no concentration data)
01.6.3	Other creams	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16 – except mozzarella	QS	–	–	Not taken into account (no concentration data)
01.7.5	Processed cheese	QS	–	–	Not taken into account (no concentration data)
01.7.6	Cheese products (excluding products falling in category 16)	QS	–	–	Not taken into account (no concentration data)
01.8	Dairy analogues, including beverage whiteners	QS	–	–	Not taken into account (no concentration data)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS	2.5	2.5	Reported use levels
02.3	Vegetable oil pan spray	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
03	Edible ices	QS	1,659	20,016	Reported use levels
04.2.1	Dried fruit and vegetables	QS	227	250	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	QS	–	–	Not taken into account (no concentration data)
04.2.4.1	Fruit and vegetable preparations excluding compote	QS	–	–	Not taken into account (no concentration data)
04.2.5.4	Nut butters and nut spreads	QS	–	–	Not taken into account (no concentration data)
04.2.6	Processed potato products	QS	–	–	Not taken into account (no concentration data)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	QS	116	278	Analytical data
05.2	Other confectionery including breath freshening microsweets	QS	11,946	30,000	Reported use levels
05.3	Chewing gum	QS	17,100	100,000	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS	–	–	Not taken into account (no corresponding FoodEx code)
06.2.2	Starches	QS	–	–	Not taken into account (no concentration data)
06.3	Breakfast cereals	QS	0.0017	0.0017	Reported use levels

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level ^(b)	
06.4.2	Dry pasta – only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS	–	–	Not taken into account (no concentration data)
06.4.4	Potato Gnocchi – except fresh refrigerated potato gnocchi	QS	–	–	Not taken into account (no concentration data)
06.4.5	Fillings of stuffed pasta (ravioli and similar)	QS	–	–	Not taken into account (no concentration data)
06.5	Noodles	QS	–	–	Not taken into account (no concentration data)
06.6	Batters	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
06.7	Pre-cooked or processed cereals	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
07.1	Bread and rolls – except products in 7.1.1 and 7.1.2	QS	30,000	31,000	Reported use levels
07.2	Fine bakery wares	QS	9,446	58,000	Reported use levels
08.3.1	Non-heat-treated meat products	QS	1,500	1,500	Reported use levels
08.3.2	Heat-treated meat products – except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	QS			
08.3.3	Casings and coatings and decorations for meat	QS	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	–	–	Not taken into account (no concentration data)
09.3	Fish roe – only processed fish roe	QS	–	–	Not taken into account (no concentration data)
10.2	Processed eggs and egg products	QS	–	–	Not taken into account (no concentration data)
11.2	Other sugars and syrups	QS	–	–	Not taken into account (no concentration data)
11.3	Honey as defined in Directive 2001/110/EC	NA	78	265	Analytical data ^(a)
11.4.1	Table Top Sweeteners in liquid form	QS	–	–	Not taken into account (no Concentration data)
12.1.2	Salt substitutes	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
12.2.2	Seasonings and condiments	QS	48,800	48,800	Reported use levels
12.3	Vinegars	QS	–	–	Not taken into account (no concentration data)
12.4	Mustard	QS	–	–	not taken into account (no concentration data)
12.5	Soups and broth	QS	3	10	Reported use levels
12.6	Sauces	QS	20,013	50,000	Reported use levels

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level ^(b)	
12.7	Salads and savoury based sandwich spreads	QS	–	–	not taken into account (no concentration data)
12.8	Yeast and yeast products	QS	44	44	Reported use levels
12.9	Protein products, excluding products covered in category 1.8	QS	–	–	Not taken into account (no concentration data)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS	390	1180	Reported use levels
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS	34,967	34,967	Reported use levels
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009 – including dry pasta	QS	–	–	Not taken into account (no concentration data)
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices – only vegetable juices	QS	420	880	Analytical data
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products – only vegetable nectars	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.1.4	Flavoured drinks	QS	4,820	12,700	Analytical data
14.1.5.2	Other – excluding unflavoured leaf tea; including flavoured instant coffee	QS	–	–	Not taken into account (no concentration data)
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	NA	6,491	9,300	Analytical data ^(a)
14.2.3	Cider and perry	QS	3,068	4,580	Analytical data ^(a)
14.2.4	Fruit wine and made wine	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.5	Mead	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008 – except whisky or whiskey	QS	–	–	Not taken into account (no concentration data)
14.2.7.1	Aromatised wines	QS	8,065	23,600	Analytical data ^(a)
14.2.7.2	Aromatised wine-based drinks	QS			
14.2.7.3	Aromatised wine-product cocktails	QS			
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	QS	3,890	660	Analytical data ^(a)
15.1	Potato-, cereal-, flour- or starch-based snacks	QS	–	–	Not taken into account (no concentration data)
15.2	Processed nuts	QS	–	–	Not taken into account (no concentration data)

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level ^(b)	
16	Desserts excluding products covered in category 1, 3 and 4	QS	6	6	Reported use levels
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS	125,000	330,000	Reported use levels
17.2	Food supplements supplied in a liquid form	QS			
17.3	Food supplements supplied in a syrup-type or chewable form	QS			
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	QS	–	–	Not taken into account (no concentration data)

MPL: maximum permitted level; QS: *quantum satis*.

NA: not authorised according to Annex II to Regulation No 1333/2008.

(a): Levels taken into account when estimating exposure from glycerol (E 422) as a food additive and due to carry-over (Annex III) and from natural sources (i.e. honey, wines and other alcoholic beverages) *(data set 2).

(b): Maximum of reported use levels or p95 of analytical data.

Appendix E – Summary of total estimated exposure of glycerol (E 422) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Infants							
Bulgaria (NUTRICHILD)	659	138	457	126	435	83	266
Germany (VELS)	159	128	442	109	392	74	257
Denmark (IAT 2006_07)	826	115	302	105	271	100	259
United Kingdom (DNSIYC_2011)	1,366	78	269	66	223	46	153
Italy (INRAN_SCAI_2005_06)	12	10	–	9	–	6	–
Toddlers							
Belgium (Regional_Flanders)	36	583	–	453	–	308	–
Bulgaria (NUTRICHILD)	428	390	769	330	701	220	415
Germany (VELS)	348	453	938	356	796	222	418
Denmark (IAT 2006_07)	917	290	484	235	400	223	368
Spain (enKid)	17	158	–	138	–	89	–
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	300	601	229	451	153	307
United Kingdom (DNSIYC_2011)	1,314	221	497	175	406	123	261
Italy (INRAN_SCAI_2005_06)	36	178	–	161	–	95	–
Netherlands (VCP_kids)	322	425	785	324	674	236	413
Children							
Austria (ASNS_Children)	128	349	689	278	535	190	326
Belgium (Regional_Flanders)	625	463	856	360	674	240	402
Bulgaria (NUTRICHILD)	433	453	910	369	786	238	460
Czech Republic (SISP04)	389	351	727	277	594	176	349
Germany (EsKiMo)	835	269	510	210	420	155	285
Germany (VELS)	293	462	877	353	654	220	372
Denmark (DANSDA 2005-08)	298	318	552	242	421	212	351
Spain (enKid)	156	268	550	215	487	147	274
Spain (NUT_INK05)	399	278	489	216	421	157	269
France (INCA2)	482	326	632	270	528	131	246
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	331	596	242	449	162	282
Greece (Regional_Crete)	838	251	518	220	472	112	222
Italy (INRAN_SCAI_2005_06)	193	239	518	199	449	131	276
Latvia (EFSA_TEST)	187	281	688	225	554	131	318
Netherlands (VCP_kids)	957	402	740	305	620	214	357
Netherlands (VCPBasis_AVL2007_2010)	447	480	852	358	652	239	384
Sweden (NFA)	1,473	424	783	303	561	183	316
Adolescents							
Austria (ASNS_Children)	237	217	444	175	361	122	232
Belgium (Diet_National_2004)	576	259	515	201	399	125	227
Cyprus (Childhealth)	303	128	283	101	217	74	149
Czech Republic (SISP04)	298	283	629	224	500	144	298

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Germany (National_Nutrition_Survey_II)	1,011	199	470	161	398	108	227
Germany (EsKiMo)	393	221	458	178	387	127	228
Denmark (DANSDA 2005-08)	377	186	339	148	287	118	210
Spain (AESAN_FIAB)	86	147	311	125	260	78	149
Spain (enKid)	209	212	481	165	370	121	265
Spain (NUT_INK05)	651	200	364	153	288	114	203
France (INCA2)	973	194	402	158	337	87	168
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	207	384	157	303	100	175
Italy (INRAN_SCAI_2005_06)	247	154	326	121	289	88	200
Latvia (EFSA_TEST)	453	210	455	165	369	110	238
Netherlands (VCPBasis_AVL2007_2010)	1,142	327	608	248	473	162	290
Sweden (NFA)	1,018	263	500	195	369	123	222
Adults							
Austria (ASNS_Adults)	308	154	335	129	302	82	152
Belgium (Diet_National_2004)	1,292	181	411	145	325	95	198
Czech Republic (SISP04)	1,666	142	315	120	263	85	166
Germany (National_Nutrition_Survey_II)	10,419	154	344	126	286	84	166
Denmark (DANSDA 2005-08)	1,739	117	229	94	197	79	143
Spain (AESAN)	410	111	249	93	208	61	127
Spain (AESAN_FIAB)	981	109	241	90	206	59	126
Finland (FINDIET2012)	1,295	129	274	102	225	73	149
France (INCA2)	2,276	130	263	105	218	70	142
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	121	258	93	203	63	125
Hungary (National_Repr_Surv)	1,074	103	218	89	186	78	150
Ireland (NANS_2012)	1,274	147	289	111	225	82	154
Italy (INRAN_SCAI_2005_06)	2,313	89	186	73	153	59	125
Latvia (EFSA_TEST)	1,271	126	288	105	242	77	168
Netherlands (VCPBasis_AVL2007_2010)	2,057	186	379	143	312	100	189
Romania (Dieta_Pilot_Adults)	1,254	68	161	57	136	50	110
Sweden (Riksmaten 2010)	1,430	135	287	106	229	69	138
The elderly							
Austria (ASNS_Adults)	92	139	268	119	265	78	135
Belgium (Diet_National_2004)	1,215	121	247	97	208	71	130
Germany (National_Nutrition_Survey_II)	2,496	129	269	109	242	76	139
Denmark (DANSDA 2005-08)	286	89	168	75	137	68	123
Finland (FINDIET2012)	413	111	246	90	210	64	133
France (INCA2)	348	111	225	91	202	71	148
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	106	204	83	166	54	99
Hungary (National_Repr_Surv)	286	93	186	81	154	71	124
Ireland (NANS_2012)	226	120	239	99	209	68	130

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Italy (INRAN_SCAI_2005_06)	518	84	169	72	151	62	133
Netherlands (VCPBasis_AVL2007_2010)	173	132	244	101	196	75	120
Netherlands (VCP-Elderly)	739	124	211	93	165	73	119
Romania (Dieta_Pilot_Adults)	128	76	171	65	156	61	146
Sweden (Riksmaten 2010)	367	114	235	93	192	57	102

—: 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).

Appendix F – Refined estimated exposure assessment scenario considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008), food categories which may contain glycerol due to carry-over and natural sources for which data were available (i.e. wines and other alcoholic beverages, and honey): data set 2

	Infants (12 weeks– 11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Brand-loyal scenario						
Mean	9–126	138–453	199–369	102–248	59–151	66–124
95th percentile	223–435	400–796	420–786	217–500	141–325	156–274
Non-brand- loyal scenario						
Mean	6–100	89–308	112–240	74–163	51–104	57–83
95th percentile	153–267	261–418	222–460	149–298	116–201	102–154

Appendix G – Main food categories contributing to exposure to glycerol (E 422) using data set 2

Table G.1: Main food categories contributing to the exposure to glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 and from natural sources using the *brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) ^(a)					
03	Edible ices	–	14.0 (1)	8.8 (1)	–	–	–
05.2	Other confectionery including breath refreshing microsweets	–	–	41.2 (1)	45.8 (1)	8.0 (1)	–
07.1	Bread and rolls	30.4–93.0 (5)	11–84.7 (10)	22.6–66.7 (17)	29.1–55.5 (16)	32.4–72.4 (17)	34.8–84.1 (14)
07.2	Fine bakery wares	19.4–42.8 (3)	14.1–51.5 (9)	16.9–62.2 (16)	14.8–53.5 (15)	5.4–38.4 (16)	10.4–41 (12)
08.3	Meat products	7.7 (1)	5.6 (1)	–	–	–	–
12.6	Sauces	14.4–30.9 (3)	5.9–11.7 (3)	5–11.6 (7)	5.2–10.5 (9)	6.6–14 (9)	5.3–12.1 (7)
14.1.4	Flavoured drinks	8.4–29.4 (2)	6.5–40.3 (7)	7.8–40.8 (16)	8.6–43.1 (17)	5.1–31.7 (16)	6.3–9.8 (7)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	–	–	–	–	5.6–8.9 (5)	5.4–16 (8)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	36.2–76.1 (2)	27.6 (1)	9.2 (1)	–	5.1–9.7 (3)	5.9–9.4 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Table G.2: Main food categories contributing to exposure to glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 and from natural sources using the *non-brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) ^(a)					
05.2	Other confectionery including breath refreshing microsweets	–	–	36.8 (1)	41.0 (1)	5.7 (1)	–
07.1	Bread and rolls	47.9–94.7 (5)	23.3–86.3 (10)	6.3–74.8 (18)	45.5–74.5 (16)	47.8–80.7 (17)	54.1–86.9 (14)
07.2	Fine bakery wares	8.7–16.7 (3)	8.9–20.3 (9)	8.4–29.9 (16)	7.5–23.7 (15)	8.2–15.2 (14)	6.1–16.6 (11)
08.3	Meat products	16.9 (1)	12.0 (1)	5.0 (1)	–	–	–
12.6	Sauces	13.5–22.5 (3)	6.1–10.3 (5)	5.0–15.0 (10)	6.6–10.6 (10)	6.0–12.8 (10)	5.0–11.9 (9)
14.1.4	Flavoured drinks	6.6–17.6 (2)	5.4–26.6 (7)	5.7–28.8 (17)	7.8–33.3 (17)	6.2–20.6 (15)	5.3–7.6 (6)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	–	–	–	–	5.2–9.7 (9)	6.0–16 (10)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	26.0–65.2 (2)	27.8 (1)	10.3 (1)	–	5.7 (1)	5.1–6.3 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Appendix H – Mean and 95th percentile of acute^(a) exposure to glycerol (E 422) per food category and population group (mg/kg bw per meal)

Food category number	Food category name	High level ^(b) used in the calculation (in mg/kg)	Infants		Toddlers		Children		Adolescents		Adults		The elderly	
			Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	2.5 ^(c)					< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
02.2	Fat and oil emulsions mainly of type water-in-oil	2.5 ^(c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
03	Edible ices	20,000 (milk-based) 930 (water-based) ^(c)	25.4	129.0	55.1	127.3	49.1	124.2	25.9	64.2	17.7	44.9	15.9	37.1
04.2	Processed fruit and vegetables – dried fruit	250 ^(d)	0.2	0.9	0.2	0.6	0.2	0.4	0.1	0.2	0.1	0.3	0.1	0.3
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	278 ^(d)	0.2	0.4	0.2	0.6	0.2	0.5	0.1	0.4	0.1	0.3	0.1	0.2
05.2	Other confectionery including breath refreshing microsweeteners	30,000 ^(c)	36.9	90.5	45.3	125.0	24.6	81.8	18.1	52.9	14.1	42.9	7.0	23.6
05.3	Chewing gum	100,000 ^(c)			5.3	11.3	12.1	31.3	7.1	22.6	7.2	21.7	7.0	24.1
06.3	Breakfast cereals	0.0017 ^(c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
07.1	Bread and rolls	31,000 ^(c)	119.5	204.4	87.9	170.3	67.0	148.3	41.8	96.0	31.4	69.3	28.4	60.4
07.2	Fine bakery wares	58,000 ^(c)	145.2	320.2	148.5	394.2	135.3	375.3	81.9	236.3	60.7	181.7	46.6	155.4
12.2	Herbs, spices, seasonings	48,800 ^(c)	1.4	4.6	2.2	6.9	2.2	5.9	1.5	4.2	1.7	5.1	1.6	5.1
12.5	Soups and broths	10 ^(c)	1.7	10.8	2.9	11.4	2.4	8.3	0.5	2.4	0.4	2.0	0.2	1.0
12.6	Sauces	50,000 ^(c)	5.4	12.6	6.3	16.9	31.5	99.8	28.7	96.1	26.1	90.1	23.3	80.0
12.8	Yeast and yeast products	44 ^(c)	0.3	1.7	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.1	< 0.01
14.1.2	Fruit and vegetable juices	880 ^(d)	5.1	11.6	5.5	14.2	5.2	11.8	4.3	10.2	2.5	5.9	2.0	3.9
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	880 ^(d)					7.3	13.5	3.9	7.6	5.6	21.6	2.2	5.2

Food category number	Food category name	High level ^(b) used in the calculation (in mg/kg)	Infants		Toddlers		Children		Adolescents		Adults		The elderly	
			Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
14.1.4	Flavoured drinks	12,700 ^(d)	178.0	331.3	135.2	254.0	91.0	224.1	66.8	155.5	51.7	120.0	34.6	80.4
16	Desserts excluding products covered in category 1, 3 and 4	6 ^(c)			2.5	12.9	0.3	2.2	0.5	1.6	1.6	3.1	0.2	1.9

p95: 95th percentile.

(a): Acute exposure was calculated by combining a high concentration of glycerol (E 422) with the consumed amount of the food category per individual and meal.

(b): Max of the usage data or p95 of the analytical data.

(c): Usage levels.

(d): Analytical data.

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Data collection on co-formulants used in representative plant protection product formulations in the context of the EFSA peer review process for approval/renewal of approval of active substances

European Food Safety Authority (EFSA)

Abstract

EFSA collected data on co-formulants contained in plant protection product (PPP) formulations for representative uses as part of applications for approval or renewal of active substances, to collate the information available during the peer review process. Information was extracted from pesticide active substances dossiers for which a peer review output was issued between January 2019 and March 2022, in which a total of 182 co-formulants were found. Most of the data submitted on the identity and physicochemical properties of the co-formulants are complete as required by Regulation (EU) 284/2013. The data related to (eco)toxicological properties mainly refers to the safety data sheet of the individual co-formulants and, if available, to registration dossiers under Regulation (EC) 1907/2006 (REACH). Fifty-three % of the identified co-formulants have been registered under REACH and about 80% of the 53% are registered for more than 1000 tonnes a year for which registrants are required to submit the most complete data set under REACH. For the remaining percentage, a more limited range of toxicological information is available: ECHA investigated whether they are exempted from registration under REACH. Co-formulants contained in plant protection products may also be subject to other European legislation such as the regulations on biocides, food/feed additives, food contact materials, or are included in lists of excipients or cosmetic ingredients. Observations highlighted in the present report can serve Member State risk assessors and risk managers to harmonise the assessment of co-formulants in collaboration with the EU agencies (EFSA, ECHA).

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Key words: co-formulants, peer review, risk assessment, representative plant protection product formulations, pesticide

Requestor: EFSA

Question number: EFSA-Q-2022-00487

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Amendment: Further investigations have been conducted with the collaboration of ECHA leading to the addition of Annex A on the outcome of the ECHA's compliance check, examination of testing proposals and substance evaluation on the REACH registration dossiers of the 61 identified co-formulants and Annex B on eligibility for potential exemptions from registration under REACH for the 45 co-formulants not regulated under EU legal frameworks (listed in Appendix D).

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1. Introduction

Regulation (EC) No 1107/2009¹ (hereafter 'the PPP Regulation') provides for a two-step system for the regulation of plant protection products in the European Union (EU): EFSA and Member States (MSs) evaluate active substances used in plant protection products (PPPs) and the formulation(s) for representative use(s) submitted in application dossiers, and MSs evaluate and authorise all PPPs uses at national level, as set out in Chapter II (active substance) and Chapter III (Plant Protection Products) of the PPP Regulation. Before an active substance can be used in a plant protection product in the EU, it must be approved by the European Commission. Approvals are limited in time and a new evaluation is conducted before an approval is renewed.

The dossier submitted by an applicant supporting the (renewal of) approval of an active substance, which includes information for at least one formulated product for the supported representative use(s), is subject to a first assessment by the Rapporteur Member State (RMS) and, if applicable, a co-RMS, which issue a draft or renewal assessment report (DAR/RAR). EFSA then carries out a peer review of the DAR/RAR in collaboration with Member States and provides independent scientific advice issuing an EFSA Conclusion. Finally, risk managers (European Commission and Member States) take a regulatory decision whether or not to approve the substance taking into account the EFSA output, which includes all background documents and the conclusion.

Following the approval of the active substance, producers can submit applications to Member States for authorisation of uses of plant protection product(s) containing the approved active substances. The concerned national competent authorities perform the risk assessments for the intended uses of PPPs, including their co-formulants, and decide on the authorisation at national level. The composition of authorised PPPs may change over time: Article 45 of the PPP Regulation covers the amendments on request of the authorisation holder, including a change in the chemical composition of a plant protection product after authorisation.

On February 2021, EFSA received a letter co-signed by more than 100 political leaders regarding the role of taking into account the cumulative effects of substances in pesticide formulations (EFSA, 2022). From June to September 2021, discussions between non-governmental organisations (NGOs), Members of the European Parliament (MEPs) and EFSA took place to provide clarifications on the methods and data used to establish the safety of formulations of pesticides (letters and minutes of the technical meetings available on the EFSA website (EFSA, 2022)).

On March 2021, Regulation (EU) 2021/383² included 144 substances into Annex III to the PPP Regulation, which are not acceptable for inclusion as co-formulants in plant protection products as soon as possible but no later than 24 March 2023.

Requirements regarding co-formulants contained in plant protection products for representative use(s) in the EFSA peer review process:

In Article 2(3) of the Regulation, co-formulants are defined as 'substances or preparations which are used or intended to be used in a plant protection product or adjuvant but are neither active substances nor safeners or synergists'. In the EU peer review framework, co-formulants in a product for the representative use(s) must be declared by the applicant(s) in the application for (renewal of) approval of an active substance. They should be assessed, and the results included by the RMS in the DAR or RAR.

¹ Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1-50.

² Commission Regulation (EU) 2021/383 of 3 March 2021 amending Annex III to Regulation (EC) No 1107/2009 of the European Parliament and of the Council listing co-formulants which are not accepted for inclusion in plant protection products. OJ L 74, 4.3.2021, p. 7-26.

Data requirements on co-formulants are defined in Regulation (EU) 284/2013³:

- in Section 1 of Part A and Part B on identity of the plant protection product (sections 1.4.1 and 1.4.3.) data to allow correct identification of a co-formulant, e.g. chemical name, content, structure, CAS and EC numbers and for co-formulants which are mixtures, the composition shall be provided. Also, the safety data sheet (SDS), the function and the description of the formulation process shall be indicated in the dossier.
- in Section 5 of Part A on the analytical methods (section 5.1.1) for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities.
- in Section 7 of Part A and Part B on toxicological studies (section 7.4), the registration number in accordance with Article 20(3) of Regulation (EC) No 1907/2006, the study summaries included in the technical dossier submitted in accordance with Article 10(a)(vi) of Regulation (EC) No 1907/2006⁴ and the safety data sheet referred to in Article 31 of Regulation (EC) No 1907/2006.
- Furthermore, point 1.11 of the Introduction to the Annex empowers Member States to require further information on co-formulants to the same extent as for active substances as set out in Commission Regulation (EU) No 283/2013.

1.1. Terms of Reference as provided by EFSA

EFSA initiated a data collection related to co-formulants contained in plant protection products for representative use(s) in the context of applications for (renewal of) approval of active substances, with the aim of providing an overview of the available data and the main considerations for the assessment of co-formulants made during the pesticide peer review process. The data collection aimed at addressing the following points:

- 1) Which co-formulants are present in the plant protection products for representative use(s) submitted by the applicant(s) and are identified in the assessment reports prepared by the RMS and co-RMS during the EFSA peer review process?
- 2) What are the main comments and aspects examined in the assessment of co-formulants during the EFSA peer review process?
- 3) Which EU regulatory frameworks other than pesticides applies to the substances which are used as co-formulants in the plant protection products for representative use(s)?

EFSA undertook a collection of main data on co-formulants contained in all plant protection products for representative use(s) of all active substances dossiers for which an EFSA output was finalised between January 2019 and March 2022. The timeframe was identified to provide an overview of the most recent applications assessed by EFSA and the MSs during the peer review process. All the information collected was compiled into a database. The main findings were presented to the SCoPAFF (Standing Committee on Plants, Animals, Food and Feed)⁵ in its meeting on 17-18 May 2022, before publication in August 2022.

³ Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 85–152.

⁴ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

⁵ The PAFF Committee on pesticide is composed of representatives of Member States risk managers and presided by the European Commission representative. Discussions are regularly organised on various topics related to pesticides, including voting on the European Commission proposal for an approval/non-approval of a pesticide active substance.

2. Data and Methodologies

2.1. Collection of data submitted on pesticide co-formulants in the plant protection products for representative use(s) and overview of their assessment during the EFSA peer review process

In the dossiers submitted by the applicant(s), the document J includes all the confidential information related to co-formulants: sections 'CP 1.4.3. information on safeners, synergists and co-formulants', 'CP 7.4 available toxicological data relating to co-formulants', as well as the safety data sheets for the co-formulants and for the formulated plant protection product. The sections are structured as presented in the Annex to Regulation (EU) 284/2013 and as recommended in the SANCO Guidance (European Commission, 2021b). In the case of pesticide dossiers with multiple applicants, separate Volumes 4 are prepared for confidentiality reasons.

On the basis of the information provided by the applicant(s) in the document J, the RMS and co-RMS evaluate and prepare the confidential Volume 4 of the DAR or RAR submitted to EFSA for the peer review process.

For the data collection reported in this document, information on co-formulants was retrieved from Volume 4 of the DAR or RAR and from the safety data sheets for the substances for which a pesticide peer review output was issued from January 2019 to March 2022. The information was collected from the final version of Volumes 4, in cases where revisions were needed during the peer review process. The following information was collected:

- Information on the identity of each co-formulant: chemical name, CAS number, EC number,
- Function of the co-formulant declared by the applicant,
- Number of co-formulants used in PPPs for representative uses,
- Type of data submitted.

During the peer review process, Member States, applicant(s), the public and EFSA⁶ are invited to submit comments on the content and the RMS's conclusions of the DAR or RAR. These comments are compiled and made available as background documents. For each selected dossier, comments provided by Member States, applicant(s) and EFSA related to co-formulants section are retrievable in the background documents.

In this exercise, it is acknowledged that some information reported in Volume 4 must be kept confidential in accordance with Article 63(2) of Regulation (EC) No 1107/2009. For this reason, the complete composition of the representative products and the link between a co-formulant and its representative product or active substance are not disclosed in this Technical Report.

2.2. Relevant data sources from other EU legal frameworks for the assessment of co-formulants

Substances used as co-formulants may also be subject to other EU regulations. Data and assessments on these substances may also be relevant for the evaluation of co-formulants contained in the pesticide products. Therefore, for those substances declared as co-formulants in products for the representative use(s) it was examined if they are regulated already by other legal frameworks and had already been registered or approved (see Table 1).

⁶ Because confidential information is reported in Volume 4 and its disclosure might undermine the commercial interest of the applicant(s), the public is not invited to provide comments on Volumes 4 of the assessment report. However, the sanitised version of the confidential background documents is made available to the public on the EFSA public interface [Open EFSA](#), at the end of the peer review process for pre-Transparency Regulation applications. The comments provided by Member States, the applicant(s) and EFSA during the peer review process can be retrieved in the 'peer review report' which is the compilation of all the comments (and follow-up actions) provided during the peer review process and experts' discussions.

Table 1: EU legal framework in which co-formulants may be regulated

Regulated substance	Source ^(a)
Chemical	- Regulation (EC) No 1907/2006⁷ (REACH Regulation) ECHA website: registered Substances Factsheets (ECHA, 2022a); dossier Evaluation status (ECHA, 2022b)
Biocide active substance	- Regulation (EU) No 528/2012⁸ (BPR) ECHA website: database on biocide active substances (ECHA, 2022c)
Cosmetic ingredient	- Regulation (EC) No 1223/2009⁹ : - Annex II: list of substances prohibited in cosmetic products - Annex III: list of substances which cosmetic products must not contain except subject to the restrictions laid down - Annex IV: list of colorants allowed in cosmetic products - Annex V: list of preservatives allowed in cosmetic products - Annex VI: list of UV filters allowed in cosmetic products EC website: cosmetic ingredient database (CosIng) (European Commission, 2022a) - The European Union list of fragrance allergens requiring labelling on cosmetic and detergent products (EMA, 2017)
Feed additive ^(b)	Regulation (EC) No 1831/2003¹⁰ on feed additives for use in animal nutrition, in particular the list of approved feed additives (Annex I)
Food additive ^(b)	- Regulation (EC) No 1333/2008¹¹ (Annex II) - Regulation (EU) No 231/2012¹² laying down specifications for food additives listed in Annex II and III to Regulation (EC) 1333/2008 EC website: Food additive database (European Commission, 2022b)
Food contact material ^(b)	- Directive 2007/42/EC¹³ (Annex II) relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs - Regulation (EU) 2011/10¹⁴ (Annex I) on plastic materials and articles intended to come into contact with food
Excipient with a known action or effect	EMA website: Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668) (European Commission, 2019)
	C&L Inventory:

⁷ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

⁸ Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1–123.

⁹ Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59–209.

¹⁰ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29–43.

¹¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

¹² Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

¹³ Commission Directive 2007/42/EC of 29 June 2007 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. OJ L 172, 30.6.2007, p. 71–82.

¹⁴ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

Classification under CLP regulation	– Harmonised classification according to Annex VI of Regulation (EC) No 1227/2008¹⁵ (CLP Regulation). – Classification provided by companies to ECHA in REACH registrations joint submission
Listed as co-formulants which are not accepted for inclusion in PPPs as referred to in Article 27	• Commission Regulation (EU) 2021/383 of 3 March 2021 amending Annex III to Regulation (EC) No 1107/2009 of the European Parliament and of the Council listing co-formulants which are not accepted for inclusion in plant protection products. OJ L 74, 4.3.2021, p. 7–26.

^(a) The information from the sources listed in Table 1 was collected in the period between March 2022 and April 2022.

^(b) Data on food additives, feed additives or food contact materials are directly accessible to EFSA.

For all the co-formulants contained in the products and identified for this report, the registration status under REACH was verified. A possible link to other EU regulatory frameworks was also examined.

From October 2022 to February 2023, ECHA conducted further investigations of the outcome of the dossier and substance evaluations of co-formulants registered under REACH and on the potential exemptions applicable to co-formulants not regulated by any EU legal framework.

¹⁵ Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

3. Results

3.1. Overview of data submitted on co-formulants in the framework of Regulation (EC) No 1107/2009

3.1.1. Key figures on pesticide co-formulants

General overview

DAR or RAR for which a pesticide peer review output was issued from January 2019 to March 2022 cover a total of 58 active substances and 82 plant protection products for representative use(s), containing a total of 182 declared co-formulants. The list of the 182 co-formulants is available in **Appendix A¹⁶**.

In the co-formulants-list, the chemical name, CAS number and EC number are indicated as well as the frequency with which the substance was indicated as co-formulant in a product for representative use(s). The most frequently declared co-formulants are reported in Table 2. For example, aromatic hydrocarbon (solvent naphtha (petroleum), heavy aromatic) was declared as a co-formulant in 11 PPPs for representative use(s).

Table 2: Most frequently declared co-formulants in representative products (≥5 times)

Chemical name ^(a)	CAS number	EC number	Frequency of indication as a co-formulant
Aromatic hydrocarbon (solvent naphtha (petroleum), heavy aromatic)	64742-94-5	265-198-5	11
1,2-benzisothiazol-3(2H)-one	2634-33-5	220-120-9	10
1,2-propanediol (Propane-1,2-diol)	57-55-6	200-338-0	10
Xanthan gum	11138-66-2	234-394-2	9
Silicon dioxide	7631-86-9	231-545-4	8
Lignosulfonic acid, sodium salt	8061-51-6	617-124-1	7
Sodium hydroxide (sodium salt)	1310-73-2	215-185-5	7
Silicon dioxide	112926-00-8	601-214-2	7
Lignosulfonic acid, calcium salt	8061-52-7	617-125-7	6
Hydrated aluminium silicate: Kaolin clay	1332-58-7	310-194-1	6
Sorbitan monooleate	1338-43-8	215-665-4	5
2-ethylhexan-1-ol	104-76-7	203-234-3	5

^(a) As distinct chemical names, CAS numbers and EC numbers can be linked to different substances, co-formulants were identified by the unique combination of CAS and EC numbers for accurate substance identification (SID). Finally, the most common chemical names have been reported.

¹⁶ It is noted that one substance was identified as a safener and was not included in the list according to the definition of co-formulants as referred in Article 2(3)(c) of the Regulation 'substances or preparations which are used or intended to be used in a plant protection product or adjuvant, but are neither active substances nor SAFENERS or synergists, referred as 'co-formulants'.'

Declared functions of co-formulants

According to Regulation (EU) 284/2013, the function of a co-formulant shall be specified from among a list of 22 functions¹⁷.

The same co-formulant can be declared in several products for representative use(s) and a different function can be claimed. A total of 182 co-formulants were listed: counting duplicates for different functions, 339 entries (one entry corresponding to a substance associated with a single function) have been recorded in the database.

The most declared functions are reported in Table 3.

Table 3: Most frequently declared function of co-formulants

Function of the co-formulant ^(a)	Number of times the type of function has been declared
Wetting agent (synonym surfactant)	47
Emulsifier	42
Dispersing agent	27
Solvent	25
Preservative	22
Carrier	21

A definition of the above functions is provided by ECHA for the co-formulants intended to be used in biocidal products (ECHA, 2021).

Distribution of co-formulants per representative product

Among the 58 RAR/DARs considered, the number of co-formulants contained in the products for the representative use(s) was counted and an overview is given in Figure 1. As an example, 13 of the 82 products for the representative use(s) contain only one co-formulant.



Figure 1: Distribution of pesticide co-formulants by representative product

The analysis of the concentration of co-formulants in the PPP has not been performed for the time being, and it will be subject of a future activity.

3.1.2. Type of data reported in the pesticide assessment reports

Qualitative information on the data submitted was retrieved from the sections dedicated to co-formulants of Volumes 4 of the DAR or RAR.

The analysis shows that data on substance identity (SID) and use are reported, specifically the chemical name, CAS or EC numbers, trade name, structure and function of the co-formulants. In addition,

¹⁷ adhesive (sticker), antifoaming agent, antifreeze, binder, buffer, carrier, deodorant, dispersing agent, dye, emetic, emulsifier, fertiliser, preservative, odourant, perfume, propellant, repellent, solvent, stabiliser, thickener, wetting agent and miscellaneous.

information on the content (in g/L and/or % w/w) is given for each co-formulant contained in the product for representative use(s).

Physico-chemical data such as molecular mass (g/mol) and molecular formula, are frequently reported in volume 4. SDS data are submitted by the applicant(s) together via the original dossier. Reference to the REACH dossier registration numbers, if available, is made in most Volumes 4.

Finally, the harmonised classification for the (eco)toxicological properties of the co-formulants according to Regulation (EC) No 1272/2008¹⁸ is frequently indicated. References to other EU regulatory status are rarely reported in the selected Volumes 4. It is noted that summaries of (eco)toxicological properties of co-formulants from other regulations are not directly reported in Volumes 4. However, details of (eco)toxicological data can be found on the ECHA website by reference to the REACH dossier registration number. In the REACH registration dossiers, a number of studies are summarised depending on the tonnage-related information requirements. The content of REACH registration dossiers varies from a basic data package (e.g. genotoxicity, acute toxicity studies) to a more extensive one (e.g. long term and repeated dose toxicity studies) (see section 3.3.1). None of the examined dossiers in the scope of this report contained data on co-formulants specifically generated for purposes of the PPP Regulation.

Co-formulants are tested in combination with the active substance in the tests required for the products for representative use(s) in Volume 3 CP B6 (mammalian toxicity) and Volume 3 CP B9 (ecotoxicology) of the assessment report.

3.2. Main comments raised on the assessment of co-formulants during the pesticide peer review process

Based on the comments submitted from Member States, applicant(s) and EFSA during the peer review process, the main comments raised on the evaluation of co-formulants are:

- Substance identification (SID) and composition: request for additional information to correctly identify co-formulants (e.g. when the content in g/L or CAS number is missing).
- Harmonised classification and labelling: if one or more co-formulant(s) have a harmonised classification, the classification and labelling of the product for the representative uses(s) will be calculated according to the requirements of the CLP Regulation, taking into account the presence and the concentrations of all components of the product.
- Hazard assessment (in some cases, risk assessment): request for additional information due to concerns about hazard identification. If this is the case, the necessity to conduct an exposure assessment and/or to set reference values is recommended in the commenting.

Following the commenting phase, further data were requested to the applicant when needed. The additional information when submitted by the applicant(s), was assessed by the RMS and included in a revised version of Volume 4 of the assessment report.

3.3. Source of data on pesticide co-formulants from other EU legal framework than Regulation (EC) No 1107/2009

3.3.1. Co-formulants registered under Regulation (EC) No 1907/2006 (REACH Regulation)

As set out in the REACH Regulation, all substances manufactured or imported above 1 tonne a year must be registered with a registration dossier, unless they are exempted, e.g. substances listed in Annexes IV and V to the REACH Regulation or polymers.

Based on the list of co-formulants retrieved, 96 out of 182 co-formulants have been registered under REACH (registration from 1 tonne a year (circa 53 %)).

¹⁸ Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

REACH registration dossiers contain different levels of data depending on the tonnage. The REACH Regulation defines several tonnage bands, each with the corresponding information requirements (see Table 4).

Table 4: Overview of tonnage band and the respective standard data requirements (ECHA, 2022d)

	Registration for 1 to < 10 tonne a year	Registration for 10 to <100 tonnes a year	Registration for 100 to < 1000 tonnes a year	Registration for 1000 or more tonnes a year
Annexes of the REACH Regulation	Annex VII	Annexes VII + VIII	Annexes VII + VIII + IX	Annexes VII + VIII + IX + X
Overview of data requirements from the Annexes	Physical chemical tests, <i>in vitro</i> (eco) toxicity studies, gene mutation in bacteria, skin sensitisation, short-term toxicity, etc.	<u>In addition to data required in Annex VII:</u> <i>Additional in vitro</i> genotoxicity studies, acute toxicity studies, etc.	<u>In addition to data required in Annex VII and VIII:</u> sub-chronic (eco) toxicity studies, bioaccumulation studies, degradation studies, etc.	<u>In addition to data required in Annex VII, VIII and IX:</u> Long-term repeated dose toxicity studies, Extended One-Generation Reproductive Toxicity, carcinogenicity studies (if triggered), etc.

The tonnage associated with REACH registration dossiers of co-formulants contained in the pesticide products was looked at: about 80% of the co-formulants linked to a REACH registration dossier were identified to be registered for more than 1000 tonnes a year, which means that an extensive data package should be available (see Table 5).

Table 5: Number of co-formulants registered under REACH according to the tonnage band

REACH registration dossiers	Number of co-formulants having a REACH registration dossier (/182)	% of the sub-set of co-formulants registered under REACH
Registration for 1000 tonnes or more a year	77	80.2
Registration for 100 to 1000 tonnes a year	10	10.4
Registration for 10-100 tonnes a year	5	5.2
Registration for 1-10 tonnes a year	1	1.0
Registration as cease manufacture	1	1.0
Registration as intermediate use only ^(a)	2	2.1
Total	96	53

(a) The two co-formulants registered as 'intermediate only' under REACH, clearly not compatible with co-formulant use, are from a pesticide dossier for which the active substance was not approved under Regulation (EC) No 1107/2009.

Under the REACH Regulation, a company manufacturing or importing a substance is responsible for preparing and submitting a REACH registration dossier and keeping it up to date. A screening of all substances registered under REACH is performed by ECHA to identify the ones requiring a compliance check. Based on this prioritisation exercise, further data may be requested (under compliance check procedure) if needed for concluding on the risks (and in accordance with the tonnage bands information requirements). In addition, ECHA evaluates the testing proposals made by the companies when they intended to perform a new test listed in Annexes VI and X to REACH. Finally, when a potential critical concern is identified, an additional evaluation of the substance concerned is performed by an assigned

Member State (substance evaluation). Information on the compliance checks of the REACH registration dossiers, the examination of testing proposals and the substance evaluations have been examined in this report, however several other cross regulation activities are also undertaken by ECHA (e.g. testing proposals ARNs (assessment of regulatory needs for group of substances)).

In the context of this work, for 61 out of 96 co-formulants linked to a REACH registration dossier at least one compliance check, an examination of testing proposals or substance evaluation was performed by ECHA on the entire or a part of the REACH registration dossier. According to the procedure, if a REACH registration dossier is not complete, additional information is requested by ECHA.

The outcome of the compliance checks, examinations of testing proposals and substance evaluations of the 61 co-formulants registered under REACH is available in **Annex A**.

The list of co-formulants registered under REACH is available in **Appendix B**.

3.3.1. Co-formulants subject to other European legislations

For co-formulants contained in the plant protection product for representative use(s), it was investigated under which other legal obligations the substance may be subject to as any data from other regulations may be relevant to the assessment of co-formulants contained in the pesticide products.

From the 86 co-formulants that do not have a REACH registration dossier, 41 co-formulants were regulated by at least one of the following European legislations:

- As a biocide active substance under Regulation (EU) No 528/2012,
- As an ingredient in cosmetic products under Regulation (EC) No 1223/2009,
- As a feed additive under Regulation (EC) No 1831/2003,
- As a food additive under Regulation (EC) No 1333/2008,
- As a food contact material as listed in Annex II of Directive 2007/42/EC or as listed in Annex I of Regulation (EU) 2011/10,
- As excipient with a known action or effect which should be stated on the label and outlines the information for those which must appear on the package leaflet as reported in Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'.
- Listed as unacceptable co-formulants in Annex III of Regulation (EC) No 1107/2009

It was also noted that some co-formulants are also substances intended to be consumed as food (food stuff).

For example, the substance declared as co-formulant 'gum arabic' (CAS number 9000-01-5, EC number 232-519-5) is not registered under REACH, however this substance is listed as approved feed additive (gum arabic, code: E 414) under Regulation (EC) No 1831/2003, approved food additive (ACACIA GUM, code: E 414) under Regulation (EC) No 231/2012, as plastic food contact material in Annex I of Regulation (EU) 2011/10.

The list of co-formulants linked to the EU regulations mentioned above is available in **Appendix C**.

This is a non-exhaustive overview of the regulations under which these co-formulants may be regulated.

The list of the remaining 45 co-formulants not registered under REACH nor as biocides, ingredient in cosmetic products, excipients, feed additives, food additives or food contact materials under the EU legal frameworks is available in **Appendix D**. It must still be verified whether these substances benefit from relevant exemptions under REACH (e.g. natural substance listed in Annex IV or V of REACH, polymer)

ECHA conducted an additional investigation to determine whether exemptions under REACH are potentially applicable on the 45 co-formulants listed in Appendix D (see **Annex B**)

Finally, information on the classification of physical, health and environmental hazards under the CLP Regulation was collected for each co-formulant. The CLP Regulation requires manufacturers and importers in all industrial sectors to notify ECHA of the classification, labelling and packaging of substances and mixtures.

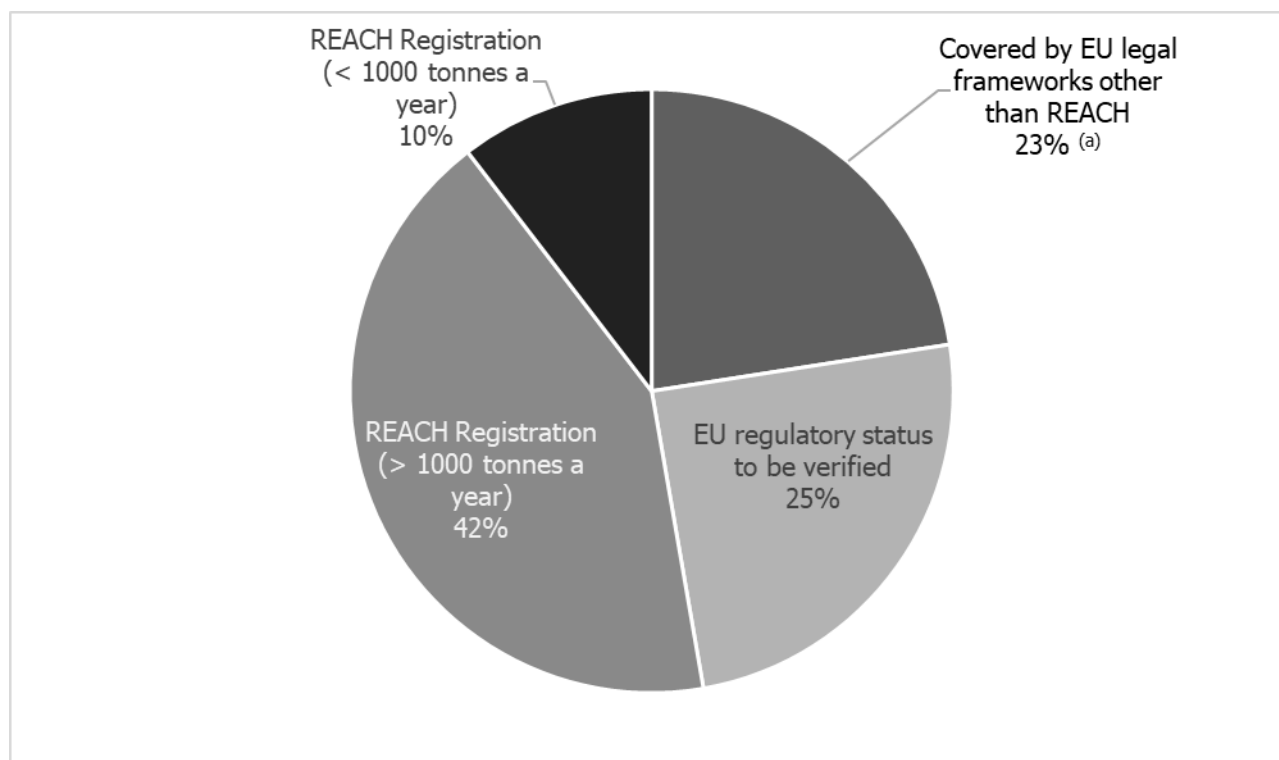
The following information was extracted from the C&L inventory:

- the classification derived from REACH registrations joint submission, notified by manufacturers and importers of substances placed on the EU/EEA market to ECHA. The information is not revised by ECHA and may be subject to change at any time.
- the harmonised classification and labelling (CLH) according to Annex VI of CLP Regulation with the specific concentration limits where applicable. The CLH of a substance may be proposed by Member States, manufacturers, importers or downstream users and can only be revised by Member States. It is a legally binding classification and labelling agreed at EU level.

The harmonised classification and the classification resulting from REACH registration dossiers for each co-formulant are available in **Appendix E**.

4. Conclusions

Key figures on data pertaining to co-formulants were generated from the information submitted for formulated products for representative use(s) contained in the pesticide active substance dossiers. A first descriptive analysis of the data declared by the applicant(s) in the framework of Regulation (EC) No 1107/2009 was provided. The number of co-formulants declared per PPP for representative use(s) ranged from 1 to 13. The most significant data package on co-formulants comes from REACH. Slightly more than half of the examined co-formulants have a REACH registration dossier, most of them are registered for a high tonnage per year, and most were checked by ECHA for compliance with data requirements, testing proposals and some substances of potential concern were re-evaluated by Member States. Figure 2 and Table 6 illustrate the different EU regulatory status of the 182 co-formulants identified in this report, noting that a given substance may be subject to several of EU regulations.



(a) Regulations concerning biocides, food contact materials, feed additives, food additives, listed as ingredient in cosmetic products and/or listed as excipients with a known action or effect.

Figure 2: Overview of regulatory status of co-formulants

Table 6: Overview of co-formulants regulated by EU legal frameworks

Legal framework	% (/182 co-formulants)	Number of co-formulants
Registration under REACH	52.7	96
Approved as Biocide	11.0	20
Approved as food additive	21.4	39
Approved as feed additive	19.2	35
Approved as food contact material	30.2	55
Listed as excipient with a known action or effect	8.2	15
Listed as cosmetic	12.1	22
EU regulatory status to be verified	24.7	45

This data collection represents a baseline for further activities in this area, with the aim of improving and harmonising the evaluation of co-formulants at EU and national level. A better understanding of the roles and responsibilities of the different actors involved in the evaluation of co-formulants and formulations for representative uses is particularly desirable. The EFSA Pesticide Steering Network¹⁹ is one of the relevant fora to progress on this subject and will be involved in this discussion, including the possibility to reinforce the reporting of the assessment of co-formulants in the DAR/RARs and in the EFSA conclusions. It is important to ensure that different legislations operate in a harmonised context, under the principles of One Substance One Assessment²⁰.

¹⁹ The Pesticide Steering Network is the forum set up by EFSA in 2008 to communicate issues to Member States risk assessors. The main goals of the PSN are to improve dialogue among participants, to build mutual understanding of risk assessment principles, to enhance knowledge on and confidence in the scientific assessments carried out in the EU, to provide increased transparency in the current process among Member States and EFSA and to raise the harmonisation level of the risk assessments developed in the EU.

²⁰ In the context of the Chemical Strategy for Sustainability (CSS) as part of the EU Green Deal, one of the objectives set by the European Commission is the simplification and consolidation of the legal frameworks, including the review on how to better use EU's agencies and scientific bodies to move towards a process of 'one substance one assessment' (OSOA). The OSOA concept foresees a central coordination mechanism of the activities across different EU agencies, a better coordination and harmonised methodologies for risk assessment and the access to all available data in the same structured format for all EU authorities.

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Abbreviations

ATE	Acute Toxicity Estimates
BPR	Biocidal Product Regulation
CAS number	Chemical Abstracts Service Registry Number
CLP	Classification and Labelling
CRA	Cumulative Risk Assessment
Co-RMS	Co-Rapporteur Member State
CosIng	Cosmetic Ingredient database
DAR	Draft Assessment Report
DG SANTE	Directorate-General for Health and Food Safety
ECHA	European Chemicals Agency
EC number	European Community number
EEA	European Economic Area
EMA	European Medicines Agency
EU	European Union
GSH	Globally Harmonised System
MEP	Member of the European Parliament
NGO	Non-governmental organizations
OSOA	One Substance One Assessment
PAFF Committee	The Standing Committee on Plants, Animals, Food and Feed
RAR	Renewal Assessment Report
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RMS	Rapporteur Member State
SDS	Safety Data Sheets
SID	Substance Identification

Appendix A – List of co-formulants contained in PPPs for representative uses by frequency of indication as co-formulant

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
1	11	Aromatic hydrocarbon (solvent naphtha (petroleum), heavy aromatic)	64742-94-5	265-198-5
2	10	1,2-benzisothiazol-3(2H)-one	2634-33-5	220-120-9
3	10	1,2-propanediol (Propane-1,2-diol) (Propylene Glycol)	57-55-6	200-338-0
4	9	Xanthan gum	11138-66-2	234-394-2
5	8	Hydrated amorphous silica (silicon dioxide)	7631-86-9	231-545-4
6	7	Sodium hydroxide (sodium salt)	1310-73-2	215-185-5
7	7	Lignosulfonic acid, sodium salt	8061-51-6	617-124-1
8	7	Silicon dioxide	112926-00-8	601-214-2
9	6	Hydrated aluminium silicate: Kaolin clay	1332-58-7	310-194-1
10	6	Lignosulfonic acid, calcium salt	8061-52-7	617-125-7
11	5	2-ethylhexan-1-ol	104-76-7	203-234-3
12	5	Sorbitan monooleate	1338-43-8	215-665-4
13	4	2,6-Di-tert-butyl-4-methylphenol (Butylated hydroxytoluene BHT)	128-37-0	204-881-4
14	4	di-2-ethylhexyl sulfosuccinate of sodium (docusate sodium) (Polyoxyethylene alkylether)	577-11-7	209-406-4
15	4	Castor oil ethoxylate (Ethoxylated vegetable oil)	61791-12-6	500-151-7
16	4	Non-ionic aqueous solution of polydimethylsiloxanes (Polysiloxanes, di-Me)	Not allocated	Not allocated
17	4	Maltodextrin	9050-36-6	232-940-4
18	4	Benzenesulphonic acid, 4-C10-14-alkyl derivs., calcium salts	90194-26-6	290-635-1
19	3	1,2,3-Propanetriol (glycerol)	56-81-5	200-289-5
20	3	Sodium dodecyl sulphate (Sodium lauryl sulfate)	151-21-3	205-788-1
21	3	Flours and sugar-food grade, corn flour, wheat flour	Not allocated	Not allocated
22	3	Sodium 4,7-diisopropyl-naphthalene-2-sulfonate	1322-93-6	215-343-3
23	3	Titanium dioxide	13463-67-7	236-675-5
24	3	3(2H)-Isothiazolone, 5-chloro-2-methyl-, mixt. with 2-methyl-3(2H)-isothiazolone	55965-84-9	611-341-5
25	3	Agnique MBL 530B: Solvent naphtha (petroleum), heavy arom (Heavy aromatic solvent naphtha)	Not allocated	Not allocated
26	3	Anhydrous sodium sulphate	7757-82-6	231-820-9
27	3	Soy lecithin (lecithins)	8002-43-5	232-307-2
28	3	Polysorbate 20, Tween 20: Sorbitan monolaurate, ethoxylated	9005-64-5	500-018-3

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
29	3	Agnique MBL 510H (<i>from a dossier for which the active substance was not approved</i>)	Not allocated	Not allocated
30	3	Fatty alcohol ethoxylate	68131-39-5	500-195-7
31	3	Gum arabic	9000-01-5	232-519-5
32	3	Ethene, homopolymer	9002-88-4	618-339-3
33	2	Naphthalene sulfonic acid, sodium salt	9084-06-4	618-665-6
34	2	(Copolymer of) styrene	100-42-5	202-851-5
35	2	Poly(oxy-1,2-ethanediyl), α -phenyl- ω -hydroxy-, styrenated	104376-75-2	600-560-1
36	2	1-Hexanol	111-27-3	203-852-3
37	2	Methyl laurate	111-82-0	203-911-3
38	2	Ascorbic acid	50-81-7	200-066-2
39	2	(Copolymer of) n-butylacrylate	141-32-2	205-480-7
40	2	Potassium (E,E)-hexa-2,4-dienoate (Potassium sorbate)	24634-61-5	246-376-1
41	2	2-Propenoic acid, 2-methyl-, polymer	25751-21-7	639-553-3
42	2	Polyether	Not allocated	Not allocated
43	2	Toluene diisocyanate	26471-62-5	247-722-4
44	2	2-Methyl-2H-isothiazol-3-on	2682-20-4	220-239-6
45	2	sodium benzoate	532-32-1	208-534-8
46	2	Sucrose (alpha-D-Glucopyranoside, beta-D-Fructofuranosyl) (Saccharose)	57-50-1	200-334-9
47	2	2-Propenoic acid, butyl ester, polymer with 1,4- diethenylbenzene and ethenylbenzene	Not allocated	Not allocated
48	2	Acetic acid	64-19-7	200-580-7
49	2	Ethoxylated alcohol, 90%	34398-01-1	500-84-3
50	2	Alcohols, C12-14, ethoxylated	68439-50-9	500-213-3
51	2	Diatomaceous earth (Kieselguhr)	91053-39-3	293-303-4
52	2	Benzenesulfonic acid, mono-C11-13-branched alkyl derivs., calcium salts	68953-96-8	273-234-6
53	2	Hydrated aluminium magnesium silicate (palygorskite) (attapulgit)	12174-11-7	601-805-5
54	2	Potassium-Di-Hydrogen Phosphate (phosphoric acid, monopotassium salt)	7778-77-0	231-913-4
55	2	Trimethylolpropane (Propylidynetrimethanol)	77-99-6	201-074-9
56	2	Polysaccharide (scleroglucan)	39464-87-4	254-464-6
57	2	Acrylic copolymer in aqueous dispersion	Not allocated	Not allocated
58	2	Benzoic acid, 4-hydroxy-, methyl ester (Methyl 4-hydroxybenzoate) (Methylparaben)	99-76-3	202-785-7
59	2	Polyvinyl alcohol, partly saponified (Acetic Acid Ethenyl Ester Co-Polymer with Ethenol)	25213-24-5	607-648-9
60	2	Skimmed Milk	Not allocated	Not allocated
61	2	Hydrocarbons, C10 aromatics, <1% naphthalene	1189173-42-9	824-107-4

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
62	2	Disodium maleate (<i>from a mixture of sodium polycarboxylate</i>) (<i>from a dossier for which the active substance was not approved</i>)	371-47-1	206-738-1
63	2	Sodium alkyl naphthalene formaldehyde condensate (alkylated naphthalene sulfonate sodium salt)	68425-94-5	614-476-8
64	1	2,2'-iminodi(ethylamine) (N-2-aminoethyl-1,2-ethanediamine) (<i>from a mixture of sodium polycarboxylate</i>) (<i>from a dossier for which the active substance was not approved</i>)	111-40-0	203-865-4
65	1	Yeast	Not allocated	Not allocated
66	1	Polyxypropylene-polyoxyethylene (2-methyloxirane)	9003-11-6	618-355-0
67	1	Dextrose (glucose)	50-99-7	200-075-1
68	1	Carboxymethyl cellulose (CMC)/Cellulose polymer	9004-32-4	618-378-6
69	1	4-nitrophenol (<i>from a dossier for which the active substance was not approved</i>)	100-02-7	202-811-7
70	1	Hexamethylene tetramine	100-97-0	202-905-8
71	1	Sodium alkyl naphthalene sulphonate	203-632-7	Not allocated
72	1	Tetradecanoic acid, isopropyl ester (Isopropyl myristate)	110-27-0	203-751-4
73	1	Hexa-2,4-dienoic acid (sorbic acid)	110-44-1	203-768-7
74	1	Methyl oleate	112-62-9	203-992-5
75	1	Dimethyl ether	115-10-6	204-065-8
76	1	Polyoxyethylene styrylphenyl ether sulfate ammonium salt	119432-41-6	601-612-6
77	1	Mica (aluminium, magnesium, iron and potassium silicates)	Not allocated	Not allocated
78	1	Nonyl aldehyde	124-19-6	204-688-5
79	1	Riboflavin 5'-(sodium hydrogen phosphate)	130-40-5	204-988-6
80	1	Potassium hydroxide	1310-58-3	215-181-3
81	1	5-hydroxy-1,3-dioxane (1,3-dioxan-5-ol)	4740-78-7	225-248-9
82	1	4-hydroxymethyl-1,3-dioxane (1,3-dioxolan-4-ylmethanol)	5464-28-8	226-758-4
83	1	Xylene	1330-20-7	215-535-7
84	1	Pumice	1332-09-8	603-719-3
85	1	Benzenesulfonic acid, C10-13-alkyl derivs., calcium salt	90194-36-8	290-646-1
86	1	Ammonium hydroxide	1336-21-6	215-647-6
87	1	Oxirane, 2-methyl-, polymer with oxirane, mono[3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]-1-disilo-xanyl]propyl] ether	134180-76-0	603-798-4
88	1	bis(2-ethylhexyl) maleate	142-16-5	205-524-5
89	1	Sodium L-glutamate	16177-21-2	240-313-1

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
90	1	D-glucose mono-hydrate / dextrose monohydrate	14431-43-7	604-408-5
91	1	Copper phthalocyanine	147-14-8	205-685-1
92	1	Magnesium silicate	14807-96-6	238-877-9
93	1	Calcium stearate (calcium distearate)	1592-23-0	216-472-8
94	1	2-tert-Butylhydroquinone	1948-33-0	217-752-2
95	1	Octadecanoic acid, 2-ethylhexyl ester	22047-49-0	244-754-0
96	1	Polypropyleneglycol	25322-69-4	500-039-8
97	1	benzenesulfonic acid, dodecyl-, calcium salt	26264-06-2	247-557-8
98	1	Sodium methylene polymethylnaphthyl sulphonate (Naphthalenesulfonic acid, methyl-, polymer with formaldehyde, sodium salt)	81065-51-2	617-192-2
99	1	Polyalkyleneoxide modified heptamethyltrisiloxane (3-(2-methoxyethoxy)propyl-methyl-bis(trimethylsilyloxy)silane)	27306-78-1	608-078-3
100	1	Black Dye, Tris(N,N,N-tributyl-1-butanaminium)[[2,2''6',2''-terpyridine]-4,4',4''-tricarboxylato(3-)-N1,N1',N1'']tris(thiocyanato-N)hydrogen ruthenate	Not allocated	Not allocated
101	1	2-(3-tert-butyl-2-hydroxy-5-methylphenyl)-5-chloro-2H-benzotriazole (Bumetrizole)	3896-11-5	223-445-4
102	1	Calcium dipropionate	4075-81-4	223-795-8
103	1	3,5,7-Triaza-1-azoniatricyclo [3.3.1.1 ^{3,7}]decane ,1-(3-chloro-2-propenyl)-, chloride (Methenamine 3-chloroallylochloride)	4080-31-3	223-805-0
104	1	Calcite (calcium carbonate)	471-34-1	207-439-9
105	1	2-Bromo-2-nitropropane-1,3-diol (Bronopol)	52-51-7	200-143-0
106	1	Aminoacetic acid (glycine)	56-40-6	200-272-2
107	1	Polyurea	Not allocated	Not allocated
108	1	Polyoxyethylene sorbitol hexaeloate	57171-56-9	611-475-4
109	1	Edetic acid (EDTA) - Ethylenediaminetetraacetic acid	60-00-4	200-449-4
110	1	Ethoxylated lanolin	61790-81-6	612-384-2
111	1	Ethanol	64-17-5	200-578-6
112	1	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-88-4	265-090-8
113	1	Petroleum distillate, solvent refined hydrotreated light paraffinic oil	64742-55-8	265-158-7
114	1	2-Naphthalenecarboxamide, 3-hydroxy-N-(2-methylphenyl)-4-[2-(2,4,5-trichlorophenyl)diazenyl]-	6535-46-2	229-440-3
115	1	Benzoic Acid	65-85-0	200-618-2
116	1	2-propanol	67-63-0	200-661-7

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
117	1	Polyoxyethylene alkyl ether sulfate ammonium salt (Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-[bis(1-phenylethyl)phenoxy]-, ammonium salt)	59891-11-1	680-224-9
118	1	Fatty acids, C16-18 and C18-unsaturated, Me esters	67762-38-3	267-015-4
119	1	Polyglycol esters (Soitem 251/C)	Not allocated	Not allocated
120	1	Fatty acids, tall-oil, ethoxylated, propoxylated (Ethylene oxide, propylene oxide copolymer, tall oil fatty acid ester)	67784-86-5	614-134-8
121	1	Alkylpolyglycoside (D-Glucopyranose, oligomers, decyl octyl glycosides)	68515-73-1	500-220-1
122	1	Oxirane, methyl-, polymer with oxirane, monobutyl ether	9038-95-3	618-542-7
123	1	Tetrapotassium pyrophosphate	7320-34-5	230-785-7
124	1	Ethylene oxide	75-21-8	200-849-9
125	1	Propylene oxide (Methyloxirane)	75-56-9	200-879-2
126	1	Di-Sodium Hydrogen Phosphate	7558-79-4	231-448-7
127	1	Sodium Chloride	7647-14-5	231-598-3
128	1	DL-alpha-tocopheryl acetate	7695-91-2	231-710-0
129	1	Ammonium sulphate	7783-20-2	231-984-1
130	1	Anhydrous citric acid	77-92-9	201-069-1
131	1	Iso-tridecyl alcohol polyglycol ether with 8 EO (Alcohols, C11-14-iso-, C13-rich, ethoxylated)	78330-21-9	934-084-3
132	1	3,6-dimethyloct-4-yne-3,6-diol	78-66-0	201-131-8
133	1	Isobutanol (2-methylpropan-1-ol)	78-83-1	201-148-0
134	1	Propionic acid	79-09-4	201-176-3
135	1	Malt	Not allocated	Not allocated
136	1	Highly refined mineral oil	8042-47-5	232-455-8
137	1	9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylum chloride (<i>from a dossier for which the active substance was not approved</i>)	81-88-9	201-383-9
138	1	Gelatins	9000-70-8	232-554-6
139	1	Dodecylethoxylate (Dodecylphenol, ethoxylated)	9014-92-0	500-023-0
140	1	Ethoxylated sorbitan monooleate	9005-65-6	500-019-9
141	1	Polyoxyethylene Sorbitan Monostearate	9005-67-8	500-020-4
142	1	Polyoxyethylene tridecyl ether phosphate	9046-01-9	618-558-4
143	1	Glycerides, palm-oil mono-, hydrogenated	67254-73-3	614-041-2
144	1	Propylparaben (propyl 4-hydroxybenzoate)	94-13-3	202-307-7
145	1	Vegetable oil (sunflower oil refined)	84625-38-7	283-413-0
146	1	Lauroyl N-methylglycine (N-lauroylsarcosine)	97-78-9	202-608-3

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
147	1	Polyarylphenol ethoxylate propoxylate (block polymer)	Not allocated	Not allocated
148	1	Barley grain	Not allocated	Not allocated
149	1	Corn syrup solids	68131-37-3	268-616-4
150	1	diethyl malonate	105-53-3	203-305-9
151	1	linalool	78-70-6	201-134-4
152	1	d-limonene	5989-27-5	227-813-5
153	1	4-(4-hydroxyphenyl)butan-2-one	5471-51-2	226-806-4
154	1	hexyl acetate	142-92-7	205-572-7
155	1	Potato flakes	Not allocated	Not allocated
156	1	Reaction mass of dimethyl adipate and dimethyl glutarate and dimethyl succinate	Not allocated	906-170-0
157	1	Sawdust	Not allocated	Not allocated
158	1	Sodium salt of sulphonated kraft lignin	Not allocated	Not allocated
159	1	Bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate	52829-07-9	258-207-9
160	1	2H-1,3,5-oxadiazine-2,4,6(3H,5H)-trione,3,5-bis(6-isocyanatohexyl)-polymer (Polyharnstoff to 2:1-D)	Not allocated	Not allocated
161	1	Cellulose (Ahlstom #601 Filter Paper)	9004-34-6	232-674-9
162	1	Antifoam emulsion (<i>the chemical, CAS/EC numbers or trade name was not reported in Volume 4/SDS data</i>)	Not allocated	Not allocated
163	1	Benzyloxy methanol	14548-60-8	238-588-8
164	1	Citronellol	106-22-9	203-375-0
165	1	D-Glucose, hydrate	77938-63-7	616-580-9
166	1	Ethylbenzene	100-41-4	202-849-4
167	1	Ethylenediaminetetraacetic acid (EDTA), tetrasodium salt	64-02-8	200-573-9
168	1	Polyethylene terephthalate polymer (Poly(oxy-1,2-ethanedioylcarbonyl-1,4-phenylenecarbonyl))	25038-59-9	607-507-1
169	1	Heavy aromatic solvent naphtha	Not allocated	938-406-3
170	1	Naphthalene	91-20-3	202-049-5
171	1	Polyethylene glycol mono (tristylphenyl) ether (tristylphenyl) ether (Poly(oxy-1,2-ethanediyl), α-[tris(1-phenylethyl)phenyl]-ω-hydroxy-)	99734-09-5	619-457-8
172	1	Propylene carbonate	108-32-7	203-572-1
173	1	2-methoxyethanol	9004-74-4	618-394-3
174	1	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-89-5	265-091-3
175	1	Solvesso 100: Solvent naphtha (petroleum), light arom.	64742-95-6	265-199-0
176	1	Soybean oil, epoxidised	8013-07-8	232-391-0
177	1	Styrene/(Meth)Acrylate-Copolymer	27136-15-8	608-050-0
178	1	α-methyl-ω-hydroxy poly(oxy-1,2-ethandiyl)	9003-13-8	500-003-1

N°	Frequency of indication as co-formulant	Chemical name ^(a)	CAS number	EC / List No
179	1	Dibutyl-naphthalene sulfonic acid, sodium salt	25417-20-3	246-960-6
180	1	Silicic acid, calcium salt	1344-95-2	215-710-8
181	1	Trehalose (α -D-Glucopyranoside, α -D-glucopyranosyl, hydrate (1:2))	6138-23-4	612-140-5
182	1	Sodium dioctyl sulfosuccinate	577-11-7	209-406-4

(a) As several chemical names can be associated with the same CAS and/or EC number, the most common chemical names have been reported.

Appendix B – List of co-formulants registered under REACH (and the status of the dossier evaluation including the compliance check and the examination of testing proposals)

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
1	Sodium hydroxide (sodium salt)	1310-73-2	≥ 10 000 000 to < 100 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15566	No	- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
2	Fatty acids, C16-18 and C18-unsaturated, Me esters	67762-38-3	≥ 10 000 000 to < 100 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15554	Yes	None
3	Sodium dodecyl sulphate (Sodium lauryl sulfate)	151-21-3	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2126	Yes	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
4	Hexamethylene tetramine	100-97-0	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15907	No	- approved as preservative (Annex V of Regulation (EC) No 1223/2009) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
5	1-Hexanol	111-27-3	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13265	Yes	None
6	Methyl laurate	111-82-0	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14160	No	None
7	Dimethyl ether	115-10-6	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15974	Yes	None
8	2,6-Di-tert-butyl-4-methylphenol (Butylated hydroxytoluene BHT)	128-37-0	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15975	Yes	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as feed additive (Regulation (EC) No 1831/2003)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
						- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10) - listed as food contact material (Directive 2007/42/EC)
9	Potassium hydroxide	1310-58-3	≥ 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15804	Yes	- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
10	Copper phthalocyanine	147-14-8	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15491	Yes	- approved as colorant (Annex IV of Regulation (EC) No 1223/2009) - listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009
11	Polypropyleneglycol	25322-69-4	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15083	No	None
12	sodium benzoate	532-32-1	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15083	Yes	- approved as biocide (Regulation (EU) No 528/2012)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
				ration-dossier/-/registered-dossier/14966		- approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Directive 2007/42/EC)
13	Alkylpolyglycoside (D-Glucopyranose, oligomers, decyl octyl glycosides)	68515-73-1	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14947	Yes	None
14	2,2'-iminodi(ethylamine) (N-2-aminoethyl-1,2-ethanediamine) (<i>from a mixture of sodium polycarboxylate</i>) (<i>from a dossier for which the active substance was not approved</i>)	111-40-0	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15883	Yes	- listed as food contact material (Regulation (EU) 2011/10)
15	Tetrapotassium pyrophosphate	7320-34-5	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-	Yes	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
16	Di-Sodium Hydrogen Phosphate	7558-79-4	≥ 10 000 to < 100 000 tonnes	/registered-dossier/14700 https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14974	Yes	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
17	Anhydrous citric acid	77-92-9	≥ 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15451	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10) - listed as food contact material (Directive 2007/42/EC)
18	Trimethylolpropane (Propylidynetrimethanol)	77-99-6	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15407	Yes	- listed as food contact material (Regulation (EU) 2011/10)
19	Isobutanol (2-methylpropan-1-ol)	78-83-1	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15092	Yes	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
20	Highly refined mineral oil	8042-47-5	≥ 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15514	Yes	- pesticide active substance (Regulation (EC) No 1107/2009)
21	Reaction mass of dimethyl adipate and dimethyl glutarate and dimethyl succinate	Not allocated	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14563	Yes	None
22	Ethylenediaminetetraacetic acid (EDTA), tetrasodium salt	64-02-8	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15231	Yes	None
23	Propylene carbonate	108-32-7	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/16088	Yes	None
24	Soybean oil, epoxidised	8013-07-8	≥ 10 000 to < 100 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15408	Yes	- listed as food contact material (Regulation (EU) 2011/10)
25	(Copolymer of) styrene	100-42-5	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-	Yes	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
				/registered-dossier/15565		- approved as feed additive (Regulation (EC) No 1831/2003) - listed as food contact material (Regulation (EU) 2011/10)
26	Toluene diisocyanate	26471-62-5	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15073	Yes	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009
27	Calcite (calcium carbonate)	471-34-1	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16050	Yes	- approved as colorant (Annex IV of Regulation (EC) No 1223/2009) - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Directive 2007/42/EC) - pesticide active substance (Regulation (EC) No 1107/2009)
28	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-88-4	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15697	No	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009 - listed in Annex III on co-formulants which are not accepted for inclusion in plant protection products as referred to in Article 27 of Regulation (EC) 1107/2009
29	Petroleum distillate, solvent refined hydrotreated light paraffinic oil	64742-55-8	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15697	No	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
				/registered-dossier/15621		- listed in Annex III on co-formulants which are not accepted for inclusion in plant protection products as referred to in Article 27 of Regulation (EC) 1107/2009
30	propylene oxide (Methyloxirane)	75-56-9	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/16091	Yes	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009 - listed as food contact material (Regulation (EU) 2011/10)
31	Ammonium sulphate	7783-20-2	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15571	No	- initial application for approval in progress as biocide (Regulation (EU) No 528/2012) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
32	Ethylbenzene	100-41-4	≥ 1 000 000 to < 10 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15377	No	None
33	1,2,3-Propanetriol (glycerol)	56-81-5	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14481	Yes	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - listed as food contact material (Directive 2007/42/EC) - listed as food contact material (Regulation (EU) 2011/10)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
34	Tetradecanoic acid, isopropyl ester (Isopropyl myristate)	110-27-0	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16077	Yes	- approved as feed additive (Regulation (EC) No 1831/2003)
35	Hexa-2,4-dienoic acid (sorbic acid)	110-44-1	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11476	No	- initial application for approval in progress as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10) - listed as food contact material (Directive 2007/42/EC)
36	Xylene	1330-20-7	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15448	Yes	None
37	bis(2-ethylhexyl) maleate	142-16-5	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15448	Yes	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
38	Magnesium silicate	14807-96-6	≥ 1 000 to < 10 000 tonnes	/registered-dossier/14675 https://echa.europa.eu/de/registration-dossier/-/registered-dossier/18727	Yes	- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10) - pesticide active substance (Regulation (EC) No 1107/2009)
39	Potassium (E,E)-hexa-2,4-dienoate (Potassium sorbate)	24634-61-5	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11008	No	- approved as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
40	2-(3-tert-butyl-2-hydroxy-5-methylphenyl)-5-chloro-2H-benzotriazole (Bumetrizole)	3896-11-5	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5785	Yes	- listed as food contact material (Regulation (EU) 2011/10)
41	Aminoacetic acid (glycine)	56-40-6	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14675	No	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
42	di-2-ethylhexyl sulfosuccinate of sodium (docusate sodium) (Polyoxyethylene alkylether)	577-11-7	≥ 10 000 tonnes	/registered-dossier/14889 https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16066	Yes	- listed as food contact material (Directive 2007/42/EC)
43	2-Naphthalenecarboxamide, 3-hydroxy-N-(2-methylphenyl)-4-[2-(2,4,5-trichlorophenyl)diazanyl]-	6535-46-2	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14453	Yes	- approved as colorant (Annex IV of Regulation (EC) No 1223/2009) - listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009
44	Benzenesulfonic acid, mono-C11-13-branched alkyl derivs., calcium salts	68953-96-8	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14538	No	None
45	DL-alpha-tocopheryl acetate	7695-91-2	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13377	Yes	- approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
46	Benzoic acid, 4-hydroxy-, methyl ester (Methyl 4-hydroxybenzoate) (Methylparaben)	99-76-3	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14310	Yes	- approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
47	linalool	78-70-6	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14501	Yes	- listed as food contact material (Regulation (EU) 2011/10) - listed in the European Union list of fragrance allergens requiring labelling on cosmetic and detergent products - listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009 - approved as feed additive (Regulation (EC) No 1831/2003)
48	d-limonene	5989-27-5	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15256	Yes	- listed in the European Union list of fragrance allergens requiring labelling on cosmetic and detergent products - listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as feed additive (Regulation (EC) No 1831/2003) - pesticide active substance (Regulation (EC) No 1107/2009)
49	hexyl acetate	142-92-7	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13766	Yes	- approved as feed additive (Regulation (EC) No 1831/2003)
50	Citronellol	106-22-9	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13766	Yes	- listed in the European Union list of fragrance allergens requiring labelling on cosmetic and detergent products

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
				/registered-dossier/14242		- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as feed additive (Regulation (EC) No 1831/2003)
51	Silicic acid, calcium salt	1344-95-2	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14771	Yes	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
52	α-methyl-ω-hydroxy poly(oxy-1,2-ethandiyl)	9003-13-8	≥ 1 000 to < 10 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/2120	Yes	None
53	2-ethylhexan-1-ol	104-76-7	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15194	Yes	- approved as feed additive (Regulation (EC) No 1831/2003) - listed as food contact material (Regulation (EU) 2011/10)
54	Hydrated amorphous silica (silicon dioxide)	7631-86-9	≥ 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15556	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10) - pesticide active substance (Regulation (EC) No 1107/2009)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
55	Titanium dioxide	13463-67-7	≥ 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15560	Yes	- approved as colorant and UV filter (Annex IV and Annex VI of Regulation (EC) No 1223/2009) - listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
56	(Copolymer of) n-butylacrylate	141-32-2	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15779	Yes	- listed as food contact material (Regulation (EU) 2011/10)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
57	1,2-propanediol (Propane-1,2-diol) (Propylene Glycol)	57-55-6	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16001	Yes	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Directive 2007/42/EC) - listed as food contact material (Regulation (EU) 2011/10)
58	Ethanol	64-17-5	≥ 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16105	Yes	- initial application for approval in progress as biocide (Regulation (EU) No 528/2012) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Directive 2007/42/EC) - listed as food contact material (Regulation (EU) 2011/10)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
59	Acetic acid	64-19-7	≥ 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15549	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Directive 2007/42/EC) - listed as food contact material (Regulation (EU) 2011/10) - pesticide active substance (Regulation (EC) No 1107/2009)
60	Benzoic Acid	65-85-0	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13124	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - pesticide active substance (Regulation (EC) No 1107/2009)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
61	2-propanol	67-63-0	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15339	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as feed additive (Regulation (EC) No 1831/2003)
62	Alcohols, C12-14, ethoxylated	68439-50-9	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16040	Yes	None
63	ethylene oxide	75-21-8	≥ 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15813	No	- initial application for approval in progress as biocide (Regulation (EU) No 528/2012) - listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009 - listed as food contact material (Regulation (EU) 2011/10) - listed in Annex III on co-formulants which are not accepted for inclusion in plant protection products as referred to in Article 27 of Regulation (EC) 1107/2009
64	Sodium Chloride	7647-14-5	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15467	Yes	- pesticide active substance (Regulation (EC) No 1107/2009)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
65	Anhydrous sodium sulphate	7757-82-6	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15539	Yes	None
66	Potassium-Di-Hydrogen Phosphate (phosphoric acid, monopotassium salt)	7778-77-0	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15471	No	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
67	Propionic acid	79-09-4	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14128	Yes	- approved as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - approved as feed additive (Regulation (EC) No 1831/2003) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
68	Aromatic hydrocarbon (solvent naphtha (petroleum), heavy aromatic)	64742-94-5	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15728	Yes	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
69	naphthalene	91-20-3	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15924	Yes	- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation(EC) No 1223/2009
70	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-89-5	≥ 100 000 to < 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15604	No	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009 - listed in Annex III on co-formulants which are not accepted for inclusion in plant protection products as referred to in Article 27 of Regulation (EC) 1107/2009
71	Solvesso 100: Solvent naphtha (petroleum), light arom.	64742-95-6	≥ 1 000 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15237	No	- listed in Annex III on co-formulants which are not accepted for inclusion in plant protection products as referred to in Article 27 of Regulation (EC) 1107/2009
72	Nonyl aldehyde	124-19-6	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5471	No	None
73	Calcium stearate (calcium distearate)	1592-23-0	≥ 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/18930	No	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
74	2-tert- Butylhydroquinone	1948-33-0	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5612	No	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
75	Octadecanoic acid, 2-ethylhexyl ester	22047-49-0	≥ 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13584	No	None
76	benzenesulfonic acid, dodecyl-, calcium salt	26264-06-2	≥ 100 tonnes to < 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/17919	No	None
77	2-Bromo-2-nitropropane-1,3-diol (Bronopol)	52-51-7	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11419	No	- Initial application for approval in progress as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
78	Edetic acid (EDTA) - Ethylenediaminetetraacetic acid	60-00-4	≥ 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14698	Yes	- listed as food contact material (Regulation (EU) 2011/10)
79	Castor oil ethoxylate (Ethoxylated vegetable oil)	61791-12-6	≥ 1 000 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14599	No	- listed as excipient in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
80	Polysorbate 20, Tween 20: Sorbitan monolaurate, ethoxylated	9005-64-5	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13525	No	- approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
81	Propylparaben (propyl 4-hydroxybenzoate)	94-13-3	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13890	Yes	- approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as food contact material (Regulation (EU) 2011/10)
82	Lauroyl N-methylglycine (N-lauroylsarcosine)	97-78-9	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13890	No	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
83	diethyl malonate	105-53-3	≥ 100 to < 1 000 tonnes	/registered-dossier/5710 https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/5774	No	- approved as feed additive (Regulation (EC) No 1831/2003)
84	4-(4-hydroxyphenyl)butan-2-one	5471-51-2	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/12633	No	- approved as feed additive (Regulation (EC) No 1831/2003)
85	Fatty alcohol ethoxylate	68131-39-5	≥ 100 to < 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/12510	Yes	None
86	Bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate	52829-07-9	≥ 1 000 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14267	Yes	None
87	1,2-benzisothiazol-3(2H)-one	2634-33-5	≥ 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/22961/1/2	No	- Initial application for approval in progress as biocide (Regulation (EU) No 528/2012)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
88	2-Methyl-2H-isothiazol-3-on	2682-20-4	≥ 10 to < 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/23868	No	- approved as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009) - listed as food contact material (Regulation (EU) 2011/10)
89	Calcium dipropionate	4075-81-4	≥ 10 to < 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5706	Yes	- approved as preservative (Annex V of Regulation (EC) No 1223/2009) - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012)
90	3(2H)-Isothiazolone, 5-chloro-2-methyl-, mixt. with 2-methyl-3(2H)-isothiazolone	55965-84-9	≥ 10 to < 100 tonnes	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/23870	No	- approved as biocide (Regulation (EU) No 528/2012) - approved as preservative (Annex V of Regulation (EC) No 1223/2009)
91	Polyoxyethylene Sorbitan Monostearate	9005-67-8	≥ 10 to < 100 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/11403	No	None
92	Trehalose (α-D-Glucopyranoside, α-D-glucopyranosyl, hydrate (1:2))	6138-23-4	≥ 10 to < 100 tonnes	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/26864	No	None
93	Sodium 4,7-diisopropyl-naphthalene-2-sulfonate	1322-93-6	≥ 1 to < 10 tonnes	https://echa.europa.eu/de/regist	Yes	None

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name ^(a)	Associated CAS number	Substance registration: total tonnage band	Link to the registration dossier	Dossier evaluation performed on the REACH registration dossier? (Yes/No)	Other EU regulatory status (i.e. as biocides, cosmetics, excipients, feed additives, food additives, food contact materials or pesticide active substances)
94	4-nitrophenol (<i>from a dossier for which the active substance was not approved</i>)	100-02-7	Intermediate use only	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/28175	No	None
95	Ammonium hydroxide	1336-21-6	Cease manufacture	https://echa.europa.eu/de/registration-dossier/-/registered-dossier/7510	No	- listed as substance whose use in cosmetic products in the European Union is banned, except under certain conditions as indicated in Annex III of Regulation (EC) No 1223/2009 - approved as food additive (Regulation (EC) 1333/2008 and Regulation (EU) No 231/2012) - listed as food contact material (Regulation (EU) 2011/10)
96	9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride (<i>from a dossier for which the active substance was not approved</i>)	81-88-9	Intermediate use only	https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/26187	No	- listed as substances prohibited in cosmetic products as indicated in Annex II of Regulation (EU) No 1223/2009

(a) As several chemical names can be associated with the same CAS and/or EC number, the most common chemical names have been reported.

Appendix C – List of co-formulants subject to various EU legal frameworks

N°	Chemical name (a)	CAS number	Approved as biocide	Approved as food additive	Approved as feed additive	Listed as food contact material	Listed as food contact material	Listed as cosmetic	Listed as excipient	Other status
			Regulation (EU) No 528/2012	Regulation (EC) 1333/2008 Regulation (EU) No 231/2012	Regulation (EC) No 1831/2003	Regulation (EU) 2011/10	Directive 2007/42/EC	Regulation (EC) No 1223/2009	Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'	
1	Yeast	Not allocated			X					- food stuff
2	Dextrose (glucose)	50-99-7				X				- food stuff
3	Carboxymethyl cellulose (CMC) /Cellulose polymer	9004-32-4		X	X					
4	Xanthan gum	11138-66-2		X	X					
5	Ascorbic acid	50-81-7	X	X	X	X	X			- pesticide active substance (Regulation (EC) No 1107/2009)
6	Riboflavin 5'- (sodium hydrogen phosphate)	130-40-5		X	X			Colorant (Annex IV)		
7	Flours, sugar- food grade	Not allocated								- food stuff

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name (a)	CAS number	Approved as biocide	Approved as food additive	Approved as feed additive	Listed as food contact material	Listed as food contact material	Listed as cosmetic	Listed as excipient	Other status
			Regulation (EU) No 528/2012	Regulation (EC) 1333/2008 Regulation (EU) No 231/2012	Regulation (EC) No 1831/2003	Regulation (EU) 2011/10	Directive 2007/42/EC	Regulation (EC) No 1223/2009	Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'	
8	Sorbitan monooleate	1338-43-8		X		X				
9	Sodium L- glutamate	16177-21-2			X					
10	Silicon dioxide	112926-00-8	X							
11	3,5,7-Triaza-1- azoniatricyclo [3.3.1.1 ^{3,7}]deca ne, 1-(3-chloro- 2-propenyl)-, chloride	4080-31-3	X			X				
12	Sucrose (Saccharose)	57-50-1				X				- food stuff - pesticide active substance (Regulation (EC) No 1107/2009)
13	Non-ionic aqueous solution of polydimethyl siloxanes	Not allocated		X						
14	Hydrated aluminium silicate: Kaolin clay	1332-58-7			X	X				- pesticide active substance (Regulation (EC) No 1107/2009)

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name (a)	CAS number	Approved as biocide	Approved as food additive	Approved as feed additive	Listed as food contact material	Listed as food contact material	Listed as cosmetic	Listed as excipient	Other status
			Regulation (EU) No 528/2012	Regulation (EC) 1333/2008 Regulation (EU) No 231/2012	Regulation (EC) No 1831/2003	Regulation (EU) 2011/10	Directive 2007/42/EC	Regulation (EC) No 1223/2009	Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'	
15	Ethoxylated alcohol, 90%	34398-01-1						Registered as surfactant in CosIng		
16	Diatomaceous earth (Kieselguhr)	91053-39-3			X					
17	Soy lecithin (lecithins)	8002-43-5		X	X	X				- pesticide active substance (Regulation (EC) No 1107/2009)
18	Malt	Not allocated								- food stuff
19	Lignosulfonic acid, sodium salt	8061-51-6			X					
20	Lignosulfonic acid, Calcium salt	8061-52-7			X					
21	Gelatins	9000-70-8				X	X			- food stuff
22	Polysaccharide (scleroglucan)	39464-87-4								- food stuff
23	Polyoxyethylene tridecyl ether phosphate	9046-01-9				X				
24	Maltodextrin	9050-36-6								- pesticide active

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name (a)	CAS number	Approved as biocide	Approved as food additive	Approved as feed additive	Listed as food contact material	Listed as food contact material	Listed as cosmetic	Listed as excipient	Other status
			Regulation (EU) No 528/2012	Regulation (EC) 1333/2008 Regulation (EU) No 231/2012	Regulation (EC) No 1831/2003	Regulation (EU) 2011/10	Directive 2007/42/EC	Regulation (EC) No 1223/2009	Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'	
										substance (Regulation (EC) No 1107/2009)
25	Glycerides, palm- oil mono-, hydrogenated	67254-73-3								- food stuff
26	Skimmed Milk	Not allocated								- food stuff
27	Barley grain	Not allocated								- food stuff
28	Corn syrup solids	68131-37-3								- food stuff
29	Gum arabic	9000-01-5		X	X	X				
30	Potato flakes	Not allocated								- food stuff
31	Cellulose(Ahlsto m #601 Filter Paper)	9004-34-6		X		X				
32	Benzoyloxy methanol	14548-60-8	X					Preservative (annex V)		
33	Ethene, homopolymer	9002-88-4				X				
34	2- methoxyethanol	9004-74-4		X						
35	Sodium dioctyl sulfosuccinate	577-11-7					X			

Data collection on co-formulants used in representative plant protection product formulations

N°	Chemical name (a)	CAS number	Approved as biocide	Approved as food additive	Approved as feed additive	Listed as food contact material	Listed as food contact material	Listed as cosmetic	Listed as excipient	Other status
			Regulation (EU) No 528/2012	Regulation (EC) 1333/2008 Regulation (EU) No 231/2012	Regulation (EC) No 1831/2003	Regulation (EU) 2011/10	Directive 2007/42/EC	Regulation (EC) No 1223/2009	Annex to the EC guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'	
36	Styrene/(Meth)A crylate- Copolymer	27136-15-8				X				
37	D-glucose mono- hydrate / dextrose monohydrate	14431-43-7								- food stuff
38	Vegetable oil (sunflower oil refined)	84625-38-7								- food stuff
39	D-glucose, hydrate	77938-63-7								- food stuff
40	Ethoxylated sorbitan monooleate	9005-65-6				X				
41	Dibutylphthalate sulfonic acid, sodium salt	25417-20-3		X						

(a) As several chemical names can be associated with the same CAS and EC number, the most common chemical names have been reported.

Appendix D – List of co-formulants not registered under REACH, not approved as biocides, feed additives, food additives and food contact materials, and not listed as cosmetics, excipients with a known action or effect under the EU legal frameworks

N°	Chemical name ^(a)	CAS number	EC number
1	Polyvinyl alcohol, partly saponified (Acetic Acid Ethenyl Ester co-Polymer with Ethenol)	25213-24-5	607-648-9
2	Polyxypropylene-polyoxyethylene (2-methyloxirane)	9003-11-6	618-355-0
3	Naphthalene sulfonic acid, sodium salt	9084-06-4	618-665-6
4	Poly(oxy-1,2-ethanediyl), α -phenyl- ω -hydroxy-, styrenated	104376-75-2	600-560-1
5	Sodium alkyl naphthalene sulphonate	203-632-7	Not allocated
6	Methyl oleate	112-62-9	203-992-5
7	Polyoxyethylene styrylphenyl ether sulfate ammonium salt	119432-41-6	601-612-6
8	Mica (aluminium, magnesium, iron and potassium silicates)	Not allocated	Not allocated
9	5-hydroxy-1,3-dioxane (1,3-dioxan-5-ol)	4740-78-7	225-248-9
10	4-hydroxymethyl-1,3-dioxane (1,3-dioxolan-4-ylmethanol)	5464-28-8	226-758-4
11	Pumice	1332-09-8	603-719-3
12	Benzenesulfonic acid, C10-13-alkyl derivs., calcium salt	90194-36-8	290-646-1
13	Oxirane, 2-methyl-, polymer with oxirane, mono[3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]-1-disilo-xanyl]propyl] ether	134180-76-0	603-798-4
14	2-Propenoic acid, 2-methyl-, polymer	25751-21-7	639-553-3
15	Polyether	Not allocated	Not allocated
16	Sodium methylene polymethylnaphthyl sulphonate (Naphthalenesulfonic acid, methyl-, polymer with formaldehyde, sodium salt)	81065-51-2	617-192-2
17	Polyalkyleneoxide modified heptamethyltrisiloxane (3-(2-methoxyethoxy)propyl-methyl-bis(trimethylsilyloxy)silane)	27306-78-1	608-078-3
18	Black Dye: Tris(N,N,N-tributyl-1-butanaminium)[[2,2''6',2''-terpyridine]-4,4',4''-tricarboxylato(3-)-N1,N1',N1'']tris(thiocyanato-N)hydrogen ruthenate	Not allocated	Not allocated
19	Polyurea	Not allocated	Not allocated
20	Polyoxyethylene sorbitol hexaeolate	57171-56-9	611-475-4
21	2-Propenoic acid, butyl ester, polymer with 1,4-diethenylbenzene and ethenylbenzene	Not allocated	Not allocated
22	Ethoxylated lanolin	61790-81-6	612-384-2
23	Agnique MBL 530B: Solvent naphtha (petroleum), heavy arom (Heavy aromatic solvent naphtha)	Not allocated	Not allocated
24	Polyoxyethylene alkyl ether sulfate ammonium salt (Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-[bis(1-phenylethyl)phenoxy]-, ammonium salt)	59891-11-1	680-224-9
25	Polyglycol esters (Soitem 251/C)	Not allocated	Not allocated

N°	Chemical name ^(a)	CAS number	EC number
26	Fatty acids, tall-oil, ethoxylated, propoxylated (Ethylene oxide, propylene oxide copolymer, tall oil fatty acid ester)	67784-86-5	614-134-8
27	Hydrated aluminium magnesium silicate (palygorskite) (attapulgit)	12174-11-7	601-805-5
28	Oxirane, methyl-, polymer with oxirane, monobutyl ether	9038-95-3	618-542-7
29	Iso-tridecyl alcohol polyglycol ether with 8 EO (Alcohols, C11-14-iso-, C13-rich, ethoxylated)	78330-21-9	934-084-3
30	3,6-dimethyloct-4-yne-3,6-diol	78-66-0	201-131-8
31	Dodecylethoxylate (Dodecylphenol, ethoxylated)	9014-92-0	500-023-0
32	Acrylic copolymer in aqueous dispersion	Not allocated	Not allocated
33	Polyarylphenol ethoxylate propoxylate (block polymer)	Not allocated	Not allocated
34	Agnique MBL 510H (<i>from a dossier for which the active substance was not approved</i>)	Not allocated	Not allocated
35	Hydrocarbons, C10 aromatics, <1% naphthalene	1189173-42-9	824-107-4
36	Sawdust	Not allocated	Not allocated
37	Disodium maleate (<i>from a mixture of sodium polycarboxylate</i>) (<i>from a dossier for which the active substance was not approved</i>)	371-47-1	206-738-1
38	Sodium salt of sulphonated kraft lignin	Not allocated	Not allocated
39	2H-1,3,5-oxadiazine-2,4,6(3H,5H)-trione,3,5-bis(6-isocyanatohexyl)-polymer (Polyharnstoff to 2:1-D)	Not allocated	Not allocated
40	Antifoam emulsion (<i>the chemical, CAS/EC numbers or trade name was not reported in Volume 4/SDS data</i>)	Not allocated	Not allocated
41	Benzenesulphonic acid, 4-C10-14-alkyl derivs., calcium salts	90194-26-6	290-635-1
42	Polyethylene terephthalate polymer (Poly(oxy-1,2-ethanediylloxycarbonyl-1,4-phenylenecarbonyl))	25038-59-9	607-507-1
43	Polyethylene glycol mono (tristylphenyl) ether (Poly(oxy-1,2-ethanediyl), α-[tris(1-phenylethyl)phenyl]-ω-hydroxy-)	99734-09-5	619-457-8
44	Heavy aromatic solvent naphtha	Not allocated	938-406-3
45	Sodium alkyl naphthalene formaldehyde condensate (alkylated naphthalene sulfonate sodium salt)	68425-94-5	614-476-8

(a) As several chemical names can be associated with the same CAS and EC number, the most common chemical names have been reported.

Appendix E – Harmonised classification according to Annex VI of Regulation (EC) No 1227/2008 (CLP Regulation) and classification provided by companies to ECHA in REACH registrations joint submission

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
1	Hydrated amorphous silica (silicon dioxide)	7631-86-9	231-545-4	Not classified		Not classified	
2	Aromatic hydrocarbon (solvent naphtha (petroleum), heavy aromatic)	64742-94-5	265-198-5	Asp. Tox. 1 - H304 - May be fatal if swallowed and enters airways.		Asp. Tox. 1 - H304 - May be fatal if swallowed and enters airways. Skin Irrit. 2 – H315 – Causes skin irritation. STOT SE 3 – H336 – May cause drowsiness or dizziness (other: central nervous system; inhalation). Aquatic Chronic 2 – H411 – toxic to aquatic life with long lasting effects. Flam. Liq. 3 – H226 – Flammable liquid and vapour. Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Skin Sens. 1B – H317 – May cause an allergic skin reaction. Eye Irrit. 2.- H319 – Causes serious eye irritation.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
3	1,2-benzisothiazol-3(2H)-one	2634-33-5	220-120-9	Acute Tox. 4 - H302 – Harmful if swallowed.	Skin Sens. 1; H317: C ≥ 0,05 %	Acute Tox. 3 – H331 – Toxic if inhaled. STOT SE 3 – H335 – May cause respiratory inhalation (other: Respiratory tract; inhalation). STOT SE 3 – H340 – May cause genetic defects. STOT SE 3 – H350 – May cause cancer. Repr. 2 – H361 – Suspected of damaging fertility or the unborn child. STOT RE 1 – H372 – Causes damage to organs. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects. Acute Tox. 1 – H330 – Fatal if inhaled. Carc. 2 – H351 – Suspected of causing cancer (inhalation, oral). Acute Tox. 4 – H332 – Harmful if inhaled.	M = 10 M(Chronic) = 1 M = 1

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				Skin Irrit. 2 - H315 – Causes skin irritation. Eye Dam. 1 - H318 – Causes serious eyes damage. Skin Sens. 1 - H317 – May cause an allergic skin reaction. Aquatic Acute 1 - H400 – Very toxic to aquatic life.		Skin Irrit. 2 - H315 – Causes skin irritation. Eye Dam. 1 - H318 – Causes serious eyes damage. Skin Sens. 1 - H317 – May cause an allergic skin reaction. Aquatic Acute 1 - H400 – Very toxic to aquatic life. Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	
4	1,2-propanediol (Propane-1,2-diol) (Propylene Glycol)	57-55-6	200-338-0	Not classified		Not classified	
5	Xanthan gum	11138-66-2	234-394-2	Not classified		Not classified	
6	Sodium hydroxide (sodium salt)	1310-73-2	215-185-5	Skin Corr. 1A - H314 – Causes severe skin burns and eye damage.	Eye Irrit. 2; H319: 0,5 % ≤ C < 2 % Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 %	Met. Corr. 1 – H290 – May be corrosive to metals. Skin Corr. 1A - H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage.	
7	Lignosulfonic acid, sodium salt	8061-51-6	617-124-1	Not classified		Not classified	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
8	Hydrated aluminium silicate: Kaolin clay	1332-58-7	310-194-1	Not classified		Not classified	
9	Lignosulfonic acid, calcium salt	8061-52-7	617-125-7	Not classified		Not classified	
10	2-ethylhexan-1-ol	104-76-7	203-234-3	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H332 – Harmful if inhaled. STOT SE 3 – H335 – May cause respiratory irritation (Respiratory tract irritation; inhalation).	
11	Sorbitan monooleate	1338-43-8	215-665-4	Not classified		Not classified	
12	2,6-Di-tert-butyl-4-methylphenol (Butylated hydroxytoluene BHT)	128-37-0	204-881-4	Not classified		Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	M(Chronic) = 1
13	di-2-ethylhexyl sulfosuccinate of sodium (docusate sodium) (Polyoxyethylene alkylether)	577-11-7	209-406-4	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
14	Castor oil ethoxylate (Ethoxylated vegetable oil)	61791-12-6	500-151-7	Not classified		Not classified	
15	Non-ionic aqueous solution of polydimethylsiloxa nes (Polysiloxanes, di- Me)	Not allocated	Not allocated	Not applicable		Not applicable	
16	Maltodextrin	9050-36-6	232-940-4	Not classified		Not classified	
17	Benzenesulphonic acid, 4-C10-14- alkyl derivs., calcium salts	90194-26-6	290-635-1	Not classified		Not classified	
18	1,2,3-Propanetriol (glycerol)	56-81-5	200-289-5	Not classified		Not classified	
19	Sodium dodecyl sulphate (Sodium lauryl sulfate)	151-21-3	205-788-1	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects. Flam. Sol. 2 – H228 – Flammable solid.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						Acute Tox. 4 – H332 – Harmful if inhaled. STOT SE 3 – H335 – May cause respiratory irritation (Respiratory tract irritation; inhalation).	
20	Flours and sugar- food grade, corn flour, wheat flour	Not allocated	Not allocated	Not applicable		Not applicable	
21	Sodium 4,7- diisopropyl- naphthalene-2- sulfonate	1322-93-6	215-343-3	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Skin Corr. 1 – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
22	Titanium dioxide	13463-67-7	236-675-5	Carc. 2 – H351 (inhalation) – Suspected of causing cancer.		Carc. 2 – H351 (inhalation) – Suspected of causing cancer.	
23	3(2H)- Isothiazolone, 5- chloro-2-methyl-, mixt. with 2- methyl-3(2H)- isothiazolone	55965-84-9	611-341-5	Acute Tox. 3 – H301 – Toxic if swallowed. Acute Tox. 2 – H310 – Fatal in contact with skin. Skin Corr. 1C – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage.	Eye Dam. 1: C ≥ ,6 % Eye Irrit. 2; H319: ,06 % ≤ C < ,6 % Skin Corr. 1C: C ≥ ,6 %	Not classified	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				Skin Sens. 1A – H317 – May cause an allergic skin reaction. Acute Tox. 2 – H330 – Fatal if inhaled. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	Skin Irrit. 2; H315: ,06 % ≤ C < ,6 % Skin Sens. 1A: C ≥ ,0015 % M=100 M(Chronic)=100		
24	Agnique MBL 530B: Solvent naphtha (petroleum), heavy arom (Heavy aromatic solvent naphtha)	Not allocated	Not allocated	Not applicable		Not applicable	
25	Anhydrous sodium sulphate	7757-82-6	231-820-9	Not classified		Not classified	
26	Soy lecithin (lecithins)	8002-43-5	232-307-2	Not classified		Not classified	
27	Polysorbate 20, Tween 20: Sorbitan monolaurate, ethoxylated	9005-64-5	500-018-3	Not classified		Not classified	
28	Agnique MBL 510H (<i>from a dossier for which the active</i>	Not allocated	Not allocated	Not applicable		Not applicable	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
29	<i>substance was not approved</i> Fatty alcohol ethoxylate	68131-39-5	500-195-7	Not classified		Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	M = 1
30	Gum arabic	9000-01-5	232-519-5	Not classified		Not classified	
31	Ethene, homopolymer	9002-88-4	618-339-3	Not classified		Not classified	
32	Naphthalene sulfonic acid, sodium salt	9084-06-4	618-665-6	Not classified		Not classified	
33	(Copolymer of) styrene	100-42-5	202-851-5	Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H332 – Harmful if inhaled. STOT RE 1 – H372 (hearing organs) – Causes damage to organs. Repr. 2 – H361d - Suspected of damaging the unborn child.		Flam. Liq. 3 – H226 – Flammable liquid and vapour. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H332 – Harmful if inhaled. STOT SE 3 – H335 (Nose; inhalation) – May cause respiratory irritation. Repr. 2 – H361 - Suspected of damaging the unborn child.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						STOT RE 1 – H372 (ear; inhalation) – Causes damage to organs. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
34	Poly(oxy-1,2-ethanediyl), α-phenyl-ω-hydroxy-, styrenated	104376-75-2	600-560-1	Not classified		Not classified	
35	1-Hexanol	111-27-3	203-852-3	Acute Tox. 4 – H302 – Harmful if swallowed.		Acute Tox. 4 – H302 – Harmful if swallowed. Flam. Liq. 3 – H226 – Flammable liquid and vapour. Acute Tox 4. – H312 – Harmful in contact with skin. Eye Irrit. 2 – H319 – Causes serious eye irritation. Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H336 (other: central nervous system; inhalation) – May cause drowsiness or dizziness. Aquatic Acute 1 – H400 – Very toxic to aquatic life.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
36	Methyl laurate	111-82-0	203-911-3	Not classified		Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	
37	Ascorbic acid	50-81-7	200-066-2	Not classified		Aquatic Acute 1 – H400 – Very toxic to aquatic life.	
38	(Copolymer of) n-butylacrylate	141-32-2	205-480-7	Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation. Skin Sens. 1 - H317 – May cause an allergic skin reaction. STOT SE 3 – H335 – May cause respiratory irritation.		Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	
						Not classified	
						Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation Skin Sens. 1B - H317 – May cause an allergic skin reaction. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H332 – Harmful if inhaled. STOT SE 3 – H335 (Respiratory tract irritation; inhalation). May cause respiratory irritation. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
39	Potassium (E,E)- hexa-2,4-dienoate	24634-61-5	246-376-1	Eye Irrit. 2 – H319 – Causes serious eye irritation.		Eye Irrit. 2 – H319 – Causes serious eye irritation.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
	(Potassium sorbate)						
40	2-Propenoic acid, 2-methyl-, polymer	25751-21-7	639-553-3	Not classified		Not classified	
41	Polyether	Not allocated	Not allocated	Not applicable		Not applicable	
42	Toluene diisocyanate	26471-62-5	247-722-4	Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation. Skin Sens. 1 - H317 – May cause an allergic skin reaction. Acute Tox. 2 – H330 – Fatal if inhaled. STOT SE 3 – H335 – May cause respiratory irritation. Resp. Sens. 1 – H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled. Carc. 2 – H351 – Suspected of causing cancer. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	Resp. Sens. 1; H334: C ≥ 0,1 %	Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1 - H317 – May cause an allergic skin reaction. Eye Irrit. 2A – H319 – Causes serious eye irritation. Acute Tox. 1 – H330 – Fatal if inhaled. Resp. Sens. 1 – H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled. STOT SE 3 – H335 (other: lung; inhalation) – May cause respiratory irritation. Carc. 2 – H351 – Suspected of causing cancer. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
43	2-Methyl-2H-isothiazol-3-on	2682-20-4	220-239-6	Acute Tox. 3 – H301 – Toxic if swallowed.	Skin Sens. 1A; H317: C ≥ 0,0015 %	Acute Tox. 3 – H301 – Toxic if swallowed.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				Acute Tox. 3 – H311 – Toxic in contact with skin. Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage. Skin Sens. 1A – H317 – May cause an allergic skin reaction. Acute Tox. 2 – H330 – Fatal if inhaled. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	M=10 M(Chronic)=1	Acute Tox. 3 – H311 – Toxic in contact with skin. Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Skin Sens. 1A – H317 – May cause an allergic skin reaction. Acute Tox. 2 – H330 – Fatal if inhaled. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	
44	sodium benzoate	532-32-1	208-534-8	Not classified		Eye Irrit. 2A – H319 – Causes serious eye irritation.	
45	Sucrose (alpha-D- Glucopyranoside, beta-D- Fructofuranosyl) (Saccharose)	57-50-1	200-334-9	Not classified		Not classified	
46	2-Propenoic acid, butyl ester, polymer with 1,4- diethenylbenzene and ethenylbenzene	Not allocated	Not allocated	Not applicable		Not applicable	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
47	Acetic acid	64-19-7	200-580-7	Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Corr. 1A – H314 – Causes severe skin burns and eye damage.	Eye Irrit. 2; H319: 10 % ≤ C < 25 % Skin Corr. 1A; H314: C ≥ 90 % Skin Corr. 1B; H314: 25 % ≤ C < 90 % Skin Irrit. 2; H315: 10 % ≤ C < 25 %	Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Corr. 1A – H314 – Causes severe skin burns and eye damage.	
48	Ethoxylated alcohol, 90%	34398-01-1	500-84-3	Not classified		Not classified	
49	Alcohols, C12-14, ethoxylated	68439-50-9	500-213-3	Not classified		Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	M = 1
50	Diatomaceous earth (Kieselguhr)	91053-39-3	293-303-4	Not classified		Not classified	
51	Benzenesulfonic acid, mono-C11- 13-branched alkyl derivs., calcium salts	68953-96-8	273-234-6	Not classified		Acute Tox 4. – H312 – Harmful in contact with skin. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
52	Hydrated aluminium magnesium silicate (palygorskite) (attapulgit)	12174-11-7	601-805-5	Not classified		Not classified	
53	Potassium-Di-Hydrogen Phosphate (phosphoric acid, monopotassium salt)	7778-77-0	231-913-4	Not classified		Not classified	
54	Trimethylolpropane (Propylidynetrimethanol)	77-99-6	201-074-9	Not classified		Repr. 2 – H361fd – Suspected of damaging fertility or the unborn child.	
55	Polysaccharide (scleroglucan)	39464-87-4	254-464-6	Not classified		Not classified	
56	Acrylic copolymer in aqueous dispersion	Not allocated	Not allocated	Not applicable		Not applicable	
57	Benzoic acid, 4-hydroxy-, methyl ester (Methyl 4-hydroxybenzoate) (Methylparaben)	99-76-3	202-785-7	Not classified		Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	
58	Polyvinyl alcohol, partly saponified (Acetic Acid Ethenyl Ester Co-	25213-24-5	607-648-9	Not classified		Not classified	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
59	Polymer with Ethenol) Skimmed Milk	Not allocated	Not allocated	Not applicable		Not applicable	
60	Hydrocarbons, C10 aromatics, <1% naphthalene	1189173- 42-9	824-107-4	Not classified		Not classified	
61	Disodium maleate (from a mixture of sodium polycarboxylate) (from a dossier for which the active substance was not approved)	371-47-1	206-738-1	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1 - H317 – May cause an allergic skin reaction. Eye Irrit. 2 – H319 – Causes serious eye irritation. STOT SE 3 – H335 (Respiratory system) – May cause respiratory irritation.	
62	Sodium alkylnaphthalene formaldehyde condensate (alkylated naphthalene sulfonate sodium salt)	68425-94-5	614-476-8	Not classified		Not classified	
63	2,2'- iminodi(ethylamin e) (N-2- aminoethyl-1,2-	111-40-0	203-865-4	Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin.		Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin.	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
	ethanediamine) (from a mixture of sodium polycarboxylate) (from a dossier for which the active substance was not approved)			Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Skin Sens. 1 - H317 – May cause an allergic skin reaction.		Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Skin Sens. 1B - H317 – May cause an allergic skin reaction. Acute Tox. 2 – H330 – Fatal if inhaled. STOT SE 3 – H335 (Respiratory tract irritation; inhalation). May cause respiratory irritation.	
64	Yeast	Not allocated	Not allocated	Not applicable		Not applicable	
65	Polyxypropylene- polyoxyethylene (2-methyloxirane)	9003-11-6	618-355-0	Not classified		Not classified	
66	Dextrose (glucose)	50-99-7	200-075-1	Not classified		Not classified	
67	Carboxymethyl cellulose (CMC)/Cellulose polymer	9004-32-4	618-378-6	Not classified		Not classified	
68	4-nitrophenol (from a dossier for which the a.s. was not approved)	100-02-7	202-811-7	Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Acute Tox. 4 – H332 – Harmful if inhaled. STOT RE 2 – H373 – May cause damage to organs		Acute Tox. 3 – H301 – Toxic if swallowed. Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Acute Tox. 4 – H332 – Harmful if inhaled.	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				through prolonged or repeated exposure.		STOT RE 2 – H373 – May cause damage to organs through prolonged or repeated exposure (liver, kidneys; oral).	
69	Hexamethylene tetramine	100-97-0	202-905-8	Flam. Sol. 2 – H228 – Flammable solid. Skin Sens. 1 – H317 – May cause an allergic skin reaction.		Flam. Sol. 2 – H228 – Flammable solid. Skin Sens. 1B – H317 – May cause an allergic skin reaction.	
70	Sodium alkylnaphthalene sulphonate	203-632-7	Not allocated	Not applicable		Not applicable	
71	Tetradecanoic acid, isopropyl ester (Isopropyl myristate)	110-27-0	203-751-4	Not classified		Not classified	
72	Hexa-2,4-dienoic acid (sorbic acid)	110-44-1	203-768-7	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation.	
73	Methyl oleate	112-62-9	203-992-5	Not classified		Not classified	
74	Dimethyl ether	115-10-6	204-065-8	Press. Gas Flam. Gas 1 – H220 – Extremely flammable gas.		Flam. Gas 1 – H220 – Extremely flammable gas. Press. Gas (comp.) – H280 – Contains gas under pressure; may explode if heated.	
75	Polyoxyethylene styrylphenyl ether sulfate ammonium salt	119432-41- 6	601-612-6	Not classified		Not classified	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
76	Mica (aluminium, magnesium, iron and potassium silicates)	Not allocated	Not allocated	Not applicable		Not applicable	
77	Nonyl aldehyde	124-19-6	204-688-5	Not classified		Aquatic Chronic 3 – H412 - Harmful to aquatic life with long lasting effects.	
78	Riboflavin 5'-(sodium hydrogen phosphate)	130-40-5	204-988-6	Not classified		Not classified	
79	Potassium hydroxide	1310-58-3	215-181-3	Acute Tox. 4 – H302 – Harmful if swallowed. Skin Corr. 1A – H314 – Causes severe skin burns and eye damage.	Eye Irrit. 2; H319: 0,5 % ≤ C < 2 % Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 %	Acute Tox. 4 – H302 – Harmful if swallowed. Skin Corr. 1A – H314 – Causes severe skin burns and eye damage. Met. Corr. 1 – H290 – May be corrosive to metals.	
80	5-hydroxy-1,3-dioxane (1,3-dioxan-5-ol)	4740-78-7	225-248-9	Not classified		Not classified	
81	4-hydroxymethyl-1,3-dioxane (1,3-dioxolan-4-ylmethanol)	5464-28-8	226-758-4	Not classified		Not classified	
82	Xylene	1330-20-7	215-535-7	Flam. Liq. 3 – H226 – Flammable liquid and vapour.		Not classified	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				Acute Tox 4. – H312 – Harmful in contact with skin. Skin Irrit. 2 – H315 – Causes skin irritation. Acute Tox. 4 – H332 – Harmful if inhaled.			
83	Pumice	1332-09-8	603-719-3	Not classified		Not classified	
84	Benzenesulfonic acid, C10-13-alkyl derivs., calcium salt	90194-36-8	290-646-1	Not classified		Not classified	
85	Ammonium hydroxide	1336-21-6	215-647-6	Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Aquatic acute 1 – H400 – Very toxic to aquatic life.	STOT SE 3; H335: C ≥ 5 %	Not classified	
86	Oxirane, 2- methyl-, polymer with oxirane, mono[3-[1,3,3,3- tetramethyl-1- [(trimethylsilyl)ox y]-1-disilo- xanyl]propyl] ether	134180-76- 0	603-798-4	Not classified		Not classified	
87	bis(2-ethylhexyl) maleate	142-16-5	205-524-5	Not classified		STOT RE 2 – H373 (kidney; oral) – May cause damage to	M(Chronic) = 0

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						organs through prolonged or repeated exposure. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	
88	Sodium L- glutamate	16177-21-2	240-313-1	Not classified		Not classified	
89	D-glucose mono- hydrate / dextrose monohydrate	14431-43-7	604-408-5	Not classified		Not classified	
90	Copper phthalocyanine	147-14-8	205-685-1	Not classified		Not classified	
91	Magnesium silicate	14807-96-6	238-877-9	Not classified		Not classified	
92	Silicon dioxide	112926-00- 8	601-214-2	Not classified		Not classified	
93	Calcium stearate (calcium distearate)	1592-23-0	216-472-8	Not classified		Not classified	
94	2-tert- Butylhydroquinon e	1948-33-0	217-752-2	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1 – H317 – May cause an allergic skin reaction. Eye Irrit. 2 – H319 – Causes serious eye irritation.	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						Aquatic acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.	
95	Octadecanoic acid, 2-ethylhexyl ester	22047-49-0	244-754-0	Not classified		Not classified	
96	Polypropyleneglyc ol	25322-69-4	500-039-8	Not classified		Not classified	
97	benzenesulfonic acid, dodecyl-, calcium salt	26264-06-2	247-557-8	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. Aquatic Chronic 4 – H413 – May cause long lasting harmful effects to aquatic life.	
98	Sodium methylene polymethylnaphth yl sulphonate (Naphthalenesulfo nic acid, methyl-, polymer with formaldehyde, sodium salt)	81065-51-2	617-192-2	Not classified		Not classified	
99	Polyalkyleneoxide modified	27306-78-1	608-078-3	Not classified		Not classified	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
	heptamethyltrisiloxane (3-(2-methoxyethoxy)propyl-methyl-bis(trimethylsilyloxy)silane)						
100	Black Dye, Tris(N,N,N-tributyl-1-butanaminium)[[2,2''6',2''-terpyridine]-4,4',4''-tricarboxylato(3-)-N1,N1',N1'']tris(thiocyanato-N)hydrogen ruthenate	Not allocated	Not allocated	Not applicable		Not applicable	
101	2-(3-tert-butyl-2-hydroxy-5-methylphenyl)-5-chloro-2H-benzotriazole (Bumetrizole)	3896-11-5	223-445-4	Not classified		Not classified	
102	Calcium dipropionate	4075-81-4	223-795-8	Not classified		Eye Dam. 1 – H318 – Causes serious eye damage.	
103	3,5,7-Triaza-1-azoniatricyclo [3.3.1.1 ^{3,7}]decane, 1-(3-chloro-2-	4080-31-3	223-805-0	Not classified		Not classified	

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	propenyl)-, chloride (Methenamine 3- chloroallylochlorid e)						
104	Calcite (calcium carbonate)	471-34-1	207-439-9	Not classified		Not classified	
105	2-Bromo-2- nitropropane-1,3- diol (Bronopol)	52-51-7	200-143-0	Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H335 – May cause respiratory irritation. Aquatic acute 1 – H400 – Very toxic to aquatic life.	M = 10	Acute Tox. 4 – H302 – Harmful if swallowed. Acute Tox 4. – H312 – Harmful in contact with skin. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H335 (Respiratory tract irritation; inhalation). May cause respiratory irritation. Aquatic acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	
106	Aminoacetic acid (glycine)	56-40-6	200-272-2	Not classified		Not classified	
107	Polyurea	Not allocated	Not allocated	Not applicable		Not applicable	

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108	Polyoxyethylene sorbitol hexaeloate	57171-56-9	611-475-4	Not classified		Not classified	
109	Edetic acid (EDTA) - Ethylenediamine tetraacetic acid	60-00-4	200-449-4	Eye Irrit. 2 – H319 – Causes serious eye irritation.		Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H332 – Harmful if inhaled. STOT RE 2 – H373 (other: Respiratory tract; inhalation) - May cause damage to organs through prolonged or repeated exposure.	
110	Ethoxylated lanolin	61790-81-6	612-384-2	Not classified		Not classified	
111	Ethanol	64-17-5	200-578-6	Flam. Liq. 2 – H225 – Highly flammable liquid and vapour.		Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 4 – H302 – Harmful if swallowed. STOT SE 2 – H371 (other: optic nerve) - May cause damage to organs. Carc. 1B – H350 (inhalation) – May cause cancer.	
112	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-88-4	265-090-8	Carc. 1 B – H350 – May cause cancer.		Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Carc. 1 B – H350 – May cause cancer.	

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						Repr. 2 – H361 (dermal; unborn child) – Suspected of damaging fertility or the unborn child. STOT RE 1 – H372 (adrenals, bone marrow; dermal) – Causes damage to organs.	
113	Petroleum distillate, solvent refined hydrotreated light paraffinic oil	64742-55-8	265-158-7	Carc. 1 B – H350 – May cause cancer.		Carc. 1 B – H350 – May cause cancer. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Repr. 2 – H361 (dermal; unborn child) – Suspected of damaging fertility or the unborn child. STOT RE 1 – H372 (adrenals, bone marrow; dermal) – Causes damage to organs.	
114	2- Naphthalenecarbo xamide, 3- hydroxy-N-(2- methylphenyl)-4- [2-(2,4,5- trichlorophenyl)di azenyl]-	6535-46-2	229-440-3	Not classified		Not classified	
115	Benzoic Acid	65-85-0	200-618-2	Skin Irrit. 2 – H315 – Causes skin irritation.		Skin Irrit. 2 – H315 – Causes skin irritation.	

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				Eye Dam. 1 – H318 – Causes serious eye damage. STOT RE 1 – H372 (lungs; inhalation) – Causes damage to organs.		Eye Dam. 1 – H318 – Causes serious eye damage. STOT RE 1 – H372 (lungs; inhalation) – Causes damage to organs.	
116	2-propanol	67-63-0	200-661-7	Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Eye Irrit. 2 – H319 – Causes serious eye irritation. STOT SE 3 – H336 – May cause drowsiness or dizziness.		Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Eye Irrit. 2 – H319 – Causes serious eye irritation. STOT SE 3 – H336 (other: n.a.; inhalation) – May cause drowsiness or dizziness	
117	Polyoxyethylene alkyl ether sulfate ammonium salt (Poly(oxy-1,2- ethanediyl), .alpha.-sulfo- .omega.-[bis(1- phenylethyl)phen oxy]-, ammonium salt)	59891-11-1	680-224-9	Not classified		Not classified	
118	Fatty acids, C16- 18 and C18- unsaturated, Me esters	67762-38-3	267-015-4	Not classified		Not classified	
119	Polyglycol esters (Soitem 251/C)	Not allocated	Not allocated	Not applicable		Not applicable	
120	Fatty acids, tall- oil, ethoxylated,	67784-86-5	614-134-8	Not classified		Not classified	

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	propoxylated (Ethylene oxide, propylene oxide copolymer, tall oil fatty acid ester)						
121	Alkylpolyglycoside (D- Glucopyranose, oligomers, decyl octyl glycosides)	68515-73-1	500-220-1	Not classified		Eye Dam. 1 – H318 – Causes serious eye damage.	
122	Oxirane, methyl-, polymer with oxirane, monobutyl ether	9038-95-3	618-542-7	Not classified		Not classified	
123	Tetrapotassium pyrophosphate	7320-34-5	230-785-7	Not classified		Eye Irrit. 2 – H319 – Causes serious eye irritation.	
124	Ethylene oxide	75-21-8	200-849-9	Press. Gas Flam. Gas 1 – H220 – Extremely flammable gas. Acute Tox. 3 – H301 – Toxic if swallowed. Skin Corr. 1 – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage. Acute Tox. 3 – H331 -Toxic if inhaled. STOT SE 3 – H335 – May cause respiratory irritation.		Press. Gas (Liq.) – H280 – Contains gas under pressure; may explode if heated. Flam. Gas 1 – H220, H230. Acute Tox. 3 – H301 – Toxic if swallowed. Skin Corr. 1 – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage. Acute Tox. 3 – H331 -Toxic if inhaled.	

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				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				STOT SE 3 – H336 – May cause drowsiness or dizziness. Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer. STOT RE 1 – H372 (nervous system) – Causes damage to organs. Repr. 1B – H360Fd – May damage fertility. Suspected of damaging the unborn child.		STOT SE 3 – H335 (other: respiratory tract) – May cause respiratory irritation. STOT SE 3 – H336 (nervous system) – May cause drowsiness or dizziness. Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer. STOT RE 1 – H372 (nervous system) – Causes damage to organs. Repr. 1B – H360Fd – May damage fertility. Suspected of damaging the unborn child.	
125	Propylene oxide (Methyloxirane)	75-56-9	200-879-2	Flam. Liq. 1 – H224 – Extremely flammable liquid and vapour. Acute Tox. 4 – H302 – Harmful if swallowed. Acute tox 3 – H311 – Toxic in contact with skin. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 3 – H331 – Toxic if inhaled. STOT SE 3 – H335 – May cause respiratory irritation.		Flam. Liq. 1 – H224 – Extremely flammable liquid and vapour. Acute Tox. 4 – H302 – Harmful if swallowed. Acute tox 3 – H311 – Toxic in contact with skin. Eye Irrit. 2 – H319 – Causes serious eye irritation. Acute Tox. 3 – H331 – Toxic if inhaled. STOT SE 3 – H335 (other: respiratory tract) – May cause respiratory irritation.	

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				Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer.		Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer.	
126	Di-Sodium Hydrogen Phosphate	7558-79-4	231-448-7	Not classified		Not classified	
127	Sodium Chloride	7647-14-5	231-598-3	Not classified		Not classified	
128	DL-alpha- tocopheryl acetate	7695-91-2	231-710-0	Not classified		Not classified	
129	Ammonium sulphate	7783-20-2	231-984-1	Not classified		Not classified	
130	Anhydrous citric acid	77-92-9	201-069-1	Not classified		Eye Irrit. 2 – H319 – Causes serious eye irritation. STOT SE 3 – H335 (other: respiratory tract; inhalation) – May cause respiratory irritation.	
131	Iso-tridecyl alcohol polyglycol ether with 8 EO (Alcohols, C11-14- iso-, C13-rich, ethoxylated)	78330-21-9	934-084-3	Not classified		Not classified	
132	3,6-dimethyloct-4- yne-3,6-diol	78-66-0	201-131-8	Not classified		Not classified	

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133	Isobutanol (2-methylpropan-1-ol)	78-83-1	201-148-0	Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H335 – May cause respiratory irritation. STOT SE 3 – H336 – May cause drowsiness or dizziness		Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H335 (other: respiratory tract) – May cause respiratory irritation. STOT SE 3 – H336 (other: central nervous system) – May cause drowsiness or dizziness	
134	Propionic acid	79-09-4	201-176-3	Skin Corr. 1B – H314 – Causes severe skin burns and eye damage.	Eye Irrit. 2; H319: 10 % ≤ C < 25 % STOT SE 3; H335: C ≥ 10 % Skin Corr. 1B; H314: C ≥ 25 % Skin Irrit. 2; H315: 10 % ≤ C < 25 %	Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Flam. Liq. 3 – H226 – Flammable liquid and vapour. Skin Corr. 1B – H314 – Causes severe skin burns and eye damage. Eye Dam. 1 – H318 – Causes serious eye damage. STOT SE 3 – H335 (other: respiratory tract; inhalation) – May cause respiratory irritation.	
135	Malt	Not allocated	Not allocated	Not applicable		Not applicable	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
136	Highly refined mineral oil	8042-47-5	232-455-8	Not classified		Asp. Tox.1 – H304 – May be fatal if swallowed and enters airways.	
137	9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylum chloride (from a dossier for which the a.s. was not approved)	81-88-9	201-383-9	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Eye Dam. 1 – H318 – Causes serious eye damage. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
138	Gelatins	9000-70-8	232-554-6	Not classified		Not classified	
139	Dodecylethoxylate (Dodecylphenol, ethoxylated)	9014-92-0	500-023-0	Not classified		Not classified	
140	Ethoxylated sorbitan monooleate	9005-65-6	500-019-9	Not classified		Not classified	
141	Polyoxyethylene Sorbitan Monostearate	9005-67-8	500-020-4	Not classified		Not classified	
142	Polyoxyethylene tridecyl ether phosphate	9046-01-9	618-558-4	Not classified		Not classified	
143	Glycerides, palm-oil mono-, hydrogenated	67254-73-3	614-041-2	Not classified		Not classified	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
144	Propylparaben (propyl 4- hydroxybenzoate)	94-13-3	202-307-7	Not classified		Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
145	Vegetable oil (sunflower oil refined)	84625-38-7	283-413-0	Not classified		Not classified	
146	Lauroyl N- methylglycine (N- lauroylsarcosine)	97-78-9	202-608-3	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage. Acute Tox. 2 – H330 – Fatal if inhaled.	
147	Polyarylphenol ethoxylate propoxylate (block polymer)	Not allocated	Not allocated	Not applicable		Not applicable	
148	Barley grain	Not allocated	Not allocated	Not applicable		Not applicable	
149	Corn syrup solids	68131-37-3	268-616-4	Not classified		Not classified	
150	diethyl malonate	105-53-3	203-305-9	Not classified		Eye Irrit. 2 – H319 – Causes serious eye irritation.	
151	linalool	78-70-6	201-134-4	Skin Sens. 1B – H317 – May cause an allergic skin reaction.		Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1B – H317 – May cause an allergic skin reaction. Eye Irrit. 2 – H319 – Causes serious eye irritation.	
152	d-limonene	5989-27-5	227-813-5	Flam. Liq. 3 – H226 – Flammable liquid and vapour.		Flam. Liq. 3 – H226 – Flammable liquid and vapour.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
				Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1 – H317 – May cause an allergic skin reaction. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.		Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1 – H317 – May cause an allergic skin reaction. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
153	4-(4-hydroxyphenyl)butan-2-one	5471-51-2	226-806-4	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed.	
154	hexyl acetate	142-92-7	205-572-7	Not classified		Flam. Liq. 3 – H226 – Flammable liquid and vapour.	
155	Potato flakes	Not allocated	Not allocated	Not applicable		Not applicable	
156	Reaction mass of dimethyl adipate and dimethyl glutarate and dimethyl succinate	Not allocated	906-170-0	Not classified		Not classified	
157	Sawdust	Not allocated	Not allocated	Not applicable		Not applicable	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
158	Sodium salt of sulphonated kraft lignin	Not allocated	Not allocated	Not applicable		Not applicable	
159	Bis(2,2,6,6- tetramethyl-4- piperidyl) sebacate	52829-07-9	258-207-9	Not classified		Eye Dam. 1 – H318 – Causes serious eye damage. Repr. 2 – H361 – Suspected of damaging fertility or the unborn child. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects.	M = 1
160	2H-1,3,5- oxadiazine- 2,4,6(3H,5H)- trione,3,5-bis(6- isocyanatohexyl)- polymer (Polyharnstoff to 2:1-D)	Not allocated	Not allocated	Not applicable		Not applicable	
161	Cellulose (Ahlstom #601 Filter Paper)	9004-34-6	232-674-9	Not classified		Not classified	
162	Antifoam emulsion (<i>the chemical or trade name was not reported in Volume 4</i>)	Not allocated	Not allocated	Not applicable		Not applicable	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
163	Benzyloxy methanol	14548-60-8	238-588-8	Not classified		Not classified	
164	Citronellol	106-22-9	203-375-0	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Skin Sens. 1B – H317 – May cause an allergic skin reaction. Eye Irrit. 2 – H319 – Causes serious eye irritation.	
165	D-Glucose, hydrate	77938-63-7	616-580-9	Not classified		Not classified	
166	Ethylbenzene	100-41-4	202-849-4	Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Acute Tox. 4 – H332 – Harmful if inhaled. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. STOT RE 2 – H373 (hearing organs) – May cause damage to organs through prolonged or repeated exposure.		Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Acute Tox. 4 – H332 – Harmful if inhaled. STOT RE 2 – H373 (hearing organs) – May cause damage to organs through prolonged or repeated exposure. Aquatic Chronic 3 – H412 – Harmful to aquatic life with long lasting effects.	
167	Ethylenediamine tetraacetic acid (EDTA), tetrasodium salt	64-02-8	200-573-9	Acute Tox. 4 – H302 – Harmful if swallowed. Eye Dam. 1 – H318 – Causes serious eye damage.		Acute Tox. 4 – H302 – Harmful if swallowed. Eye Dam. 1 – H318 – Causes serious eye damage. Acute Tox. 4 – H332 – Harmful if inhaled.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						STOT RE 2 – H373 (other: respiratory tract; inhalation) – May cause damage to organs through prolonged or repeated exposure	
168	Polyethylene terephthalate polymer (Poly(oxy-1,2- ethanediylloxycarb onyl-1,4- phenylenecarbony l))	25038-59-9	607-507-1	Not classified		Not classified	
169	Heavy aromatic solvent naphtha	Not allocated	938-406-3	Not classified		Not classified	
170	Naphthalene	91-20-3	202-049-5	Acute Tox. 4 – H302 – Harmful if swallowed. Carc. 2 – H351 – Suspected of causing cancer. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects.		Acute Tox. 4 – H302 – Harmful if swallowed. Carc. 2 – H351 (inhalation) – Suspected of causing cancer. Aquatic Acute 1 – H400 – Very toxic to aquatic life. Aquatic Chronic 1 – H410 – Very toxic to aquatic life with long lasting effects. Flam. Sol. 2 – H228 – Flammable solid. Aquatic acute 1 – H400 – Very toxic to aquatic life.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
171	Polyethylene glycol mono (tristyphenyl) ether (tristyphenyl) ether (Poly(oxy-1,2-ethanediyl), α-[tris(1-phenylethyl)phenyl]-ω-hydroxy-)	99734-09-5	619-457-8	Not classified		Not classified	
172	Propylene carbonate	108-32-7	203-572-1	Eye Irrit. 2 – H319 – Causes serious eye irritation.		Eye Irrit. 2 – H319 – Causes serious eye irritation.	
173	2-methoxyethanol	9004-74-4	618-394-3	Not classified		Not classified	
174	Solvent-refined (severe) light and heavy paraffinic petroleum oil	64741-89-5	265-091-3	Carc. 1B – H350 – May cause cancer.		Carc. 1B – H350 – May cause cancer. Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways.	
175	Solvesso 100: Solvent naphtha (petroleum), light arom.	64742-95-6	265-199-0	Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer.		Asp. Tox. 1 – H304 – May be fatal if swallowed and enters airways. Muta. 1B – H340 – May cause genetic defects. Carc. 1B – H350 – May cause cancer. Flam. Liq. 2 – H225 – Highly flammable liquid and vapour. Skin Irrit. 2 – H315 – Causes skin irritation.	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
						STOT RE 3 – H336 (other: central nervous system; inhalation) – May cause drowsiness or dizziness. Repr. 2 – H361 (Fertility; unborn child) – Suspected of damaging fertility or the unborn child. Aquatic Chronic 2 – H411 – Toxic to aquatic life with long lasting effects. Flam. Liq. 3 – H226 – Flammable liquid and vapour. Flam. Liq. 1 – H224 – Extremely flammable liquid and vapour.	
176	Soybean oil, epoxidised	8013-07-8	232-391-0	Not classified		Not classified	
177	Styrene/(Meth)Acr ylate-Copolymer	27136-15-8	608-050-0	Not classified		Not classified	
178	α -methyl- ω - hydroxy poly(oxy- 1,2-ethandiyl)	9003-13-8	500-003-1	Not classified		Acute Tox. 4 – H302 – Harmful if swallowed. Skin Irrit. 2 – H315 – Causes skin irritation. Eye Irrit. 2 – H319 – Causes serious eye irritation.	
179	Dibutyl-naphthalen e sulfonic acid, sodium salt	25417-20-3	246-960-6	Not classified		Not classified	

N°	Chemical name (a)	CAS number	EC / List No	Harmonised classification approved by the European Union		Classification provided by companies to ECHA in REACH registrations joint submission	
				Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors, ATE	Hazard class, category code(s) and hazard statement	Specific concentration limits, M- Factors
180	Silicic acid, calcium salt	1344-95-2	215-710-8	Not classified		Not classified	
181	Trehalose (α-D- Glucopyranoside, α-D- glucopyranosyl, hydrate (1:2))	6138-23-4	612-140-5	Not classified		Not classified	
182	Sodium dioctyl sulfosuccinate	577-11-7	209-406-4	Not classified		Skin Irrit. 2 – H315 – Causes skin irritation. Eye Dam. 1 – H318 – Causes serious eye damage.	

Annex A – Outcome of the ECHA’s compliance check, examination of testing proposals and substance evaluation on the REACH registration dossiers of the 61 identified co-formulants

Annex B – Eligibility for potential exemptions from registration under REACH for the 45 co-formulants not regulated under EU legal frameworks (listed in Appendix D)

Annex A and Annex B can be found in the online version of this output (‘Supporting information’ section).

FOREWORD

INTRODUCTION

GLYCEROL
CAS N°: 56-81-5

SIDS Initial Assessment Report

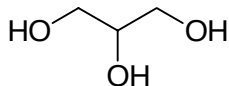
For

SIAM 14

Paris, France, 26-28 March 2002

1. **Chemical Name:** Glycerol
2. **CAS Number:** 56-81-5
3. **Sponsor Country:** United Kingdom
SIDS Contact Point:
Dr Steve Robertson
Environment Agency
National Centre for Ecotoxicology & Hazardous Substances
Isis House, Howbery Park, Wallingford OX10 8BD, UK
Fax: +44 1491 828 556
e-mail: steve.robertson@environment-agency.gov.uk
4. **Shared Partnership with:**
5. **Roles/Responsibilities of the Partners:**
 - Name of industry sponsor /consortium The industry contact point is Dr L. Hughes, ICI Uniqema, Wilton Centre, Wilton, Redcar, United Kingdom
 - Process used
6. **Sponsorship History**
 - How was the chemical or category brought into the OECD HPV Chemicals Programme ? This substance is sponsored by the UK under the ICCA Initiative and is submitted for first discussion at SIAM 14.
7. **Review Process Prior to the SIAM:** The industry consortium collected new data and prepared the updated IUCLID, and draft versions of the SIAR and SIAP. UK government peer-reviewed the documents, audited selected studies and conducted separate literature searches.
8. **Quality check process:**
9. **Date of Submission:** February 2002
10. **Date of last Update:**
11. **Comments:** No testing (X) Testing ()

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	56-81-5
Chemical Name	1,2,3-Propanetriol (Glycerol)
Structural Formula	 <chem>OCC(O)CO</chem>
RECOMMENDATIONS The chemical is currently of low priority for further work.	
SUMMARY CONCLUSIONS OF THE SIAR Human Health All SIDS health endpoints are fulfilled. It should be noted that much of the data on glycerol is historic and of rather low quality compared to current guideline requirements. Nevertheless, there is an overall consistency within the available data that allows conclusions to be drawn. Glycerol is absorbed following ingestion and metabolised by glycerokinase in the liver to carbon dioxide and water or incorporated in the standard metabolic pathways to form glucose and glycogen. The weight of evidence indicates that glycerol is of low toxicity when ingested, inhaled or in contact with the skin. Glycerol is of a low order of acute oral and dermal toxicity with LD₅₀ values in excess of 4000 mg/kg bw. At very high dose levels, the signs of toxicity include tremor and hyperaemia of the gastro-intestinal tract. Skin and eye irritation studies indicate that glycerol has low potential to irritate the skin and the eye. The available human and animal data, together with the very widespread potential for exposure and the absence of case reports of sensitisation, indicate that glycerol is not a skin sensitiser. Repeated oral exposure to glycerol does not induce adverse effects other than local irritation of the gastro-intestinal tract. The 2-year study of Hine (1953) was chosen to establish the overall NOEL after prolonged treatment with glycerol of 10,000 mg/kg bw/day (20% in diet), which is in agreement with the findings in other studies. At this dose level no systemic or local effects were observed. For inhalation exposure to aerosols, the NOAEC for local irritant effects to the upper respiratory tract is 165 mg/m³ and 662 mg/m³ for systemic effects. Glycerol is free from structural alerts, which raise concern for mutagenicity. Glycerol does not induce gene mutations in bacterial strains, chromosomal effects in mammalian cells or primary DNA damage <i>in vitro</i>. Results of a limited gene mutation test in mammalian cells were of uncertain biological relevance. <i>In vivo</i>, glycerol produced no statistically significant effect in a chromosome aberrations and dominant lethal study. However, the limited details provided and the absence of a positive control, prevent any reliable conclusions to be drawn from the <i>in vivo</i> data. Overall, glycerol is not considered to possess genotoxic potential. The experimental data from a limited 2 year dietary study in the rat does not provide any basis for concerns in relation to carcinogenicity. Data from non-guideline studies designed to investigate tumour promotion activity in male mice suggest that oral administration of glycerol up to 20 weeks had a weak promotion effect on the incidence of tumour formation. No effects on fertility and reproductive performance were observed in a two generation study with glycerol administered by gavage (NOAEL 2000 mg/kg bw/day). No maternal toxicity or teratogenic effects were seen in the rat, mouse or rabbit at the highest dose levels tested in a guideline comparable teratogenicity study (NOEL 1180 mg/kg bw/day).	

Environment

All SIDS environmental endpoints are fulfilled. It should be noted that much of the data on glycerol is historic and of rather low quality compared to current guideline requirements. However, the weight of evidence indicates that glycerol is of low toxicity to aquatic organisms and this conclusion is supported by QSAR predictions. The lowest LC₅₀ for fish is a 24-h LC₅₀ of >5000 mg/l for *Carassius auratus* (Goldfish) and for aquatic invertebrates, a 24h EC₅₀ of >10000 mg/l for *Daphnia magna* is the lowest EC₅₀. Several tests on algae are available, which suggest very low toxicity to a range of species, however their validity is uncertain. A QSAR prediction for the 96h EC₅₀ to algae was 78000 mg/l. No toxicity towards the microorganism *Pseudomonas putida* was observed at 10000 mg/l after exposure for 16 hours. No long-term aquatic toxicity data is available. Screening studies are available on frog and carp embryos which indicate some effects on growth and hatching rates respectively at very high concentrations of glycerol, >7000 mg/l. However, their ecological relevance is not clear.

In view of the limited robustness of the studies present, it was decided to derive a tentative PNEC for aquatic organisms using QSAR predictions of acute toxicity. The tentative PNEC for aquatic organisms is calculated to be 780 mg/L, based on the lowest QSAR value (calculated for algae EC₅₀ 77,712 mg/L) and applying an assessment factor of 100 in accordance with the OECD guidance. An assessment factor of 1000 for the aquatic PNEC compartment could also be considered to reflect the uncertainty in the use of QSAR-predicted values. There are no sediment or terrestrial effect data, but partitioning to both soil and sediment is expected to be very low, based on the very low log K_{ow} of glycerol. The equilibrium partitioning method was used to calculate tentative PNECs for soil and sediment based on the PNEC_{aqueatic} of 777 mg/l, PNEC_{sediment} = 479 mg/kg wwt and PNEC_{soil} = 92.1 mg/kg wwt.

Exposure

The worldwide market for glycerol for the year 2000 was 500,000 tonnes. Glycerol has widespread use and can be found in industrial, professional and consumer products. Glycerol is used as a constituent in numerous products and as an intermediate in industrial applications for the manufacture of products such as soaps/detergents and glycerol esters. It is found in consumer products such as pharmaceuticals, cosmetics, tobacco, food and drinks and is present in numerous other products such as paints, resins and paper.

There is a potential for occupational exposure through inhalation and skin contact. Consumers may be exposed to glycerol by the oral and dermal routes of exposure. Smoking may lead to an additional glycerol uptake by inhalation.

There is potential exposure to the aquatic compartment arising from the production and processing of this substance. Glycerol will enter the aqueous and terrestrial environment from end uses such as in consumer products and down hole lubricants for oil and gas fields.

Glycerol is a liquid with a calculated vapour pressure of 0.000106 hPa (at 25°C), is fully miscible with water and has a Log K_{ow} of -1.76 (measured). It has a calculated half-life for photo-oxidation of ~7 hours and is not susceptible to hydrolysis. The experimental data indicate that glycerol is readily biodegradable under aerobic conditions. Fugacity modelling (Mackay Level III) predicts that glycerol will partition to the aquatic compartment (100%). Based on the low Log Kow, it has a low potential for sorption to soil and is not expected to bioaccumulate.

NATURE OF FURTHER WORK RECOMMENDED

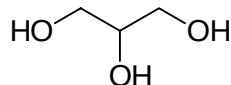
No further work is indicated, because of the low hazard potential of this substance.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 56-81-5
 Chemical Name: 1,2,3-Propanetriol
 Molecular Formula: $C_3H_8O_3$
 Structural Formula:



Molecular Weight: 92
 Synonyms: Glycerol; glycerine; glycerin; glyceryl alcohol; trihydroxypropane, 1,2,3-trihydroxypropane; Citifluor AF 2; Glycerin mist; Glyceritol; Clyzerin, wasserfrei (German); Grocolene; Moon; Osmoglyn; Star

1.2 Physico-Chemical properties

Glycerol (CAS no. 56-81-5) is a liquid at room temperature having the following physical–chemical properties and characteristics, which have been obtained from various reference sources (see the IUCLID dataset for further details).

Table 1 Summary of physico-chemical properties

PROPERTY	VALUE
Physical form	Liquid
Purity	95 – 99.5% (water as an impurity with trace levels of polyglycerol)
Melting point	18°C
Boiling point	290°C at 1013 hPa
Relative density	1.26 at 20°C
Vapour pressure	0.000106 hPa at 25 Deg C (calculated) and 0.0033 hPa at 50°C (measured)
n-octanol –water partition coefficient	log K_{ow} - 1.76
Water solubility	Miscible
Dissociation constant	0.07E-13
Flash point	160°C
Autoflammability	393°C
Viscosity	1410 mPa s at 20°C
Surface tension	63.4 mN/m at 20°C

For vapour pressure a measured value at 50°C is available. At this temperature vapour pressure is very low. It is expected that at room temperature this value will even be lower. This is confirmed by

model calculations with the Syracuse programme (EPIWIN vs 3.04) indicating a vapour pressure of 0.000106 hPa at 25°C. This value is used in model calculations.

Model calculations on the octanol-water partition coefficient differ by about one order of magnitude (see IUCLID dataset). Since a measured value of Log Kow = -1.76 is available, this has been selected as the key value. This measured value is supported by a QSAR prediction using KOWWIN version 1.66, predicted Log Kow = - 1.65.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Estimated Production or Import Volume

In 2000 the estimated world production of glycerol was 500,000 tonnes. The amount imported and/or produced in Europe was 227,000 tonnes for 1999 and for the UK, around 28,000 tonnes (data from APAG).

Uses

Glycerol has a ubiquitous use pattern and can be found in industrial, professional and consumer products. Glycerol is used as a constituent in numerous products and as an intermediate in industrial applications for the manufacture of products such as soaps/detergents and glycerol esters. It is found in consumer products such as pharmaceuticals, cosmetics, tobacco, food and drinks and is present in numerous other products such as paints, resins and paper. For example, it is used as a down hole lubricant in oil and gas fields and as a wetting agent in pesticide formulations. There is no single use which dominates the use pattern.

Table 2.1.1 gives an approximate breakdown of end uses and is derived from industry data in Europe (APAG, 1999) and from the Danish Products Register. More detailed information is available in the SIDS Dossier.

The NIOSH NOES Survey of 1981 – 1983 estimated that 137,302 workers were potentially exposed to glycerol in the United States.

The number of manufacturing and processing sites for glycerol will be significant based on the ubiquitous use of this substance and the source and quantity of releases will vary depending on the nature and pattern of use.

Table 2.1.1: Overview of Use

Type of end use	% of production volume (approx.)	Specific applications
Pharmaceuticals	<10	Excipient and formulation aid.
Chemical intermediate, nitration , and esters	15	Chemical synthesis
Cosmetic and Toiletries	20	Cosmetics including fragrances, bath and hair preparations, eye makeup, soaps and skin care preparations.
Resins, polyols and polyurethanes	20	Intermediate and monomer
Industrial Fluids	<10	Antifreezes, lubricants and hydraulic fluids
Tobacco	<10	Humectant
Cellulose films	<10	Intermediate.
Food	<10	Food additive
Other chemical uses	<10	

Table 2.1.2: Typical Routes of Exposure

Environmental exposure	Aquatic	Production
	Aquatic/terrestrial	Processing/ Industrial use
	Aquatic	Consumer use
Consumer exposure	Dermal	Cosmetics and pharmaceuticals
		Paints, printing inks and resins
		Paper and plastics
	Oral	Pharmaceuticals
		Cosmetics
		Cellulose films (meat casing, sausage skin)
		Food and drinks
	Inhalation	Smoking
Worker exposure	Inhalation	Production/Processing
	Dermal	Paints, printing inks, resins

2.2 Environmental Exposure and Fate

Glycerol is completely miscible with water, has a vapour pressure of 0.000106 hPa at 25°C and a calculated Henry's Law constant of 9.75E-6 Pa.m³/mol. The Henry's Law constant was calculated using the maximum solubility permitted in the EUSES model (100,000 mg/L). The following values were used in environmental fate and distribution modelling:

Parameter	Value	Discussion
Vapour pressure	0.000106 hPa	This value is obtained from Syracuse EPIWIN. Measured values were all obtained at higher temperatures. The differences have a negligible effect on modelling output.
Solubility	100,000 mg/L	Glycerol is completely miscible. For modelling the maximum solubility permitted in EUSES has been used.
Log K _{ow}	-1.76	Measured value
Biodegradability	readily	Based on a test according to OECD 301.

2.2.1 Sources of Environmental Exposure

During production, processing and use glycerol may be released to the environment.

There will be small amounts of glycerol released from the production and processing, which will typically be treated by the site wastewater treatment plant. Glycerol will enter the aqueous and terrestrial environment from end uses such as in cosmetics and pharmaceutical products and down hole lubricants for oil and gas fields.

There is little likelihood of emissions to the atmosphere from production, processing or downstream use.

2.2.2 Photodegradation

The calculated half-life for the photo-oxidation (reaction with hydroxyl radicals) of glycerol in air is 6.8 hours (EPIWIN vs 3.04).

2.2.3 Stability in Water

The stability of glycerol in water was not assessed. This is considered acceptable, because the molecule does not contain functional groups that are expected to react with water.

2.2.4 Transport between Environmental Compartments

From the EQC model (Mackay level III), it can be deduced that 100% of glycerol will end up in the water phase. Negligible amounts will be distributed towards soil, air and sediment.

Results of fugacity modelling	
Compartment	Distribution
Water	100 %
Air	0 %
Soil	0 %

From the measured log K_{ow} of -1.76 the log K_{oc} was determined to be 0.65 (EU Technical Guidance Document QSAR for alcohols, chapter 4 section 4.3, ¹) indicating a low potential for sorption to soil.

The distribution in a sewage treatment plant has been estimated using the SimpleTreat model to be 87% degraded, 13% to water, based on ready biodegradability, log $K_{ow} = -1.76$, water solubility = 1×10^5 mg/L and vapour pressure = 0.000106 hPa.

Conclusion

Based on the relevant physical-chemical properties and the fact that glycerol is readily biodegradable, glycerol will partition primarily to water (Mackay level III modelling shows 100% in water). In the sewage treatment plant glycerol will undergo a substantial degree of degradation.

2.2.5 Biodegradation

A number of biodegradation assays have been carried out with glycerol. In a Closed Bottle test (performed according to OECD 301) 92% biodegradation was reported after 30 days. More than 60% biodegradation measured as ThOD was reached within the 10-day window (Henkel 2001). Glycerol is considered to be readily biodegradable.

In addition, the relationship between BOD₅, COD and ThOD was determined. The standard dilution method for a period of 5 days (BOD₅) and the standard potassium dichromate method (COD) were used for the determinations. Both tests were performed according to APHA and ASTM guidelines, respectively. BOD₅ was 82% of ThOD and 86% of COD. It can be concluded that glycerol has the potential to be rapidly biodegraded in a wastewater treatment plant (Bridie 1979). The BOD₅/COD ratio is 0.86 and the fact that it is >0.5 further supports the ready biodegradability of glycerol.

¹ $\text{Log}K_{oc} = 0.39 \text{ log}K_{ow} + 0.50$ (alcohols)

Several other studies using adapted activated sludge or effluent from a sewage treatment plant demonstrates rapid biodegradation of glycerol (Matsui 1975, Matsui 1988, Pitter 1976, and Belly 1976).

Under anaerobic conditions, using microorganisms adapted to acetate, glycerol was biodegradable (Chou 1978).

Conclusion

Glycerol is considered to be readily biodegradable in the aquatic environment. Pre-adapted microorganisms can degrade glycerol rapidly under both aerobic and anaerobic conditions.

2.2.6 Bioaccumulation

The calculated bioconcentration factor is 3.162 (EPIWIN vs 3.04).

Conclusions

Based on Log K_{ow} -1.76, glycerol will have a low bioaccumulation potential and is not expected to bioaccumulate.

2.3 Human Exposure

2.3.1 Occupational Exposure

There is potential for occupational exposure through inhalation and dermal exposure. Occupational exposure to glycerol can occur during production, during processing or during use of products containing glycerol. The dermal route is considered to be the most relevant exposure route although inhalation exposure may occur to aerosols released from the spray application of resins or paints

For occupational exposure to glycerol mist, typically an exposure limit is applied based on the low toxicity of the aerosol. This value is 10 mg/m³ as an 8-hour time weighted average. (Belgium, Netherlands, Ireland, USA, UK).

2.3.2 Consumer Exposure

Glycerol is used extensively in cosmetics, toiletries and pharmaceutical products. It is used as a component in formulations mainly to provide emolliency and other performance benefits to the formulation. Consumer exposure to glycerol will occur principally through its use in food, cosmetics, toiletries and pharmaceuticals mainly through dermal exposure although oral exposure will occur as a consequence of use in foods as a direct food additive and indirectly from cellulose films used for food applications, orally administered drugs and oral hygiene products. There will be limited consumer dermal exposure through contact with paints, printing inks, resins and matrices containing glycerol.

- Glycerol has undergone review and approval for use as a direct food additive, indirect food additive and is recognised as generally safe for use in food. The use in food has been subject to review by expert assessment by organisations such as WHO, JECFA and the European SCF. (Joint FAO/WHO Expert Committee on Food Additives, 19th report. WHO Food Additive Series.8 1975.)
- SCF Reports of the Scientific Committee for Food, 33rd Series, European Commission, 1995.

The use of glycerol in tobacco products may lead to inhalation exposure as a constituent of tobacco smoke.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Data from studies in humans and animals indicate glycerol is rapidly absorbed in the intestine and the stomach, distributed over the extracellular space (Lin 1977, Tourtelotte 1970) and excreted. Glycerol is phosphorylated to alpha-glycerophosphate by glycerol kinase predominantly in the liver (80-90%) and kidneys (10-20%) and incorporated in the standard metabolic pathways to form glucose and glycogen (Tao 1983, Lin 1977). Glycerol kinase is also found in intestinal mucosa, brown adipose tissue, lymphatic tissue, lung and pancreas. Glycerol may also be combined with free fatty acids in the liver to form triglycerides (lipogenesis) which are distributed to the adipose tissues. The turnover rate is directly proportional to plasma glycerol levels (Bortz 1972).

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

No data available

Dermal

No deaths were observed in a group of 6 rabbits after occlusive dermal application for 8 hours of synthetic or natural glycerol at 18,700 mg/kg bw. (Hine 1953).

Oral

In a study with limited reporting, twelve female rats received 27,260 mg natural or synthetic glycerol/kg bw by gavage (Jansson and de Rooy). Cageside observations included muscle spasms and convulsions and survivors appeared normal within 2.5 h of dosing. The number of deaths was not reported. Macroscopic examination of decedents and survivors showed hyperaemia of the pylorus, small intestine and cerebral meninges (3 animals), congestion of the lungs and pale spleen. For this study an LD₅₀ value of 27,200 mg/kg bw was reported (Hine 1953). In other studies, with limited reporting, LD₅₀s of >25,300 and >24, 000 mg/kg were derived for Sprague-Dawley rats (Bartsch 1976) and female Fischer 344 rats (Clark 1979), respectively.

Hine (1953) also investigated the acute toxicity of synthetic or natural glycerol in mice and guinea pigs. Again the reporting was limited, however both species showed similar clinical signs (tremor and convulsions) and macroscopic findings (hyperaemia of pylorus and small intestine, pale spleen, lung congestion). The LD₅₀s for mice and guinea pigs were reported to be 23, 000 and 10, 000 mg/kg, respectively.

A number of acute oral toxicity LD₅₀ values for the rat (range from >5000 to 58400 mg/kg) and the mouse (4,250 to 38,000 mg/kg) are reported in the scientific literature (see table 3.1.2), although where values are very similar, it is not always clear whether or not these are from independent studies. Original reports for several secondary reported LD₅₀ values were not available. The LD₅₀ values reported are consistent with the range of values found in the available literature except in one case, where an oral LD₅₀ value of 4250 mg/kg was reported for the mouse (Anon. 1977).

A number of studies conducted in several species on the acute oral and dermal toxicity of glycerol are included in table 3.1.2. Although the additional studies in table 3.1.2 are poorly reported, the LD₅₀ values are generally similar to those obtained by Hine (1953)

Other Routes of Exposure

In rats and mice, LD₅₀ values for glycerol were between 4,420 and 10,100 mg/kg bw after intraperitoneal administration and 4250-6700 (rabbit LD₅₀ = 53000) mg/kg bw after intravenous administration. Glycerol is much more toxic after subcutaneous administration (LD₅₀ 91-100 mg/kg bw). The key finding is that of haemolysis. Further details are available in the SIDS dossier.

Table 3.1.2 Summary of acute oral and dermal toxicity data (key studies emboldened)

Species	LD ₅₀ (mg/kg bw)	Reference
ORAL		
Rat	27,200	Hine 1953
Rat	>25,300	Bartsch 1976
Rat	>24,000	Clark 1979
Rat	26,000	Anderson 1950
Rat	27,500	Smyth 1941
Rat	>25,000	Tao 1983
Rat	58,400	Bornmann 1955, Loeser 1954,
Rat	>5000, 15750, 27500, 26250-28750,	Janssen and de Rooy
Rat	>10000, 12600	Anon, 1945
Mouse	23,000	Hine 1953
Mouse	37,950	Bartsch 1976
Mouse	4,250	Anon., 1977
Mouse	26,000	Anderson 1950
Mouse	38,000	From Bartsch 1976
Mouse	>38,000	Tao 1983
Mouse	37,763	Bornmann 1955, Loeser 1954
Mouse	25,888	Bornmann 1955, Loeser 1954
Mouse	12,500	Latven 1939
Mouse	22400, 38000, 31250, 28 000	Janssen and de Rooy
Rabbit	27000	Anon, 1959
Guinea pig	10,000	Hine 1953
Guinea pig	7,750	Smyth 1941, Sonntag
DERMAL		
Rabbit	> 18,700 mg/kg bw	Hine 1953

Studies in Humans

A single human 'lowest-lethal-dose' (LDLo) value is reported to be 1428 mg/kg (Anon., 1969). However, the reliability of this information is unknown, as the original literature reference was not available to the reviewer.

Anecdotal reports indicate that subcutaneous or intraperitoneal injection of glycerol in humans, results in albuminuria, haemoglobinuria, anaemia and renal damage.

Conclusion

Glycerol is of very low acute toxicity to mammals. The range of acute oral LD₅₀ values derived from studies in experimental animals is between >4,000 and < 38,000 mg/kg, with the majority of values being between 23,000 and 38,000 mg/kg. For acute dermal toxicity a single LD₅₀ of >18,700 mg/kg for rabbits is available. No information is available on the acute toxicity of inhaled glycerol. Glycerol is more toxic when administered intravenously, intraperitoneally or subcutaneously.

3.1.3 Irritation

Skin Irritation

There were no studies conducted to modern OECD guidelines. In a study conducted using contemporary protocols but prior to GLP standards it was demonstrated that dermal application of 0.5-mL glycerol to the rabbit's skin for 24 hours did not lead to signs of irritation 24 and 72 hours after application. Irritation scores according to Draize scale were 0-0.4, compared to a maximum score of 30 (Weil 1971).

Another test with similar methodology gave a similar outcome (Clark 1979) and there was no evidence of irritation in rabbits following repeated applications of 4 mL over 30% of the surface area 8 h/day for 90 days (Hine 1953).

Conclusion

No OECD guideline studies are available, however, the data available indicate that glycerol is not irritating when in contact with the skin.

Eye Irritation

In a study conducted to a contemporary protocol and prior to GLP standards, 0.1 mL undiluted glycerol was instilled in the eyes of 6 rabbit) caused no evidence of irritation after 1, 24 and 72 hours and after 7 days. The overall irritation score using the Draize system was 0-2 on a scale up to a maximum of 110 (Weil 1971). In another study of similar design, using 4 rabbits, irritation of unspecified severity observed at 1 h after instillation of glycerol was absent after 24 h (Hine 1953). Another test with a similar design on a glycerol/water mixture (not further specified) gave a similar result and reactions, which were reversible within 24 h (Clark 1979). Due to the methodology and scoring systems used in these non-OECD guidelines it is not possible to directly compare the results to internationally agreed criteria for assessing eye irritation. However, it is apparent from these studies that glycerol has a very low potential to irritate the eyes.

In an OECD guideline study reported in a secondary source, slight to moderate corneal irritation was observed in all rabbits after 1h, however the effects were found to regress after 24h and were fully reversed by 48h (Janssen and de Rooy).

In a secondary source, there is an anecdotal report that in workers that glycerol caused a burning and stinging sensation with tear production but without injury (Grant 1974).

Conclusion

The data available indicate that glycerol is not irritating to the eyes.

3.1.4 Sensitisation

Studies in Animals

No studies conducted to current OECD guidelines are available. A group of male 24 Guinea pigs receiving 10 0.1 mL injections of 0.1 % synthetic or natural glycerol in isotonic saline every alternate day over 20 days showed no indication of sensitisation following challenge with further 0.05 mL injections of 0.1 % glycerol after a 2 week exposure-free period (Hine 1953). However, it is unclear from the protocol and reporting whether or not the study was capable of detecting sensitisation since it was not apparent whether maximal dose-levels were used, and the use of positive controls or a measure of strain sensitivity were not reported.

Human experience

Data is available from a briefly reported study, in which skin patch tests were conducted on workers in a foam rubber factory. No sensitising effects of a glycerol/water mixture became apparent (El-Nagdy 1973). Considering the extensive, widespread dermal exposure to glycerol in preparations repeatedly applied to the skin, the absence of case reports of humans showing skin reactions is consistent with glycerol having a very low skin sensitisation potential.

Conclusion

Based on the available information, there is no human or animal data that indicates glycerol to be a skin sensitizer. Considering the extensive, widespread dermal exposure to glycerol in preparations repeatedly applied to the skin, the absence of case reports of humans showing skin reactions is consistent with glycerol having a very low skin sensitisation potential.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

Two key studies were identified which had some limitations in the reporting and of the study protocols compared to current OECD guidelines (a reduced number of haematology and clinical chemistry investigations, and range of organs examined). However, these inhalation studies provide useful information as they used test and control groups of rats exposed to respirable aerosols of glycerol at measured concentrations and histopathological examination of a range of tissues including the respiratory tract was performed.

Sprague-Dawley rats (10/sex/treatment) were exposed nose-only to a respirable aerosol of glycerol during 14 days (5 days/week, 6 hours/day). The mean exposure concentrations achieved were 0, 1000, 1930 and 3910 mg/m³. The mass median aerodynamic diameter (MMAD) was reported to be < 1.5 micrometres. Two males at 1000 mg/m³ and 1 male and 1 female at 2000 mg/m³ died (which were incidental to treatment). Body weight gain was decreased in all treated animals. This effect may be attributed to stress due to nose-only exposure. Serum glucose was decreased in treated females, but since it did not appear in males and no relationship with concentration was established, the biological relevance of this effect is not considered to be of toxicological significance. There was no effect on lung, liver, kidney, brain and heart weight nor any macroscopic findings reported. Histopathological examination of the respiratory tract, liver, kidneys and heart of controls and high dose animals revealed an increased incidence of minimal to mild squamous metaplasia of the epiglottis in all treated animals (1/10, 13/18, 16/19 and 13/14 at 0, 1000, 1930, and 3910 mg/m³, respectively). The frequency of animals with mild metaplasia was greatest at the highest exposure

concentration. No systemic effects were seen at the highest dose tested (Anderson 1950, Renne 1992).

In a further study by the same authors using a similar protocol, nose-only exposure of rats (SD 15/sex/treatment) 6h/day, 5d/w for 13 weeks to a respirable aerosol (MMAD <2 micrometres) of glycerol at measured concentrations of 0, 33, 165 and 662 mg/m³ led to decreased triglyceride levels in males at 33 (34%) and 165 mg/m³ (22%). This effect appears to be of little toxicological significance as there was no dose-response relationship and was seen in males only. There were no treatment related effects on cageside observations, haematology, organs weights or gross pathology. Microscopic evaluation of the tissues showed “minimal” (10 animals) or “mild” (one animal) squamous metaplasia of the epiglottis in 11 animals in total at the highest concentration. Since the effect on triglycerides did not show a relationship with concentration, was seen in males only and in the absence of any systemic target organ toxicity, the biological relevance of this effect is not considered to be of toxicological significance. Based on an increased incidence of “minimal” to “mild” squamous metaplasia of the epiglottis, the NOAEC for local irritant effects to the upper respiratory tract is 165 mg/m³ and 662 mg/m³ for systemic effects (Anderson 1950, Renne 1992).

Oral

No OECD guideline studies are available although there are a large number of older studies available. In the best available dietary study, groups of 22 rats (Long-Evans)/sex/treatment received 5, 10 and 20% glycerol (natural or synthetic) in their diet (males 2000, 4000 and 8000 mg/kg bw; females 2500, 5000 and 10000 mg/kg bw) for 2 years. Routine clinical observations were made, and bodyweight and food consumption was determined weekly. Deviations from the OECD guideline included the absence of clinical chemistry investigation and a limited range of haematological and urinary analyses were performed. A limited range of organs was investigated at necropsy and the liver, spleen, adrenals, small intestine, gonads and urinary bladder were examined microscopically. Glycogen and fat content of the liver was determined in surviving rats from the 0 and 20 % dose groups. No individual data were reported. For high dosed animals treatment was discontinued after 1 year (reason not stated in report, presumably as an ‘interim’ assessment for carcinogenicity; 3.1.7). No data on mortality and clinical observations were reported. Food consumption was slightly increased in males treated with 5 and 10% natural glycerol. Incidental observations considered by the report-authors to be without relationship to treatment included: bronchiectasis, pneumonia, pulmonary abscesses, hydronephrosis and pyelonephritis. Although the results were not described in detail, based on this limited dietary study it can be concluded that no adverse effects were observed at up to 10,000 mg/kg bw (Hine 1953).

A number of other studies have been incorporated in Table 3.1.5. These studies are considered less reliable indicators of the systemic effects of glycerol following repeated administration, mainly because of limited toxicity assessments and/or deficient experimental design. The effects they do report are consistent with those observed in the key studies and as such they may contribute to the overall assessment of toxicity of glycerol.

Table 3.1.5 Repeated dose oral studies (excluding key study)

Study type	Species	No. of animals	Doses(mg/kg)	Effects	NOAEL (mg/kg)	Reference
DIET						
28-day	Rat	n.i.	20% suppl. diet (= 8824 mg/kg bw)	No abnormal findings	-	Stoewsand 1966
50-wk	Dog	3	35% in diet (no reference value available to convert to mg/kg bw)	BWG↓	-	Johnson 1933
WATER						
3-mnth	Rat	n.i.	1-20% solution in drinking water (= 667-13340 mg/kg bw)	At 20%: Mortality 2/12 rats at top dose; initial growth and development ↓ (with recovery)	-	Loeser 1954, Bornmann 1955
6-mnth	Rat	5 females/treatment	5% (natural or synthetic) in drinking water (= 3335 mg/kg bw)	Mortality 1/5 (synthetic) Small thymus and spleen, calcified kidney masses	-	Anderson 1950
GAVAGE						
6-mnth	Rat	n.i.	Oral 10 ml of 50% solution/kg bw (= 6300 mg/kg bw)	No abnormal findings	-	Loeser 1954
50-day	Rat	n.i.	Oral 10 ml of 20% solution/kg bw (= 2520 mg/kg bw)	No abnormal findings	-	Loeser 1954
44-day	Rat	20 males/treatment	1, 5, 10, 20% in water (gavage) (=115-2300 mg/kg bw)	Mortality 15% in all treatment groups and controls	-	Fisher 1949
44-day	Rat	20 males/treatment	~1260 mg/kg bw (gavage)	No treatment related effects	-	Fisher 1949
21-day	Rat	8 males/treatment	~1525 mg/kg bw (gavage)	Mortality at 1525 mg/kg 5/8, O ₂ consumption ↓	-	Fisher 1949
3-day	Rat	10 females/treatment	950, 1900 and 3800 mg/kg bw (gavage)	GI-tract: hyperaemia, petechial haemorrhage or erosions (DR)	LOAEL (local) 950 mg/kg bw	Staples 1967

Study type	Species	No. of animals	Doses(mg/kg)	Effects	NOAEL (mg/kg)	Reference
3-day	Dog	1-2/ treatment	950, 1900 and 3800 mg/kg bw (gavage)	GI-tract: hyperaemia, petechial haemorrhage or erosions (DR)	NOAEL (local) 950 mg/kg bw	Staples 1967
30-40 day	Guinea pig	10	Oral 5 ml of 50% solution (= 6300 mg/kg bw)	All animals died RBC↓		Ostwald 1962

↑ = increase

BWG = body weight gain

CHO = cholesterol

CL = chloride

n.i.= not indicated

↓ = decrease

RBC=red blood cells

UV= urinary volume

WC = water consumption

DR = dose related

Human experience

In cases where glycerol was given intravenously to control cerebral oedema, there were no identified toxic signs attributed to glycerol (Meyer 1971). Chronic ingestion of glycerol gave increased levels of triglycerides (MacDonald 1970).

Conclusion

A considerable number of studies have been performed. However, many of these studies are considered to be of indeterminable reliability due to deficiencies in reporting or methodology, primarily because they were performed before internationalised guidelines were available.

Based on the studies of better quality, it can be concluded that repeated oral exposure by gavage to glycerol does not induce adverse effects other than local irritation of the gastro-intestinal tract. The lowest effect value was 950 mg/kg bw and found in a 3 day study with rats (Staples 1967). The design of this study was considered not to be representative for a repeated dose study, because the duration of exposure was only 3 days and only irritant properties were investigated. The 2-year study of Hine (1953) was chosen to establish the overall NOAEL after prolonged treatment of rats with glycerol. It was concluded that the NOEL is 10,000 mg/kg bw (20% in diet), which is in agreement with most of the findings reported in Table 3.1.3. At this dose level no systemic or local effects were observed in the parameters investigated. However, it is noted that gavage dosing with bolus administration of glycerol may enhance the local toxicity to the gastrointestinal tract compared with continuous administration via the diet, however, toxic effects are still only seen at relatively high dose levels and do not raise concern

For inhalation exposure, irritant effects were observed at 662 mg/m³. No other target organ involvement was identified. The NOAEL for local effects on the respiratory tract following exposure by inhalation is 165 mg/m³.

3.1.6 Mutagenicity

In vitro Studies

Glycerol did not induce mutations in bacteria in an Ames test, which used four *Salmonella typhimurium* strains both with and without metabolic activation (rat and hamster S-9). The test was performed in three different laboratories (Haworth 1983). No mutagenic effects were reported in an additional Ames test with 5 strains and rat S-9 as metabolic activation system (Doolittle 1988).

Glycerol was considered to be negative by the authors in a mammalian cell gene mutation test (HGPRT) since the increased number of mutations at the two highest dose levels was considered to be biologically irrelevant, because no concentration dependence was seen (Doolittle 1988). However, it is unknown why the concentrations tested were not maximised to those recommended in the OECD guideline and hence, the result is of uncertain biological relevance.

Chromosomal damage was investigated in the chromosomal aberration test using cultured mammalian cells (Chinese hamster ovary), which was reported as negative. In this test an isolated increase in number of aberrations was seen at 200 µg/mL (with metabolic activation). This finding was considered to be of no biological relevance, since there was no relationship with the concentration tested (Doolittle 1988). Glycerol did not induce sister chromatid exchanges in CHO cells (Doolittle 1988). In rat hepatocytes, the number of nuclear grains did not differ between glycerol treated and control cells (Doolittle 1988). Therefore, it can be concluded that no unscheduled DNA synthesis occurred.

The results of a bacterial recombination assay were positive, which may be attributed to the interference of glycerol with cellular surfaces or with osmotic effects (Nonakae 1989).

Other *in vitro* tests available are summarised in table 3.1.6.

Table 3.1.6 *In vitro* mutagenicity tests

Test type	Result	Reference
Ames	negative	Stolzenberg 1979, Ishidate 1984 , Clark 1979, Yamaguchi 1982,
Chromosome aberration	negative	Ishidate 1984

In vivo Studies

Two *in vivo* assays are available for glycerol (Varilyak and Kozachuk, 1985). In a rat bone marrow chromosome aberration test glycerol did not induce a statistically significant increase in chromosomal aberrations compared to controls. However, the lack of sufficient reported details on clinical toxicity and absence of a positive control limit the significance which can be attached to this result.

In the same study a rat dominant lethal assay was conducted. Although a dose related increase was seen in post implantation loss, this increase was not statistically significant compared to controls. However, the limited details on methodology, small number of animals per dose group and absence of a positive control, mean no reliable conclusions can be drawn from the data.

Conclusion

There are no structural alerts (expert judgement) which raise concern for the inherent mutagenic potential of glycerol. *In vitro*, glycerol was negative (with and without metabolic activation) in Ames tests and did not induce chromosomal effects in mammalian cells. The responses seen in a limited gene mutation study in mammalian cells are of uncertain biological relevance as the doses were not maximised. Only two *in vivo* studies are available. A negative result was observed in a chromosome aberration test, and an increase (not statistically significant) in post implantation loss was seen in a rat dominant lethal assay. However, for both assays, the limited details reported and absence of a positive control, mean no reliable conclusions can be drawn from the *in vivo* data. Thus, overall, there is no *in vitro* or *in vivo* data that indicates glycerol to have a genotoxic potential.

3.1.7 Carcinogenicity

In a limited and non OECD Guideline 12-24 month dietary study in rats, evidence of malignant neoplasms were reported in 5/26, 1/22, 5/22, 0/22, 0/21, 5/22 and 0/22 animals in controls and at 5%, 10%, 20% (natural glycerol) and at 5%, 10%, 20% (synthetic glycerol). At the top dose, the treatment period was one year. Benign neoplasms were encountered including pheochromacytomas and granulosa cell tumours in 0/26, 2/22, 1/22, 0/22, 4/21, 4/22 and 1/22 animals in controls and at 5%, 10%, 20% (natural glycerol) and at 5%, 10%, 20% (synthetic glycerol), respectively. The authors concluded that glycerol does not initiate tumour development in the rat (Hine 1953).

In male ddY mice administration of glycerol (5% in drinking water during 1-20 weeks) after a single s.c. injection with 4-nitroquinoline 1-oxide (4-NQO) was reported to enhance lung tumour development. Histopathologically most lung tumours were identified as adenomas (Nagahara 1987, Inayama 1986). The mechanism of tumour induction was independent from pulmonary cell kinetics (Nagahara 1987).

Conclusion

No studies conducted to modern regulatory guidelines are available. The studies that are available are therefore of lower quality. No increase in tumour formation was observed in a limited dietary carcinogenicity study in the rat. Data from non-guideline studies designed to investigate tumour promotion activity in male mice suggest that oral administration of glycerol up to 20 weeks had a weak promotion effect on the incidence of lung tumour formation. In the same studies, treatment with glycerol alone (administered in the drinking water) did not result in an increase in the number of tumour bearing mice relative to controls.. Overall, these data do not raise concern for carcinogenic potential.

3.1.8 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

In a two generation study not fully matching current OECD Guidelines, male and female rats (10/treatment) were dosed daily with glycerol (20% solution in water) during 8 weeks before mating. Females received glycerol throughout pregnancy or until weaning of the F1 generation (5 each). When the F1 generation was ~100 days of age, pups were killed except for 10/sex. These animals were used to produce the F2-generation. No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility, reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation. Although the data are limited, a NOAEL of 2000 mg/kg bw was identified from this study (Wegener 1953).

Intratesticular administration of glycerol decreased spermatogenesis, leading to complete loss of spermatogenic cells in rats, but did not affect sexual behaviour (Wiebe 1984). The intratesticular route of exposure is not considered appropriate to investigate effects on fertility. Furthermore, the author reports that no effects were observed following oral administration (data not shown).

Developmental Toxicity

Rats, mice and rabbits were treated daily with glycerol at dose levels up to 1310, 1280 and 1180 mg/kg bw (oral gavage), respectively, during part of the gestation period. The study protocol was in reasonable agreement with the requirements of the OECD 414 (1981). No maternal toxicity or

teratogenic effects were seen at the highest dose levels tested (NTIS 1974). From these studies a NOAEL of 1180 mg/kg bw can be derived.

The results from a Frog Embryo Teratogenesis Assay-Xenopus (FETAX, see also 4.1.4) were ambiguous. Since there is no other evidence of developmental effects especially on mammals, the results of this screening assay for developmental toxicity are not considered to be relevant to mammals. The authors considered the response to be a false positive (Bantle 1999).

Human experience

A fertility study involving 64 males workers involved in glycerol manufacture reported no significant differences in sperm quality parameters (sperm counts and percent “normal” forms) (Venable 1980).

Conclusion

Based on the available data, it can be concluded that glycerol does not have any adverse effects on reproductive parameters. There was no evidence of teratogenicity. The NOAEL for developmental toxicity is 1180 mg/kg bw. The evidence of effects on spermatogenesis following intratesticular administration are not considered relevant as an exposure route. These data do not cause concern in relation to reproductive effects from anticipated routes of exposure.

3.2 Initial Assessment for Human Health

The worldwide market for glycerol for the year 2000 was 500,000 tonnes. Glycerol has widespread use and can be found in industrial, professional and consumer products. There is a potential for occupational exposure through inhalation and skin contact. Consumers may be exposed to glycerol by the oral and dermal routes of exposure. Smoking may lead to an additional glycerol uptake by inhalation.

All SIDS health endpoints are fulfilled. It should be noted that much of the data on glycerol is historic and of rather low quality compared to current guideline requirements. Nevertheless, there is an overall consistency within the available data that allows conclusions to be drawn. Glycerol is absorbed following ingestion and incorporated in the standard metabolic pathways to form glucose and glycogen. The weight of evidence indicates that glycerol is of low toxicity when ingested, inhaled or in contact with the skin.

Glycerol is of a low order of acute oral and dermal toxicity with LD₅₀ values in excess of 4000 mg/kg bw. At very high dose levels, the signs of toxicity include tremor and hyperaemia of the gastro-intestinal tract. Skin and eye irritation studies indicate that glycerol has low potential to irritate the skin and the eye. The available human and animal data, together with the very widespread potential for exposure and the absence of case reports of sensitisation, indicate that glycerol is not a skin sensitiser.

Repeated oral exposure to glycerol does not induce adverse effects other than local irritation of the gastro-intestinal tract. The 2-year study of Hine (1953) was chosen to establish the overall NOEL after prolonged treatment with glycerol of 10,000 mg/kg bw (20% in diet), which is in agreement with the findings in other studies. At this dose level no systemic or local effects were observed. For inhalation exposure to aerosols, there is evidence of local irritant effects at and above 662 mg/m³. The NOAEL is 167 mg/m³.

Glycerol does not induce gene mutations in bacterial strains, chromosomal effects in mammalian cells or primary DNA damage in vitro. Results seen in a limited mammalian gene mutations test were of uncertain biological relevance. *In vivo*, glycerol produced no statistically significant effect

in a chromosome aberrations and dominant lethal study. However, the limited details provided and absence of a positive control prevent any reliable conclusions can be drawn from the *in vivo* data. Overall, glycerol is not considered to possess genotoxic potential.

The experimental data from a limited dietary study in the rat does not provide any basis for concerns in relation to carcinogenicity. Data from non-guideline studies designed to investigate tumour promotion activity in male mice suggest that oral administration of glycerol up to 20 weeks had a weak promotion effect on the incidence of tumour formation.

No effects on fertility and reproductive performance were observed in a two generation study with glycerol administered by oral gavage (NOAEL 2000 mg/kg bw). No maternal toxicity or teratogenic effects were seen in the rat, mouse or rabbit at the highest dose levels tested in a guideline comparable teratogenicity study (NOEL 1180 mg/kg bw).

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Data are available on the acute toxicity of glycerol to fish, daphnia, algae and microorganisms.

4.1.1 Fish and invertebrates

Species	Exposure (h)	LC/EC _x (mg/L)	Reference
<i>Leuciscus idus melanotus</i> (Golden Orfe)	n.i.	LC ₅₀ >10,000	Juhnke 1978
<i>Carassius auratus</i> (Goldfish)	24	LC ₅₀ >5,000	Bridie 1979
<i>Leuciscus idus</i> (Golden Orfe)	48	LC ₀ >250	Wierich 1968
<i>Oncorhynchus mykiss</i> (Rainbow trout)	96	LC ₁₀₀ = 51,000-57000	Johnson 1980 ^{n.r.}
Not specified	96	LC ₅₀ = 184,000	ECOSAR - QSAR
<i>Daphnia magna</i>	24	EC ₅₀ >10,000	Bringmann 1977, 1982
<i>Daphnia magna</i>	24	EC ₀ > 500	Henkel ^{n.r.}
<i>Daphnia</i>	48	EC ₅₀ = 153,000	ECOSAR - QSAR

n.i.= not indicated

n.r. = not retrievable

In a static test with the Golden orfe (*Leuciscus idus melanotus*), no mortality was reported up to a concentration of 10,000 mg/L. The test duration was not indicated and no other effects were mentioned (Juhnke, 1978). In a 24-hour test with the goldfish (*Carassius auratus*) a LC₅₀ of >5000 mg/L was established (Bridie 1979). In a further test with Golden orfe (*Leuciscus idus melanotus*), fish were exposed to glycerol concentrations of up to 250 mg/l for 48 hours and no effects were observed at the highest test concentration (Wierich, 1968).

For *Daphnia magna* a 24-hour EC₅₀ of >10,000 mg/L was found in several publications (Bringmann 1977, 1982). The studies are of rather low quality compared to current guideline requirements (non-GLP, no analytical measurement of test concentrations). However, the weight of evidence indicates that glycerol is of low acute toxicity to aquatic organisms with LC₅₀/EC₅₀ values being in excess of 5000 mg/L.

A QSAR prediction for the 96-hour LC₅₀ for fish of glycerol gave a value of 184,000 mg/L. For daphnia, a 48-hour LC₅₀ of 153,000 mg/L was calculated (ECOSAR v0.99f). These QSAR predictions are based on a calculated Log Kow of -1.65 and a calculated water solubility of 2.16 x 10⁴ mg/l using the neutral organics chemical class. They support the conclusion that glycerol is of low acute toxicity to fish and aquatic invertebrates. No information is available on toxicity to marine species.

Exposure of carp embryos in different developmental stages to a 1M glycerol solution after 5 minute or 1 hour exposure gave significantly decreased hatching rates (5 minutes 80-94%, 1 hour

14-78%, control values 91-95%) (Urbanyi, 1997). However, this study was designed to investigate the toxicity of glycerol when used as a cryoprotective substance. The result is not considered relevant to the hazard assessment since the test has a chronic endpoint but used only a short exposure duration and the exposure concentration was very high – 92 g/l. In addition, it is possible that the observed effects were due to physical effects due to high osmotic pressure.

Conclusions

Glycerol is of low acute toxicity to fish and aquatic invertebrates. LC/EC₅₀ values are all in excess of 5000 mg/L.

4.1.2 Algae

For blue algae (*Microcystis aeruginosa*) no inhibition of growth was seen at 2900 mg/L after 8 days of exposure to a glycerol solution in water. The study was not fully in accordance with the current OECD guideline mainly due to no quantification of the relationship between the measured extinction and growth inhibition, and it was not possible to derive an EC₅₀ value (Bringmann 1978, 1978, 1976, 1975). It is not possible to confirm the validity of the NOEC since it cannot be confirmed that algae were in the exponential growth phase for the test duration. An 8-day test with *Scenedesmus quadricauda* (green algae) showed a very low toxicity (EC₀ >10,000 mg/L). This study was not designed or conducted in line with current guidelines (Bringmann 1980, 1978, 1977, 1978).

An investigation of the effect of glycerol on the growth of 18 species of marine phytoplankton revealed that in the presence of light, glycerol generally enhanced growth at the lowest test concentration of 4600 mg/l. However, inhibition of growth was seen in *Agmenellum quadruplicatum* and *Anacystis marina* at a concentration of 4600 mg/l. The validity of this study could not be determined but it suggests that there is no significant difference in toxicity between the freshwater and marine species.

Based on the information available, it can be concluded from the weight of evidence that glycerol is of low toxicity towards algae. This conclusion is supported by a QSAR prediction for the 96-hour EC₅₀ for algae of glycerol, which gave a value of 77712 mg/L (ECOSAR v0.99f), based on a calculated Log Kow of -1.65 and a calculated water solubility of 2.16×10^4 mg/l using the neutral organics chemical class.

Conclusions

Based on the information available glycerol is of low acute/chronic toxicity to algae.

4.1.3 Microorganisms

Glycerol is of low toxicity towards microorganisms. In a 16 hour test with *Pseudomonas putida* no inhibition of bacterial growth was found at concentrations between 100 and 10,000 mg/L (Henkel 1994). The information is considered sufficient to conclude that glycerol is of low toxicity to bacteria. Other studies on microorganisms are summarised in table 4.3.1. A NOEC is considered to be an effect level of 10% or less on cell growth. The validity of these studies could not be determined but the results support the conclusion that glycerol is of low toxicity to microorganisms.

Table 4.3.1. Toxicity towards microorganisms – additional studies

Species	Exposure (h)	NOEC mg/L	Reference
<i>Chlimonas paramecium</i>	48	>10,000	Bringmann 1980, 1981
<i>Clostridium</i> sp.	n.i.	170,000	Dabrock 1992
<i>Entosiphon sulcatum</i>	72	3200	Bringmann 1978, 1980
<i>Pseudomonas putida</i>	16	>10,000	Bringmann 1976, 1977, 1980
<i>Uronema parduzci</i>	20	>10,000	Bringmann 1980, 1981

n.i.= not indicated

Conclusions

Glycerol is of low toxicity to bacteria with an EC₀ of 3200 - 10,000 mg/L.

4.1.4 Other

A whole-embryo developmental toxicity screening test with frog embryos showed effects on growth at concentrations of 7210 mg/L (with metabolic activation) or 9040 mg/L (without metabolic activation). The 96h EC₅₀ value for malformations is 9290 mg/L (with metabolic activation) or 9680 mg/L (without metabolic activation) (Bantle 1999). The results are ambiguous since certain test criteria suggest that glycerol is non-teratogenic but severe malformations were observed at concentrations approaching the 96h LC₅₀ with metabolic activation. The relevance of these results to the environment is not clear but effects were observed only at very high concentrations of glycerol. It is possible that the observed effects were physical effects due to high osmotic pressure. (It should be noted that no teratogenic effects were observed in a test with mammals, refer to section 3.1.6).

No conclusion can be drawn about the hazard posed by glycerol to amphibians.

4.1.5 Determination of PNEC aqua

Data are available from short term tests at 3 trophic levels. In view of the limited robustness of the studies present, the QSAR predictions are used to derive a tentative PNEC. Based on the lowest value (calculated for algae EC₅₀ 77,712 mg/L) and applying an assessment factor of 100 in accordance with the OECD guidance the resultant PNEC_{aqua} is 780 mg/L. However, an assessment factor of 1000 for the aquatic PNEC compartment could also be considered to reflect the uncertainty in the use of the QSAR predicted values.

Conclusions

Glycerol is of low hazard to the aquatic environment with a tentative PNEC_{aqua} of 780 mg/L.

4.2 Terrestrial and Sediment Effects

There are no terrestrial and sediment effect data. Glycerol is not expected to partition to soil and sediment and exposure to these compartments is likely to be very low.

Calculations using the equilibrium partitioning method in the EUSES program and the tentative aquatic PNEC of 780 mg/l gave a tentative PNEC_{sediment} of 479 mg/kg wwt and a tentative PNEC_{soil} of 92.1 mg/kg wwt.

4.3 Other Environmental Effects

Based on the very low log K_{ow} of -1.76, glycerol is not expected to bioaccumulate significantly.

4.4 Initial Assessment for the Environment

There is potential exposure to the aquatic compartment arising from the production and processing of this substance. Glycerol will enter the aqueous and terrestrial environment from end uses such as in consumer products and down hole lubricants for oil and gas fields.

Glycerol is a liquid of calculated vapour pressure 0.000106 hPa (at 25°C), is fully miscible with water and has a Log K_{ow} of -1.76 (measured). It has a calculated half-life for photo-oxidation of ~7 hours and is not susceptible to hydrolysis. The experimental data indicate that glycerol is readily biodegradable under aerobic conditions. Fugacity modelling (Mackay Level III) predicts that glycerol will partition to the aquatic compartment (100%). Based on the low Log K_{ow}, it has a low potential for sorption to soil and is not expected to bioaccumulate.

All SIDS environmental endpoints are fulfilled. It should be noted that much of the data on glycerol is historic and of rather low quality compared to current guideline requirements. However, the weight of evidence indicates that glycerol is of low toxicity to aquatic organisms and this conclusion is supported by QSAR predictions. The lowest LC₅₀ for fish is a 24-h LC₅₀ of >5000 mg/l for *Carassius auratus* (Goldfish) and for aquatic invertebrates, a 24h EC₅₀ of >10000 mg/l for *Daphnia magna* is the lowest EC₅₀. Several tests on algae are available which suggest very low toxicity to a range of species, however their validity is uncertain. A QSAR prediction for the 96h EC₅₀ to algae was 78000 mg/l. No long-term aquatic toxicity data is available.

Screening studies are available on frog and carp embryos which indicate some effects on growth and hatching rates respectively at very high concentrations of glycerol, >7000 mg/l. However, their ecological relevance is not clear.

In view of the limited robustness of the studies present, it was decided to derive a tentative PNEC for aquatic organisms using QSAR predictions of acute toxicity. The tentative PNEC for aquatic organisms is calculated to be 780 mg/L, based on the lowest QSAR value (algae EC₅₀ 77,712 mg/L) and applying an assessment factor of 100 in accordance with the OECD guidance. In view of the limited robustness of the studies present, it was decided that this approach should be used. There are no sediment or terrestrial effects data, but partitioning to both soil and sediment is expected to be very low, based on the very low log K_{ow} of glycerol. The equilibrium partitioning method was used to calculate tentative PNECs for soil and sediment based on the PNEC_{aquatic} of 780 mg/l, PNEC_{sediment} = 479 mg/kg wwt and PNEC_{soil} = 92.1 mg/kg wwt.

Glycerol is a naturally occurring substance of low hazard. The ecotoxicology data available is largely historical and reflects the quality standards of the time. The data could not be validated as thoroughly as current data however the aquatic toxicity data are considered valid using a weight of evidence approach supported by QSARs. An assessment factor of 1000 could be considered to reflect the uncertainty of using historical data underwritten by QSARs.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

There are data on all SIDS endpoints and for many other toxicological and ecotoxicological endpoints. There is considerable data on glycerol and based on the overall weight of evidence, the substance is of low concern.

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ANNEX: SEARCH CRITERIA:

For transparency, some study summaries in the SIDS dossier were transferred from a previous version of IUCLID not intended for submission to OECD. In some cases, it was not possible to retrieve the original literature using the reported citation or as a result of literature searching, as detailed below. If references in the SIDS dossier on glycerol (IUCLID) were not retrievable, this was indicated in the SIDS dossier, or where relevant, in the SIAR also. Key studies are studies with the highest reliability/adequacy. If several studies showed comparable reliability/adequacy, the study with the lowest LC/LD/EC₅₀ or NOEC/ NOAEL was indicated as the key study.

Physical-chemical properties are obtained from standard reference works such as Lide, Hawleys Condensed Chemical Dictionary, Beilstein, Sax and Merck Index, also some calculated values are given obtained mostly using Syracuse prediction software (EPIWIN).

Among others, the following databases were searched under the CAS number 56-81-5 and the name glycerol in March 2001: Medline, Toxline, Enviroline, Embase, BIOSIS (over the period 1992-2001). The search strategy was used with no cut off criteria applied to exclude historical investigations

The search profile used for environmental endpoints included the following keywords:

Environm? or ecotox? or fate; air or soil or water or aquatic? or sedim?; photo? or stab? or distribut? or degrad? or transp? or monitor? or BOD or COD or accumul?; solub? or partition? or Kow or Pow or Koc or hydrol?; fish? or invert? or daphn? or alg? or plant? or kinet? or acute or chronic?; vertebrat? or microorg? or micro-org? or bacter? or ?dwelling? or tranform? or terrestr?

The search profile used for human health endpoints included the following keywords:

toxic?; human and (epidem? or case-rep? or (field and stud?) or volunt?); mutag? or DNA or gentox? or Ames? or carcin?; placenta and transfer; reproduct? or terato? or prenatal? or fertil?; repeat? and expos?; animal; inhal? or ?acute? or ?chron? or dermat?; (kinet? or metabol? or endocrin?); (PBPK or PB-PK); (irritat? or sensitiz? or sensitiz? or sensibilis? or sensibiliz?);(vitro)

I U C L I D

Data Set

Existing Chemical : ID: 56-81-5
CAS No. : 56-81-5
EINECS Name : glycerol
EC No. : 200-289-5
TSCA Name : 1,2,3-Propanetriol
Generic name : glycerine
Molecular Formula : C₃H₈O₃
Structural Formula : CH₂OH CH(OH) CH₂OH
Substance Group : Not applicable
Molecular Weight : 92

Producer related part
Company : Notox
Creation date : 26.04.2001

Substance related part
Company : Notox
Creation date : 26.04.2001

Status :
Memo : Revised including robust summaries

Printing date : 29.01.2002
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Reliability (profile) : Reliability: without reliability, 1, 2, 3, 4
Flags (profile) : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE),
Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type : cooperating company
Name : other: ICI Uniqema
Contact person : L.Hughes
Date :
Street :
Town :
Country :
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

Flag : confidential
08.01.2002

Type : sponsor country
Name : UK
Contact person : Dr. S. Robertson
Date :
Street :
Town :
Country :
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

08.01.2002

08.01.2002

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR**1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION**

IUPAC Name : Glycerine
Smiles Code :
Molecular formula : CH₂OH CH(OH) CH₂OH
Molecular weight : 92
Petrol class :

1. GENERAL INFORMATION

ID: 56-81-5

DATE: 29.01.2002

08.01.2002

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : Organic
Physical status : Liquid
Purity : > 95 % v/v
Colour :
Odour :

08.01.2002

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES**

glycerol; glycerine; glycerin; glyceryl alcohol; trihydroxypropane; 1,2,3-trihydroxypropane; Citifluor AF 2; Glycerin mist; Glyceritol; Glyzerin, wasserfrei (German); Grocolene; Moon; Osmoglyn; Star

08.01.2002

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name : Water
Molecular formula : H₂O
Value : .5 - 5 % v/v

25.01.2002

1.4 ADDITIVES**1.5 TOTAL QUANTITY**

Quantity : ca. 500000 - tonnes in 2000

08.01.2002

1.6.1 LABELLING

Labelling : as in Directive 67/548/EEC
Specific limits :
Remark : None

1. GENERAL INFORMATION

ID: 56-81-5

DATE: 29.01.2002

08.01.2002

1.6.2 CLASSIFICATION

Classified : no classification required (no dangerous properties)
Class of danger :
R-Phrases :
Specific limits :

08.01.2002

1.6.3 PACKAGING**1.7 USE PATTERN**

Type of use : type
Category : Use in closed system

08.01.2002

Type of use : type
Category : Use resulting in inclusion into or onto matrix

08.01.2002

Type of use : type
Category : Wide dispersive use

08.01.2002

Type of use : industrial
Category : Chemical industry: used in synthesis

08.01.2002

Type of use : industrial
Category : Polymers industry

08.01.2002

Type of use : use
Category : Cleaning/washing agents and disinfectants

08.01.2002

Type of use : use
Category : Cosmetics

08.01.2002

Type of use : use
Category : Food/foodstuff additives

1. GENERAL INFORMATION

ID: 56-81-5

DATE: 29.01.2002

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Type of use : use
Category : Pharmaceuticals

08.01.2002

1.7.1 DETAILED USE PATTERN**1.7.2 METHODS OF MANUFACTURE****1.8 REGULATORY MEASURES****1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES**

Type of limit : MAC (NL)
Limit value : 10 mg/m³

Remark : Glycerol mist
Aerosol form to be equivalent to a low toxicity particulate.

08.01.2002

Type of limit : OES (UK)
Limit value : 10 mg/m³

Remark : Glycerol mist
Aerosol form to be equivalent to a low toxicity particulate.

08.01.2002

Type of limit : TLV (US)
Limit value : 10 mg/m³

Remark : Glycerol mist
Aerosol form to be equivalent to a low toxicity particulate.

08.01.2002

Type of limit : other: Belgium
Limit value : 10 mg/m³

Remark : Glycerol mist
Aerosol form to be equivalent to a low toxicity particulate.

08.01.2002

Type of limit : other: Ireland
Limit value : 10 mg/m³

Remark : Glycerol mist
Aerosol form to be equivalent to a low toxicity particulate.

08.01.2002

1.8.2 ACCEPTABLE RESIDUES LEVELS

1. GENERAL INFORMATION

ID: 56-81-5

DATE: 29.01.2002

1.8.3 WATER POLLUTION**1.8.4 MAJOR ACCIDENT HAZARDS****1.8.5 AIR POLLUTION****1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES**

Type : other
 Additional information :

Remark : 10 most frequent industry groups:
 Other printing works
 Manufacture of fabricated metal products, except machinery and equipment
 Treatment and coating of metals on a fee or contract basis
 Manufacture of machinery and equipment
 Painting and glazing
 Maintenance and repair of motor vehicles
 Industrial cleaning
 Social work activities
 Private households with employed persons
 All kinds of activities

Product types containing 0-100% of glycerol:
 A02 Adhesives, binding agents: 30 products, 32 total tonnes /annum
 A38 Pesticides, agricultural: 10 products, <1 total tonnes /annum
 A43 Process regulators: 17 products, 23 total tonnes/annum
 A54 Welding and soldering agents: 8 products, <1 total tonnes /annum
 B10 Colouring agents: 33 products, 13 total tonnes /annum
 B14 Corrosion inhibitors: 10 products, <1 total tonnes /annum
 B15 Cosmetics: 75 products, 311 total tonnes /annum
 B20 Fillers: 20 products, 30 total tonnes /annum
 B31 Impregnation materials: 9 products, <1 total tonnes /annum
 B32 Insulating materials: 13 products, 32 total tonnes /annum
 B41 Pharmaceuticals: 10 products, 1 total tonne /annum
 B45 Reprographic agents: 23 products, 2 total tonnes /annum
 B50 Surface-active agents: 19 products, 80 total tonnes /annum
 B55 Others: 9 products, 45 total tonnes /annum
 C09 Cleaning/washing agents: 131 products, 23 total tonnes /annum
 C13 Construction materials: 6 products, 11 total tonnes /annum
 C39 Non-agricultural pesticides and preservatives: 12 products, 2 total tonnes /annum
 D56 Cutting fluids: 9 products, 1 total tonne /annum
 D59 Paints, laquers and varnishes: 126 products, 32 total tonnes /annum
 D61 Surface treatment: 39 products, <1 total tonnes /annum

25.01.2002

(1)

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS**1.10 SOURCE OF EXPOSURE****1.11 ADDITIONAL REMARKS****1.12 LAST LITERATURE SEARCH****1.13 REVIEWS**

2.1 MELTING POINT

Value : = 18.2 °C
Decomposition : no, at °C
Sublimation : no
Method : other
Year : 2000
GLP : no data
Test substance :

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
 19.12.2001

(2)

Value : = 18 °C
Sublimation :
Method :
Year : 1981
GLP :
Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Conclusion : Most reliable data available.
Reliability : (2) valid with restrictions
 Handbook data are considered to be from a trusted source.
Flag : Critical study for SIDS endpoint
 19.12.2001

(3)

Value : = 17.9 °C
Sublimation :
Method :
Year : 1996
GLP :
Test substance :

Conclusion : Most reliable data available.
Reliability : (2) valid with restrictions
 Handbook data are considered to be from a trusted source.
Flag : Critical study for SIDS endpoint
 19.12.2001

(4)

Value : = 18.2 °C
Sublimation :
Method :
Year : 1992
GLP :
Test substance :

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information in the report was confined to the above.
 19.12.2001

(5)

Value : ca. 18 °C
Decomposition : no, at °C
Sublimation : no
Method : other: Unknown (literature)

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Year	:		
GLP	:	no data	
Test substance	:		
Remark	:	Glycerol is seldom seen in its crystallized state, because of its tendency to supercool, and the pronounced effect of small amounts of water in depressing the melting (freezing) point.	
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
11.06.2001			(6)
Value	:	ca. 18 °C	
Decomposition	:	ambiguous, at °C	
Sublimation	:	no	
Method	:		
Year	:		
GLP	:		
Test substance	:		
Remark	:	Literature could not be retrieved.	
Source	:	Pronova Oleochemicals a.s. Sandefjord EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
01.06.1995			

2.2 BOILING POINT

Value	:	= 290 °C at 1013 hPa	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Conclusion	:	Most reliable data available.	
Reliability	:	(2) valid with restrictions Handbook data are considered to be from a reliable source.	
Flag	:	Critical study for SIDS endpoint	
19.12.2001			(3)
Value	:	= 290 °C at 1013.25 hPa	
Decomposition	:	yes	
Method	:	other	
Year	:	2000	
GLP	:	no data	
Test substance	:		
Reliability	:	(2) valid with restrictions	
Flag	:	Critical study for SIDS endpoint	
19.12.2001			(2)
Value	:	= 290 °C at	
Decomposition	:	yes	
Method	:		
Year	:	1996	
GLP	:		
Test substance	:		
Remark	:	Partly decomposition	
Reliability	:	(2) valid with restrictions Handbook data are considered to be from a reliable source.	
19.12.2001			(4)

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Value	: = 290 °C at 1013 hPa	
Result	: Boiling point = 222.4 °C at 133.3 hPa Boiling point = 149 °C at 5.3 hPa Boiling point = 166.1 °C at 13.3 hPa	
Test substance Reliability	: CAS 56-81-5 (glycerine), purity not indicated. (4) not assignable The information in the report was confined to the above.	
19.12.2001		(5)
Value	: < 180 °C at	
Decomposition	: yes	
Method	:	
Year	: 1986	
GLP	: no data	
Test substance	:	
Remark	: In temperatures higher than 180 °C glycerol decomposes to Di/Polyglycolethers and acrolein. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
19.12.2001		(7)
Value	: ca. 290 °C at	
Decomposition	:	
Method	: other	
Year	:	
GLP	: yes	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: UNION DERIVAN S.A. VILADECANS EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
21.05.1998		
Value	: = 290 °C at 1010 hPa	
Decomposition	:	
Method	:	
Year	: 1955	
GLP	:	
Test substance	: other TS	
Test substance Reliability	: CAS 56-81-5 (glycerine), purity 100%. (2) valid with restrictions Also values at other pressures were reported. The most appropriate value is included in this summary and was based on several determinations by different investigators. These data are treated as handbook data.	
19.12.2001		(8)

2.3 DENSITY

Type	: density
Value	: = 1.26 at 20 °C

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Conclusion	: Most reliable data available.	
Reliability	: (2) valid with restrictions Handbook data at ambiguous temperature.	
Flag	: Critical study for SIDS endpoint	
19.12.2001		(4) (8)
Type	: relative density	
Value	: = 1.2613 g/cm ³ at 20 °C	
Method	: other	
Year	: 2000	
GLP	: no data	
Test substance	:	
Reliability	: (2) valid with restrictions	
Flag	: Critical study for SIDS endpoint	
19.12.2001		(2)
Type	: density	
Value	: = 1.262 g/cm ³ at 25 °C	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (4) not assignable The information in the report was confined to the above.	
19.12.2001		(5)
Type	: density	
Value	: ca. 1.261 g/cm ³ at 20 °C	
Method	: other	
Year	:	
GLP	: yes	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: UNION DERIVAN S.A. VILADECANS EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
21.05.1998		
Type	: relative density	
Value	: ca. 1.2613 g/cm ³ at 20 °C	
Remark	: Literature could not be retrieved.	
Source	: Wolff Walsrode AG Walsrode EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
14.05.1998		
Type	: density	
Value	: ca. 1.2 g/cm ³ at 75 °C	
Remark	: Literature could not be retrieved.	
Source	: Pronova Oleochemicals a.s. Sandefjord EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
01.06.1995		

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .0033 hPa at 50 °C

Decomposition :

Method :

Year : 1955

GLP :

Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity 100%.

Conclusion : Most reliable data available.

Reliability : (2) valid with restrictions

Handbook data at most appropriate temperature.

Flag : Critical study for SIDS endpoint

19.12.2001

(8a)

Value : = .00022 hPa at 25 °C

Decomposition :

Method : other (measured)

Year : 1989

GLP :

Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity not indicated

Remark : The vapour pressure is cited as 0.000168 mmHg (=0.00022 hPa).

Reliability : (4) not assignable

19.12.2001

(8b)

Value : = .01 hPa at 96 °C

Remark : All values are extrapolated (beyond the region of experimental measurements).

Result : Vapour pressure = 0.1 hPa at 113 °C

Vapour pressure = 1 hPa at 136 °C

Vapour pressure = 10 hPa at 168 °C

Vapour pressure = 100 hPa at 213.4 °C

Vapour pressure = 1000 hPa at 287 °C

Reliability : (2) valid with restrictions

19.12.2001

(9)

Value : = .000106 hPa at 25 °C

Decomposition :

Method : other (calculated)

Year : 1999

GLP :

Test substance :

Reliability : (4) not assignable

19.12.2001

(10)

Value : = .0033 hPa at °C

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (2) valid with restrictions
 Handbook data are considered to be from a trusted source.
 19.12.2001 (4)

Value : = .0033 hPa at 50 °C

Result : Vapour pressure = 61 hPa at 200 °C
 Vapour pressure = 5.73 hPa at 150 °C

Vapour pressure = .26 hPa at 100 °C
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information in the report was confined to the above.
 19.12.2001 (5)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = -1.65 at °C
pH value :
Method : other (calculated)
Year : 1999
GLP :
Test substance :

Test substance : CAS 56-81-5 (Glycerine), purity not indicated.
Conclusion : Reliable calculation (method of calculation known).
Reliability : (4) not assignable
 19.12.2001 (10)

Partition coefficient : octanol-water
Log pow : = -2.184 at °C
pH value :
Method : other (calculated)
Year :
GLP :
Test substance :

Method : $\log P = 3.028(+/-0.204)\log MW - 0.498(+/-0.0023)HB - 3.649(+/-0.227)$

MW= molecular weight
 HB= maximum hydrogen-bond forming ability
Reliability : (4) not assignable
 09.01.2002 (11)

Partition coefficient :
Log pow : -2.66 - -2.47 at °C
pH value : -
Method : other (calculated)
Year : 1992
GLP :
Test substance :

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information in the report was confined to the above.

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

09.01.2002

(5)

Partition coefficient :
Log pow : = -3.07 at °C
pH value :
Method :
Year : 1998
GLP :
Test substance :

Remark : Literature could not be retrieved.
 09.01.2002

(12)

Partition coefficient :
Log pow : = -1.76 at °C
pH value :
Method : other (measured)
Year : 1988
GLP :
Test substance :

Remark : Not all literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Conclusion : Only measured value available and is supported by a valid QSAR
 prediction (reference 10).
Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint

25.01.2002

(13) (14)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1955
GLP :
Test substance :

Remark : Completely miscible with water.
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Conclusion : Most reliable data available.
Reliability : (2) valid with restrictions
 Handbook data for mixtures of water and glycerol (0-100%).
Flag : Critical study for SIDS endpoint

19.12.2001

(8)

Solubility in : Water
Value : at °C
pH value :

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

concentration	:	at °C	
Temperature effects	:		
Examine different pol.	:		
pKa	:	at 25 °C	
Description	:		
Stable	:		
Deg. product	:		
Method	:		
Year	:	1996	
GLP	:		
Test substance	:		
Result	:	Miscible	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Conclusion	:	Most reliable data available.	
Reliability	:	(2) valid with restrictions	
		Handbook data are considered to be from a trusted source.	
19.12.2001			(4)
Solubility in	:	Water	
Value	:	at °C	
pH value	:		
concentration	:	at °C	
Temperature effects	:		
Examine different pol.	:		
pKa	:	14.4 at 25 °C	
Description	:		
Stable	:		
Deg. product	:		
Method	:	other: not mentioned	
Year	:	1979	
GLP	:	no data	
Test substance	:		
Remark	:	Literature could not be retrieved.	
Source	:	Unichema Chemie B.V. Gouda	
		EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
19.12.2001			(15)
Solubility in	:		
Value	:	= at °C	
pH value	:	ca. 7	
concentration	:	at °C	
Temperature effects	:		
Examine different pol.	:		
pKa	:	at 25 °C	
Description	:	of very high solubility	
Stable	:		
Deg. product	:		
Method	:	other	
Year	:	1976	
GLP	:	no data	
Test substance	:		
Remark	:	Soluble in all proportions.	
Reliability	:	(2) valid with restrictions	
19.12.2001			(2) (16)
Solubility in	:	Water	
Value	:	at °C	

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	soluble (1000-10000 mg/L)
Stable	:	
Remark	:	Literature could not be retrieved.
Source	:	Pronova Oleochemicals a.s. Sandefjord EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
05.12.2001		
Solubility in Value	:	at °C
pH value	:	ca. 7 - 8.5
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	14.4 at 25 °C
Description	:	
Stable	:	
Remark	:	Literature could not be retrieved. Soluble in all proportions.
Source	:	Wolff Walsrode AG Walsrode EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
14.05.1998		

2.6.2 SURFACE TENSION

Test type	:	
Value	:	= 63.4 mN/m at 20 °C
Concentration	:	100 vol%
Method	:	other: not indicated
Year	:	1955
GLP	:	no
Test substance	:	other TS
Test substance	:	CAS 56-81-5 (glycerine), purity 100%.
Reliability	:	(2) valid with restrictions Handbook data at ambiguous temperature.
19.12.2001		

(8)

2.7 FLASH POINT

Value	:	= 160 °C
Type	:	closed cup
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS
Test substance	:	CAS 56-81-5 (Glycerine), purity not indicated.
Reliability	:	(2) valid with restrictions Handbook data are considered to be from a trusted source. The closed cup method is the preferred method for the determination of the

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

03.01.2002	flash point.	(17)
Value	: = 160 °C	
Type	:	
Method	:	
Year	: 1981	
GLP	:	
Test substance	: other TS	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (2) valid with restrictions Handbook data are considered to be from a trusted source.	
03.01.2002		(3)
Value	: = 170 °C	
Type	: open cup	
Method	: other: D 92-33 (Am. Soc. for Testing Materials)	
Year	: 1955	
GLP	: no	
Test substance	: other TS	
Test substance	: CAS 56-81-5 (glycerine), purity 99%.	
Reliability	: (2) valid with restrictions Handbook data are considered to be from a trusted source.	
03.01.2002		(8)
Value	: = 177 °C	
Type	: open cup	
Method	:	
Year	: 1992	
GLP	:	
Test substance	:	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (4) not assignable The information in the report was confined to the above.	
03.01.2002		(5)
Value	: ca. 160 °C	
Type	: open cup	
Remark	: Literature could not be retrieved.	
Source	: Pronova Oleochemicals a.s. Sandefjord EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
01.06.1995		
Value	: ca. 177 °C	
Type	: open cup	
Method	: other: Cleveland Open Cup (ASTM D92-85)	
Year	: 1991	
GLP	: no	
Test substance	:	
Remark	: When aqueous glycerol is tested it will not flash until enough water has evaporated to bring the glycerol concentration to about 97.5% by weight. It will then flash at 190 °C. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda	

2. PHYSICO-CHEMICAL DATA

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19.12.2001	EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	(18)
Value	: = 177 °C	
Type	: open cup	
Method	: other	
Year	: 1971	
GLP	: no data	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: Croda Universal Ltd Goole, North Humberside	
13.05.1994	EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	(19)
Value	: > 180 °C	
Type	: open cup	
Method	: other	
Year	:	
GLP	: yes	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: UNION DERIVAN S.A. VILADECANS	
21.05.1998	EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Value	: ca. 180 °C	
Type	: open cup	
Remark	: Literature could not be retrieved.	
Source	: Wolff Walsrode AG Walsrode	
14.05.1998	EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	

2.8 AUTO FLAMMABILITY

Value	: = 393 °C at	
Method	:	
Year	: 1981	
GLP	:	
Test substance	: other TS	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (2) valid with restrictions	
19.12.2001	Handbook data are considered to be from a trusted source.	(3)
Method	:	
Year	: 1991	
GLP	:	
Test substance	: other TS	
Remark	: The auto ignition temperature of glycerol is 523 °C on platinum, 429 °C on glass, and 412 °C in oxygen at 1 atm.	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Conclusion	: Most reliable data available.	
Reliability	: (2) valid with restrictions	

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

19.12.2001	Handbook data are considered to be from a trusted source.	(18) (8)
Value	: = 388 °C at	
Method	:	
Year	:	
GLP	:	
Test substance	: other TS	
Test substance	: CAS 56-81-5 (Glycerine), purity not indicated.	
Reliability	: (2) valid with restrictions	
03.01.2002	Handbook data are considered to be from a trusted source.	(17)
Value	: > 350 °C at	
Method	: other	
Year	:	
GLP	: yes	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: UNION DERIVAN S.A. VILADECANS EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
21.05.1998		
Value	: ca. 370 - 422 °C at 1013.25 hPa	
Remark	: Literature could not be retrieved.	
Source	: Wolff Walsrode AG Walsrode EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
14.05.1998		
Value	: 370 °C at	
Method	:	
Year	: 1996	
GLP	:	
Test substance	:	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (2) valid with restrictions	
19.12.2001	Handbook data are considered to be from a trusted source.	(5) (4)
Value	: = 422 °C at 1013.25 hPa	
Method	: other	
Year	: 1981	
GLP	:	
Test substance	:	
Remark	: Literature could not be retrieved.	
Source	: Value given refers to autoignition temperature (739 Deg F) Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
19.12.2001		(3)

2.9 FLAMMABILITY

Result : non flammable

Remark : It is not to be expected that glycerol:
 - will produce flammable gasses if in contact with water;
 - will show spontaneous ignition in contact with inert material and intense contact with air (i.e. pyrophoric properties).
 Literature could not be retrieved.

Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

12.06.2001

Result : non flammable
Method : other
Year :
GLP : no data
Test substance :

Remark : Literature could not be retrieved.
 See flash point data.

Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

13.05.1994

2.10 EXPLOSIVE PROPERTIES

Result : not explosive

Remark : No explosive properties are to be expected.
 Literature could not be retrieved.

Source : Unichema Chemie B.V. Gouda

03.02.1994

Result : not explosive

Remark : Literature could not be retrieved.
 No explosive properties are to be expected.

Source : Simel S.p.A. Industria Chimica Cremona
 Lever Brother Ltd. Kingston Upon Thames, Surrey
 Unichema Chemie GmbH Emmerich
 Lever GmbH Hamburg
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

03.02.1994

Result : not explosive

Remark : Literature could not be retrieved.

Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

13.05.1994

Result : not explosive

Remark : Literature could not be retrieved.

Source : Wolff Walsrode AG Walsrode
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

14.05.1998

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Remark : Literature could not be retrieved.
No explosive properties are to be expected.

Source : UNION DERIVAN S.A. VILADECANS
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

21.05.1998

2.11 OXIDIZING PROPERTIES

Result : no oxidizing properties

Remark : No oxidizing properties are to be expected.
Literature could not be retrieved.

Source : Unichema Chemie B.V. Gouda

03.02.1994

Result : no oxidizing properties

Remark : Literature could not be retrieved.
No oxidizing properties are to be expected.

Source : Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

03.02.1994

Result : no oxidizing properties

Remark : Literature could not be retrieved.

Source : Croda Universal Ltd Goole, North Humberside

EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

13.05.1994

Result : no oxidizing properties

Remark : Literature could not be retrieved.

Source : Wolff Walsrode AG Walsrode

EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

14.05.1998

Remark : Literature could not be retrieved.
No oxidising properties are to be expected.

Source : UNION DERIVAN S.A. VILADECANS
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

21.05.1998

2.12 DISSOCIATION CONSTANT

Acid-base constant : .07e-13

Method : other: not indicated

Year : 1955

GLP : no

Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity 100%.

2. PHYSICO-CHEMICAL DATA

ID: 56-81-5

DATE: 29.01.2002

Conclusion : Only data available.
Reliability : (2) valid with restrictions
Handbook data are considered to be from a trusted source.
19.12.2001 (8)

2.13 VISCOSITY

Value : = 1410 - mPa s (dynamic) at 20 °C
Result :
Method : other: not indicated
Year : 1955
GLP : no data
Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity 100%.
Reliability : (2) valid with restrictions
Handbook data at ambient temperature.
19.12.2001 (8)

2.14 ADDITIONAL REMARKS

Remark : Viscosity (20 °C) : 1410 mPa.s
Surface Tension (20 °C) : 63.4 mN/m
Solubility : Glycerol will dissolve a large number of organic and inorganic compounds and will be miscible with many other substances.. Thus, glycerol will be completely miscible with most of the lower aliphatic alcohols, phenol, ethylene, propylene, and trialkyl glycols, some glycol ethers, but only partially, or not at all, with others.
Source : Unichema Chemie B.V. Gouda
11.06.2001 (18)

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 1500000 molecule/cm³
Rate constant : = .000000001874 cm³/(molecule*sec)
Degradation : 50 % after 6.8 hour(s)
Deg. product :
Method :
Year : 1999
GLP :
Test substance :

Remark : Calculated with EPIWIN, part AOPWIN v.1.90.
Conclusion : Most reliable value available.
Reliability : (4) not assignable
Calculated value.
Flag : Critical study for SIDS endpoint
19.12.2001 (10)

Type : water
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer :
Rate constant : cm³/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (measured)
Year : 1973
GLP : no data
Test substance :

Remark : Literature could not be retrieved.
Result : Rate constant: 0.19 x 10exp10 l/mol.sec.
Source : Unichema Chemie B.V. Gouda
Test condition : - OH formed by pulsed radiolysis;
- Neutral pH;
- Oxidation by H-abstraction reaction.
19.12.2001 (20)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C

Remark : Expert Statement:
Glycerol has no hydrolysable groups and is therefore not susceptible to

Reliability : hydrolysis.
11.12.2001 : (4) not assignable

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : biota
Concentration :
Method :

Remark : - Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellularly as lipids.
- It is an important intermediate in the physiology of all forms of life.
- Glycerol is widely distributed in our food, both as natural constituent and as an additive; fat or oil molecules of either animal or vegetable origin contain about 10% glycerol by weight.
- Glycerol may be formed from sugars by microbial fermentation.

11.12.2001

(5)

Type of measurement :
Media : air
Concentration :
Method :

Remark : A validated method for the determination of glycerol in air is not available.

15.11.2001

(5)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other: air - water- soil -sediment
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: calculation
Year : 1997

Method : EQC (level III), release to surface water 1000 kg/y.
Input parameters:
Water solubility 100,000 mg/L
Vapour pressure 0.000106 hPa (at 25 degrees C)
Log Kow -1.76
Melting point 18 degC

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Result	:	Distribution air/water/soil/sediment: 0%/100%/0%/0%	
Reliability	:	(4) not assignable	
Flag	:	Critical study for SIDS endpoint	
24.01.2002			(21)
Type	:	adsorption	
Media	:	water - soil	
Air	:	% (Fugacity Model Level I)	
Water	:	% (Fugacity Model Level I)	
Soil	:	% (Fugacity Model Level I)	
Biota	:	% (Fugacity Model Level II/III)	
Soil	:	% (Fugacity Model Level II/III)	
Method	:		
Year	:	1981	
Method	:	In this report a method was described handling about the permeability of clay-soils (indicative of the leaching of test substance from the soil into the ground water). The clay-soil used in the experiment with glycerol can be described as follows. Ranger shale soil: bulk density 1.73 kg/L, 0.48 %o.m., 4% montmorillonite, void ratio 0.4-0.53)	
		A moistured 1000-2000 g sample of sieved clay soil (dry weight) was placed in a permeability column, glycerol was added and the permeability was determined at 22+/-1 C, atmospheric pressure.	
		The coefficient of permeability (K) was calculated using the following formula: $K=QL/AH$ with Q=flow of the percolate in mL per second L=height of the sample in the column in cm A=cross-sectional area of the sample in square cm H=the average head of the fluid medium on the sample in cm	
Result	:	Coefficients of permeability (K) for water and glycerol were respectively 38 E-9 and 0.9 E-9 cm/sec.	
		The swell of the soil for water and glycerol were respectively 12 and 5%	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated	
Conclusion	:	Percolation through soil: Water: 38 E-9 cm/sec Glycerol: 0.9 E-9 cm/sec	
Reliability	:	(4) not assignable The study included here is not a standard OECD-test, but provides some information on the leaching behaviour/ adsorption to soil of glycerol.	
19.12.2001			(22)

3.3.2 DISTRIBUTION

Media	:	other: Koc	
Method	:	other (calculation)	
Year	:	1999	
Result	:	Koc = 1	
Test substance	:	CAS 56-81-5 (Glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
19.12.2001			(10)

3.4 MODE OF DEGRADATION IN ACTUAL USE

Remark : Literature could not be retrieved.
When used in foods or injected as part of pharmaceutical preparations glycerol is metabolised by glycerokinase in the liver to carbon dioxide and water, or used in glucose or glycogen synthesis. Most of ingested material has been metabolised within 2.5 hours.

Source : Croda Universal Ltd Goole, North Humberside
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
13.05.1994 (23)

Remark : Literature could not be retrieved.
When used in foods or injected as part of pharmaceutical preparations Glycerol is metabolised by glycerokinase in the liver to Carbon Dioxide and Water, or used in glucose or glycogen synthesis. Most of ingested material has been metabolised within 2.5 hours.

Source : Wolff Walsrode AG Walsrode
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
07.11.2001

3.5 BIODEGRADATION

Type : aerobic
Inoculum : other: garden mould suspension
Concentration : 2 mg/l related to Test substance
4 mg/l related to Test substance

Contact time :
Degradation : 92 (±) % after 30 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 5 day(s) 57 %
15 day(s) 84 %
30 day(s) 92 %
%
%

Control substance : other: dodecylsulfate
Kinetic : 5 day(s) 66 %
15 day(s) 80 %

Deg. product :
Method : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year : 2001
GLP : no
Test substance : other TS

Method : A stock solution of 1000 mg test substance/l was prepared and aliquots were added to the test flasks to a final concentration of 2 and 4 mg/l. These were incubated with 1ml/l of garden-mould suspension (100 g garden-mould and 1 l water were shaken vigorously and filtered) resulting in a cell concentration of 10e3-10e5 cells/L . The flasks were incubated at 20 C for 30 days. A control containing only inoculum (blank) and a positive control (dodecylsulfate, ca. 2 mg/l) were included. The oxygen consumption in 2 bottles for each treatment and 4 for the blank was measured after 0, 5, 15 and 30 days with a iodometric titration. The percentage biodegradation was

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Test substance	: based on ThOD(NH3).
Conclusion	: CAS 56-81-5 (Glycerine), purity not indicated.
	: Readily biodegradable.
Reliability	: Only study available, which was conducted to an OECD guideline.
	: (2) valid with restrictions
	1. The reliability is lowered because it is not a GLP study.
	2. The information in the report is confined to the above.
	3. The report is a rewritten report; the original report dates from between 1984 and 1990.
	4. The difference between replicates is < 20% as required by OECD 301.
	5. The final inoculum concentration is not very clear reported in the test report. 1 mL of a garden mould suspension is added to 1 L test solution. We assume that the garden mould suspension contains 1E3 to 1E5 cells per mL. This corresponds to a total inoculum concentration in the test solution of 1E3-1E5 cells/L, which is comparable with the requirements of the OECD (1E4-1E6 cells/L).
Flag	: Critical study for SIDS endpoint
25.01.2002	(24)
Type	: aerobic
Inoculum	: activated sludge, industrial
Concentration	: 238 mg/l related to COD (Chemical Oxygen Demand) 88 mg/l related to DOC (Dissolved Organic Carbon)
Contact time	: 24 hour(s)
Degradation	: 94 - 97 (±) % after 24 hour(s)
Result	: inherently biodegradable
Kinetic of testsubst.	: 2 hour(s) 50 - 60 % 4 hour(s) 86 - 92 % 24 hour(s) 94 - 97 % % %
Control substance	: Ethylene glycol
Kinetic	: 24 hour(s) 92 - 93 % %
Deg. product	: not measured
Method	:
Year	: 1975
GLP	: no data
Test substance	: other TS
Method	: INOCULUM/TEST ORGANISM - Inoculum (source/concentration): activated sludge (industrial) - pretreatment: washed with tap water METHOD OF PREPARATION OF TEST SOLUTION: not indicated INITIAL TEST SUBSTANCE CONCENTRATION: 88 mg C/L (=226 mg TS/L) TEST SYSTEM - Test apparatus: fill and draw type unit; one with two aeration cylinders, each with a volume of 7 L, the other with two aeration cylinders, each with a volume of 30 L (no more information available in this report, Nanbu(1971) is given as a reference) - Number of replicates: not indicated - Aeration: yes (5 L/min) - Measuring equipment: TOC: TOC analyser COD-Mn: water sample was oxidised with KMnO4-H2SO4 at 100 C for 30 minutes (catalyst: Ag2SO4)

	DURATION OF THE TEST: 24 hours SAMPLING: 0, 2, 4, 24 hours ANALYTICAL PARAMETER: TOC and COD-Mn ThOD: 1.21 mg/g = 275 mg/L (COD = 238 mg/L) TEST CONDITIONS - Test temperature: 25 C CONTROLS: not included REFERENCE SUBSTANCE: ethylene glycol was also included in this test and can be used as a positive control
Result	: Probably no blank was included in the test, but it was stated, that the contribution of TOC and COD-Mn from the activated sludge was negligible. Removal test substance as %COD, %TOC respectively: 0 hour: 0, 0 2 hour: 50, 60 4 hour: 92, 86 24 hour: 97, 94 REFERENCE SUBSTANCE Removal test substance as %COD, %TOC respectively: 0 hour: 0, 0 2 hour: 14, 18 4 hour: 32, 38 24 hour: 92, 93
Test substance Conclusion	: CAS 56-81-5 (glycerine), purity not indicated : In this test glycerol is excellently biodegradable. However, the test used industrial activated sludge so the microorganisms may have been pre-adapted. Most reliable data available.
Reliability	: (2) valid with restrictions This test was not a standard OECD 301 test, but gives information on the biodegradability of glycerol with pre-adapted microorganisms.
25.01.2002	(25)
Type	: aerobic
Inoculum	: activated sludge, industrial, adapted
Concentration	: 220 mg/l related to Test substance related to
Contact time	:
Degradation	: 97 (±) % after 14 day(s)
Result	:
Deg. product	:
Method	: other: activated sludge degradability test
Year	: 1988
GLP	: no data
Test substance	: other TS
Method	: Aeration, neutral pH, 1 day adaptation, parameter: COD
Remark	: 94% TOC removal.
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.
Reliability	: (4) not assignable The information in the report was confined to the above.
19.12.2001	(26)
Type	: aerobic
Inoculum	: activated sludge, adapted
Concentration	: 200 mg/l related to Test substance

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	related to	
Contact time	:	
Degradation	:	= 98.7 (±) % after
Result	:	other: biodegradable under test conditions
Deg. product	:	
Method	:	other: not mentioned
Year	:	1976
GLP	:	no data
Test substance	:	
Remark	:	Rate of degradation: 85 mg COD/g.hour.
Test condition	:	- Medium stirred, containing mineral salts; - 20 °C, pH 7.2; - % removal expressed as COD.
Reliability	:	(4) not assignable The information in the report was confined to the above.
19.12.2001		(27)
Type	:	aerobic
Inoculum	:	activated sludge, industrial
Contact time	:	
Degradation	:	75.2 (±) % after 5 day(s)
Result	:	
Deg. product	:	
Method	:	other: radiorespirometric test
Year	:	1976
GLP	:	no data
Test substance	:	other TS
Method	:	INOCULUM/TEST ORGANISM - Inoculum: Fresh sludge was obtained daily from a 7.5E4 L/day capacity activated sludge unit continuously fed with photographic processing effluent (1969-1976) - Preparation of inoculum: Sludge was centrifuged and subsequently one part of sludge pellet was mixed with 2 parts of water in a homogeniser. METHOD OF PREPARATION OF TEST SOLUTION: 1-2 mL sludge, 3 mL salt solution, 0.2 mL 1 M potassium phosphate buffer (pH 7.3). After sealing 0.1-0.2 mL 14C-glycerol was added. INITIAL TEST SUBSTANCE CONCENTRATION (ug C/L): 7.7-139 TEST SYSTEM - Culturing apparatus: 30 mL serum bottle sealed with rubber stoppers - Measuring equipment: 14CO2 trapped with phenethylamine in scintillation fluid. DURATION OF THE TEST: 5 days SAMPLING: day 5 TEST CONDITIONS - Test temperature: room temperature - Other relevant factors: test was performed in the dark (pH 7.3) and stopped by the addition of 2 mL 1 M perchloric acid.
Result	:	TEST SUBSTANCE 14CO2 recovery after 5 days: 75.2% Degradation based on BOD5 values: 43.5-52.9%
		CONTROL At 0 hours recovery 14CO2 <1.5%
Test substance	:	CAS 56-81-5 (U-14C-glycerol (0.111 mC/mg)), purity not indicated.
Reliability	:	(4) not assignable 1. The information was confined to what is included in the current summary, therefore the reliability of this study cannot be assessed. 2. Inoculum was activated sludge from laboratory unit fed

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19.12.2001	continuously with photographic processing effluent.	(28)
Type	: anaerobic	
Inoculum	: anaerobic microorganisms	
Concentration	: 500 mg/l related to Test substance related to	
Contact time	:	
Degradation	: 90 (±) % after 8 day(s)	
Result	: other: anaerobic degradation	
Deg. product	:	
Method	:	
Year	: 1978	
GLP	: no data	
Test substance	: other TS:	
Method	: INOCULUM Source: well-digested domestic sludge Acclimation: fed with acetate for several years Concentration suspended solids: 1000 mg/L INITIAL TEST SUBSTANCE CONCENTRATION (mg C/L): 500-1000 mg/L; the test substance was injected in intervals. the first 6 injections yielded an initial test substance concentration of 500 mg/L, thereafter the test substance concentration was increased to 1000 mg/L TEST SYSTEM - Culturing apparatus: Serum bottles, oxygen free (purged with CO ₂ & N ₂) - Number of culture flasks per concentration: duplicate - Aeration: no, anaerobic system - Measuring equipment: manometrically DURATION OF THE TEST: not clearly indicated ANALYTICAL PARAMETER: gas production in mL TEST CONDITIONS - Composition of mineral solution: 400 mg/L NH ₄ Cl, 400 mg/L KCl, 400 mg/L MgSO ₄ ·6H ₂ O, 40 mg/L FeCl ₂ ·6H ₂ O, 4 mg/L CoCl ₃ , 80 mg/L (NH ₄) ₂ HPO ₄ , 10 mg/L cysteine, 10 mg/L KI, 10 mg/L Na hexameta phosphate, 0.5 mg/L MnCl ₂ , 0.5 mg/L NH ₄ V ₂ O ₃ , 0.5 mg/L ZnCl ₂ , 0.5 mg/L Na ₂ MoO ₄ ·2H ₂ O, 0.5 mg/L H ₃ BO ₃ , NaHCO ₃ (to maintain an alkalinity of 3000 mg CaCO ₃ /L) - Test vessels contained initially 50 mL inoculum, 100 mg acetate and 25 mg test substance	
Result	: After 8 days a lag time was reached at which 90% of glycerol was degraded. The removal rate is 200 mg/L/d.	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated	
Conclusion	: Glycerol is biodegradable under circumstances described in this test.	
Reliability	: (4) not assignable 1. This test was set up to determine whether bacteria developed on acetate substrate can metabolise other compounds (e.g. glycerol). 2. Secondary literature with information essentially confined to what is included in the current summary. 3. Since adapted inoculum (adapted to acetate) is used, nothing can be said about the potency of glycerol to be readily biodegradable.	
19.12.2001		(29)
Type	: anaerobic	
Inoculum	: anaerobic microorganisms	
Concentration	: 47 mmol/l related to Test substance related to	
Contact time	:	
Degradation	: 100 (±) % after 2 day(s)	

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Result	: other: anaerobic biodegradation test. Degradation rate based on disappearance of test substance from the test solution.
Deg. product	: yes
Method	:
Year	: 1991
GLP	: no data
Test substance	: other TS:
Deg. products	: 1,3-propanediol acetate propionate
Method	: INOCULUM/TEST ORGANISM - Inoculum (source): mixed microbial culture from a fermenter fed with waste water from an industrial distillery containing glycerol (INRA-Narbonne, France) METHOD OF PREPARATION OF TEST SOLUTION: 50/50 v/v inoculum/test medium (sulphate free or sulphate containing) INITIAL TEST SUBSTANCE CONCENTRATION (mg/L): ~47 mM = 4371 mg/L = 1692 mg C/L TEST SYSTEM - Anaerobic system with 20 mM or without sulphate DURATION OF THE TEST: 4 weeks SAMPLING: ~0, 1, 2, 3, 4, 5, 7, 12, 15, 20 days; TOC: 0, 1, 2, 3, 4, 5, 7, 12, 15 days ANALYTICAL PARAMETER: Glycerol (enzymatically), 1,3-propanediol (GC-FID), propionate and acetate; TOC; OD at 580 nm TEST CONDITIONS - Composition of mineral solution: sulphate free basal medium with anorganic salts, vitamins and trace elements - Test temperature: 37 C INTERMEDIATES / DEGRADATION PRODUCTS: see analytical parameter
Result	: Without sulphate (pH 7..2) glycerol disappeared within 2 days from the test solution. Analyses were performed on glycerol, propanediol, propionate and acetate. -1,3-propanediol was found in the reaction mixture between day 1 and day 20 with a maximum of 17-21 mM at day 2-5. -Acetate was formed between day 5 and 20 reaching a maximum concentration of 27 mM at day 12-15. -The formation of propionate was analytically confirmed between day 5 and day 20 with a maximum concentration of 16 mM at day 7. -TOC (measured): 142 mM (d 0) decreased to 118 mM (d 5-15) -TOC (calculated): 142 mM (d 0); decreased to 45 mM (d 3); increase to 118 mM (d 7), decrease to 100 mM (d 15) With sulphate (pH 7.2) glycerol disappeared within 2 days from the test solution. Analyses were performed on glycerol, propanediol, propionate and acetate. -1,3-propanediol was found in the reaction mixture up to day 7 (maximum concentration 4 mM at day 2). -Acetate was formed from day 5; maximum concentrations of 40-43 mM from day 12. -The formation of propionate was analytically confirmed between day 2 and day 20 with a maximum concentration of 20 mM at day 5. -Sulphate was maximum at day 0 (23 mM) and was around 0 mM from day 12 on. -TOC (measured): 142 mM (d 0), decreased to 12 mM (d 3), increased to 112 mM (d 12), lag phase at d 15-20 of 105 mM -TOC (calculated): 142 mM (d 0); decreased to 95 mM (d 3-15)
Test substance	: CAS 56-81-5 (glycerine), purity not indicated

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Conclusion	: Glycerol is degraded very easily to other reaction products, but ultimate biodegradation (formation CO ₂) is limited in this experiment.
Reliability	: (4) not assignable 1. Secondary literature with information essentially confined to what is included in the current summary. 2. TOC analyses show that for the glycerol degradation without sulphate between day 1-7 probably another important degradation product is formed. This product is not identified. From day 7 onwards the measured TOC is also higher than the calculated one, assuming an additional degradation product (may be accumulated CO ₂). For the glycerol degradation with sulphate the measured TOC is lower than the calculated one. This cannot be explained.
19.12.2001	(30)
Type	: aerobic
Inoculum	: other
Concentration	: .5 mg/l related to Test substance related to
Contact time	: 8 day(s)
Degradation	: 100 (±) % after 8 day(s)
Result	:
Deg. product	: yes
Method	: other: not indicated
Year	: 1988
GLP	: no data
Test substance	: other TS
Method	: Microbial transformation rate of glycerol was measured in surface water from two sites at the Great Salt Lake. Properties surface water: Site I: 22.0% salt, pH 7.8, T = 22 C. Site II: 8.5% salt, pH 8.3, T = 22 C. In surface water from both sites glycerol concentration was below the detection limit (10.0 micromol/L) as determined enzymatically. For each site 10 mL of surface water was transferred into an aerobic, sterile, 58-ml rubber-stoppered vial containing 17.5 KBq [U- ¹⁴ C] glycerol (321.9 MBq/mmol; 0.50 mg/L). These mixtures were incubated at 25 C and two replicates were stopped after 0, 3, 6, 12, 24, 96 and 192 h by quenching with 10 M sodium hydroxide. 0.4 mL gas from the headspace of acidified samples was analyzed by a combined gas chromatograph-gas proportional counting technique and corrected for dissolved ¹⁴ CO ₂ by the Bunsen solubility coefficient. The remaining labelled carbon in the liquid phase was determined by scintillation counting after neutralization with 6 M sodium hydroxide and centrifugation.
Result	: After 8 days, <= 10% of glycerol was present in the liquid phase and about 70% of the labelled substrate was recovered as ¹⁴ CO ₂ . It is assumed that 30% of labelled substrate was incorporated into cell mass, which had been separated from the liquid by centrifugation.
Test substance	: CAS 56-81-5 (glycerine), reagent grade purity.
Conclusion	: From this study it can be deduced that glycerol can be degraded.
Reliability	: (4) not assignable 1. Secondary literature. The information was essentially confined to the above summary. No information on bacteria, negative control. The study was no guideline study and no data on GLP were available. 2. The high salinity may result in poor degradation.
25.01.2002	(31)
Type	: aerobic
Inoculum	: activated sludge

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Concentration	:	100 mg/l related to Test substance related to	
Contact time	:		
Degradation	:	= 63 (±) % after 14 day(s)	
Result	:	readily biodegradable	
Deg. product	:		
Method	:	OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"	
Year	:	1992	
GLP	:	no data	
Test substance	:		
Remark	:	Degree of biodegradation is expressed as BOD. Literature could not be retrieved.	
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Test condition 19.12.2001	:	Concentration of activated sludge: 30 mg/l.	(32)
Type	:	aerobic	
Inoculum	:		
Contact time	:		
Degradation	:	= 87 (±) % after	
Result	:		
Method	:	SIMPLETREAT prediction of fate in wastewater treatment plant: Input parameters for model calculations: Molecular weight 92 Melting point 18 degC Boiling point 290 degC Vapour pressure 0.0106 Pa Log Kow -1.76 Water solubility 100,000 mg/L	
Result	:	In a Sewage Treatment Plant 87% will be degraded.	
Test substance 25.01.2002	:	CAS 56-81-5 (Glycerine)	(33)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5	
Method	: other: standard dilution method (APHA No. 219)
Year	: 1971
Concentration	: related to
BOD5	: mg/l
GLP	: no data
COD	
Method	: other: standard potassium dichromate method (ASTM D 1252-67)
Year	: 1979
COD	: 1160 mg/g substance
GLP	: no data
RATIO BOD5 / COD	
BOD5/COD	: = .86
Method	: In this test the relation between BOD, COD and ThOD is determined. The ThOD is the Theoretical Oxygen Demand. BIOLOGICAL OXYGEN DEMAND (BOD): BOD is determined using the standard dilution method at 20+/-1 C for a

3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 56-81-5

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	period of 5 days and is reported to be conducted in accordance with the guideline (APHA), with the exception that 0.5 mg/L allylthiourea is added. 500 mL test solutions were seeded with a filtered 10 mL volume of the effluent from a biological sanitary waste treatment plant. Control: mixture of glucose and glutamic acid to check the activity of the inoculum.
	CHEMICAL OXYGEN DEMAND (COD): COD is obtained using the standard potassium dichromate method. The test was reported to be conducted in accordance with the guideline (ASTM).
Result	: ThOD: 1.22 g/g BOD5: 1.00 g/g (=82% ThOD) COD: 1.16 g/g (=95% ThOD)
Conclusion	: Glycerol has the potency to be degraded in a wastewater treatment plant. The BOD5/COD ratio is >0.5 which suggests that glycerol is readily biodegradable.
Reliability	: (2) valid with restrictions 1. Although the information available in the report (secondary) is confined to what is included in the current summary, the study is still thought to be reliable (Klimisch 2). A reliability of 2 is given, because it is stated that the test is performed in accordance with (acceptable) guidelines and the deviations are clearly reported. 2. The addition of 0.5 mg/L allylthiourea is believed to have no influence on the study results. Allylthiourea is added to prevent nitrification. This is not applicable for glycerine, but it is applicable to other substances tested in this report.
25.01.2002	(34)
BOD5	
Method	: other: respirometric method according to Wagner R. Vom wasser 42: 271-305 (1974)
Year	:
Concentration	: related to
BOD5	: mg/l
GLP	: no data
COD	
Method	: other: The ThOD was used
Year	: 1977
COD	: 1216.1 mg/g substance
GLP	: no data
RATIO BOD5 / COD	
BOD5/COD	: .753
Method	: Degradation of glycerol was tested in a matrix containing municipal wastewater. The concentration of test substance was probably 6 g/L.
Result	: BOD5: 898 mg/g ThOD: 1216.1 mg/g BOD5/ThOD [%]: 75.3
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.
Reliability	: (4) not assignable The information in the report was confined to the above.
19.12.2001	(35)
BOD5	
Method	: other
Year	:
Concentration	: 100 g/l related to Test substance
BOD5	: ca. 700 mg/l

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GLP	:	
COD	:	
Method	:	other
Year	:	
COD	:	ca. 1160 mg/g substance
GLP	:	
Remark	:	Literature could not be retrieved.
Source	:	Wolff Walsrode AG Walsrode EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
15.11.2001		
BOD5	:	
Method	:	other: see remark
Year	:	
Concentration	:	related to
BOD5	:	mg/l
GLP	:	no data
Remark	:	RS: BOD5: 0.65 g/g TC: Standard dilution method using sewage as seed. RE: Gellmann, I. Masters Thesis, Rutgers University (1950).
		RS: BOD5: 0.80 g/g TC: Standard dilution method using sewage as seed. RE: Gellmann, I. PhD Thesis, Rutgers University (1952).
		RS: BOD5: 0.617 g/g TC: Standard dilution method using sewage as seed. RE: Meissner, B. Wasserwirtsch.-Wassertechn. 4 (1954), 166.
		RS: BOD5: 0.78 g/g TC: Sier method using sewage (10%) as seed. RE: Gellmann, I. Masters Thesis, Rutgers University (1950).
		RS: BOD5: 0.83 g/g TC: Warburg method using sewage (10%) as seed. RE: Gellmann, I. Masters Thesis, Rutgers University (1950).
		RS: BOD5: 0.81 g/g TC: Standard dilution method using sp. cult as seed. RE: Zobel, C.E., Biol. Bull., vol. 78 (1940), 388.
		RS: BOD5: 0.64 g/g TC: Standard dilution method using sewage as seed. RE: Burford, M.G. et al. Rpt New Engl Intern. Water Poll. Con. Comm. (1953). Literature could not be retrieved.
Source	:	Unichema Chemie B.V. Gouda
15.11.2001		

3.7 BIOACCUMULATION

BCF	:	= 3.16
Elimination	:	
Method	:	OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year	:	1999
GLP	:	

3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 56-81-5

DATE: 29.01.2002

Test substance	:		
Method	:	Calculation with Epiwin model. Input logKow -1.65	
Result 19.12.2001	:	log BCF = 0.5	(10)

3.8 ADDITIONAL REMARKS

Memo	:	An overview of rate constants concerning reactions of glycerol with hydrogen atoms and hydroxyl radicals	
Method	:	In this report an overview is given of rate constants concerning reactions of glycerol with hydrogen atoms and hydroxyl radicals in aqueous solutions. The available information on the method is limited to the following Hydrogen atoms reaction: A. pH 1, method: Fe(CN) ₆ ³⁻ , 20-25 C B. method: pulse radiolysis technique/Ag ⁺ , 20-25 C Hydroxyl radicals reaction: C. pH 7, method: pulse radiolysis technique/CNS ⁻ , 20-25 C D. pH 10.7, method: pulse radiolysis technique/CO ₃ ²⁻ , 20-25 C E. pH 9, method: PNDA, 20-25 C	
Result	:	SPECIFIC RATE CONSTANTS: Hydrogen atoms reaction: A. 2E7 B. 1.45E7 Hydroxyl radicals reaction: C. 9.5E8 D. 1.0E9 E. 1.1E9	
Test substance 15.11.2001	:	CAS 56-81-5 (glycerine), purity not indicated.	(36)
Memo	:	Microbial degradation	
Remark	:	This study was performed to test the growth of <i>Aspergillus versicolor</i> and <i>Pseudomonas aeruginosa</i> on esters and alcohols. In this test glycerol was used as the control substrate. It can be concluded that both micro-organisms (<i>A. versicolor</i> and <i>P. aeruginosa</i>) are capable of growth using glycerol as the carbon source.	
Test substance 15.11.2001	:	CAS 56-81-5 (glycerine), chemically pure (probably >99%)	(37)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : Static
Species : Leuciscus idus melanotus (Fish, fresh water)
Exposure period :
Unit : mg/l
LC0 : > 10000
LC50 : > 10000
LC100 : > 10000
Limit test :
Analytical monitoring : no data
Method :
Year : 1978
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
- Species: Leuciscus idus melanotus
- Supplier: not specified
- Age/size/weight/loading: not specified
- Feeding (pretreatment): not specified
- Feeding during test: not specified

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- no information included in the report

DILUTION WATER
- no information included in the report

TEST SYSTEM
- only information available about the test system was that this test was based on the static fish toxicity test with the Goldorfe after Mann (1975/1976)

DURATION OF THE TEST: not specified

TEST PARAMETER: mortality
OBSERVATION TIMES: not specified

Result : STATISTICAL METHOD: not specified
: RESULTS:
- Nominal concentrations (mg/L): 10000
- Mortality: none
- Other effects: no data
- Dose related effects: no data

Test substance : CAS 56-81-5 (glycerine), purity not indicated

Conclusion : Most appropriate study available.

Reliability : (4) not assignable
The report was essentially confined to what is included in the current summary. There was no information on control mortality, physical-chemical parameters, actual tested concentrations, feeding rate, number and size of fish tested and photoperiod during the test and the duration of the test.

Flag : Critical study for SIDS endpoint
19.12.2001

(38)

Type : Static

4. ECOTOXICITY

ID: 56-81-5

DATE: 29.01.2002

Species	:	Carassius auratus (Fish, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
LC50	:	> 5000
Limit test	:	
Analytical monitoring	:	Yes
Method	:	other: Standard Methods for Examination of Water and Wastewater. Am. Publ. Health Assoc. Inc., New York, Method No. 231
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Method	:	TEST ORGANISMS - Species: Carassius auratus - Size;weight;loading: 62+/-7 mm; 3.3+/-1.0 g; 1.3 g/L DILUTION WATER - Source: local tap-water - Chemistry: Alkalinity 30 mg Na+/L; 65 mg/L (Cl)-, 4 mg/L (NO3)-, 35 mg/L (SO4)2-, 0.15 mg/L (PO4)3-, 25 mg/L (HCO3)-, 25 mg/L SiO2, 0.05 mg/L Fe, 100 mg/L (Ca)2+, 8 mg/L (Mg)2+; pH 7.8 TEST SYSTEM - Test type: static - Concentrations: 5000 mg/L - Exposure vessel type: 33 L glass vessels containing 25 L of test solution. - Number of fish: 10 per replicate, 1 replicate/treatment - Test temperature: 20+/-1 C - Dissolved oxygen: >4 mg/L DURATION OF THE TEST: 24 hours TEST PARAMETER: mortality OBSERVATION TIMES: 24 hours ANALYSES: - Method: TOC analysis or extraction followed by GC analysis - Sampling times: 0 and 24 hours
Result	:	RESULTS: - Nominal concentrations (mg/L): 5000 - Measured concentrations (mg/L): not reported - Mortality: <50%
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.
Conclusion	:	Although the study has an exposure time of 24-hours, it is marked as the critical study, because the underlying publication provides relative much information on the test design.
Reliability	:	(4) not assignable 1. The information in the report (Secondary literature) was essentially confined to what is included in the current summary. Actually the report consists of an overview of the fish toxicity for a number of petrochemicals. No actual mortality rates at the tested concentration was reported. 2. The loading is with 1.3 g/L slightly higher than recommended by OECD 203 (1 g/L).
Flag	:	Critical study for SIDS endpoint
23.01.2002		(39)
Type	:	other: embryogenesis
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	
Unit	:	

Limit test :
Analytical monitoring : no data
Method : other
Year : 1997
GLP : no data
Test substance : other TS

Method : This test was set up to study the toxicity of different cryoprotective agents on carp embryos at different developmental stages. This is important for the design of cryopreservation protocols for carp embryos.

Embryos were selected at three developmental stages (morula, half-epiboly and heartbeat) and subsequently equilibrated for 5 minutes and 1 hour at 24 C in 1 M solution of glycerol in water (=92 g/L). A control group was included in which embryos were equilibrated to water of 24 C. At the end of the equilibration period, embryos were transferred into water and incubated at 24 C. Survival was defined as percentage of treated embryos that hatched.

Result : Statistical method: Chi-square method.
 : Hatching rates of carp embryos (%) for embryos exposed respectively at morula stage, half-epiboly stage and heartbeat stage:
 - Control: 95, 94, 91
 - Glycerol (5 min): 80*, 94, 86*
 - Glycerol (1 h): 14*, 14*, 78*

Test substance : *Statistically different from control treatment
 : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 1. The information in the report was confined to the above mentioned.
 2. The study was designed to test the toxicity of glycerol, when used as a cryoprotective substance. The results of this test are not relevant for ecotoxicological purposes, because the tested concentration is extremely high.

08.01.2002 (40)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : Mg/l
LC0 : > 250
Limit test :
Analytical monitoring : No
Method : other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year : 1968
GLP : No
Test substance : other TS

Remark : 250 mg/l was highest concentration tested.
 Test method conforms with OECD Guideline 203. 10 fish per tested concentration were used.

Test substance : CAS 56-81-5 (Glycerine), purity 99.5%.

02.01.2002 (41)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : Mg/l

4. ECOTOXICITY

ID: 56-81-5

DATE: 29.01.2002

LC100 : = 51000 - 57000
Limit test :
Analytical monitoring : no data
Method : other: not mentioned
Year : 1980
GLP : no data
Test substance :

Remark : Literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
Test condition : - Rainbow trout of 0.9 g;
- 12 °C, hardness 40-50 mg/l CaCO₃, alkalinity 30-35 mg/l CaCO₃, pH 7.2-7.5;
- Reconstituted dilution water;
- Unmeasured concentration.

25.01.2002

(42)

Type :
Species :
Exposure period : 96 hour(s)
Unit : Mg/l
LC50 : = 184000
Method : other: calculated
Year : 1999
GLP :
Test substance :

Remark : Calculated with EPIWIN, part ECOSAR v0.99f.
Reliability : (4) not assignable
19.12.2001

(10)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : Mg/l
EC50 : > 10000
EC100 : > 10000
Analytical monitoring : no data
Method :
Year : 1982
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
- Species: Daphnia magna STRAUS IRCHA
- Source/supplier: not indicated
- Breeding method: 20-30 Daphnia in 2L beaker glasses with at least 1.6 L water (286 mg CaCO₃/L, pH 7.6-7.7), fed daily, 9 h light, 20 °C; young daphnids were removed daily
- Age: ≤24 h
- Feeding (pretreatment): daily
- Feeding during test: not specified

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: none

	DILUTION WATER - Source: synthetic test medium in accordance with ISO 6341 (medium recommended by OECD 202) - Chemistry (sum of Ca and Mg: 2.5 mmol/L; Na/K ratio: 10/1; pH: 8.0+/- 0.2) TEST SYSTEM - Test type: static - Concentrations: not specified - Exposure vessel type: 50 mL glass beakers containing 20 mL test medium - Number of individuals: 10 per replicate, 2 replicates/treatment - Photoperiod (intensity of irradiation): not specified PHYSICAL MEASUREMENTS - Measuring times: 0 and 24 hours - Test temperature: 20 C - Dissolved oxygen: measured but not reported - pH: 8.0+/-0.2 (0 hour), measured but not reported (24 hours) - Adjustment of pH: no DURATION OF THE TEST: 24 hours TEST PARAMETER: immobility OBSERVATION TIMES: 24 hours REFERENCE SUBSTANCE: Potassium dichromate
Result	STATISTICAL METHOD: Chi-Square test : RESULTS: - Nominal concentrations (mg/L): 10 000 - Immobility: <50% - Other effects: no data - Dose related effects: no data
Test substance	RESULTS: TEST WITH REFERENCE SUBSTANCE - Results: EC50 1.3 mg/L
Conclusion	: CAS 56-81-5 (glycerine), purity not indicated
Reliability	: Most elaborate study available. : (4) not assignable The report was essentially confined to what is included in the current summary. There was no information or limited information on control mortality, physico-chemical parameters, actual tested concentrations and feeding rate.
Flag	: Critical study for SIDS endpoint
19.12.2001	(43)
Type	: static
Species	: Daphnia magna (Crustacea)
Exposure period	: 24 hour(s)
Unit	: Mg/l
EC50	: > 10000
Analytical monitoring	: No data
Method	: other: not indicated
Year	: 1977
GLP	: No data
Test substance	: other TS
Remark	: Daphnia magna collected from small pond, were exposed to several test substance concentrations for 24 h at

4. ECOTOXICITY

ID: 56-81-5

DATE: 29.01.2002

	20 - 22 degr. C (pH before addition of test substance: 7.6 - 7.7, no adjustment of pH after addition of test substance). For the experiment 10 animals per replicate and 3 replicates were used.	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (4) not assignable	
	The information in the report was confined to the above.	
19.12.2001		(44)
Type	:	
Species	: Daphnia magna (Crustacea)	
Exposure period	: 24 hour(s)	
Unit	: mg/l	
EC0	: > 500	
Analytical monitoring	: No	
Method	: other: Daphnien-Kurzzeittest, DIN 38412 Teil 11, Bestimmung der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse	
Year	:	
GLP	: No	
Test substance	: other TS: as prescribed by 1.1-1.4 (Henkel KGaA).	
Remark	: 500 mg/l was highest concentration tested. Test method conforms with OECD Guideline 202 A. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda	
15.11.2001		(45)
Type	:	
Species	: other: Daphnia	
Exposure period	: 48 hour(s)	
Unit	: mg/l	
LC50	: = 153000	
Method	: other: calculated	
Year	: 1999	
GLP	:	
Test substance	:	
Remark	: Calculated with EPIWIN, part ECOSAR v0.99f.	
Reliability	: (4) not assignable	
19.12.2001		(10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint	: other: inhibition of cell growth after 8 days
Exposure period	: 8 day(s)
Unit	: mg/l
EC3	: 2900
Limit test	: No
Analytical monitoring	: No data
Method	:
Year	: 1978
GLP	: No data
Test substance	: other TS
Method	: TEST ORGANISMS - Species: Microcystis aeruginosa - Source/supplier: not specified - Method of cultivation: stemculture and preculture were

maintained for 10 days in closed 100 mL erlenmeyer containing 20 mL medium at 27 C and relative humidity of 50% before a new culture was set up

- Initial cell concentration: equivalent to a turbidity value corresponding to TE/F/578 nm = 20

DILUTION WATER

- Source: dist. water

GROWTH/TEST MEDIUM CHEMISTRY

- Test medium contains 24.8 mg/L NaNO₃, 2.0 mg/L K₂HPO₄, 75 mg/L MgSO₄·7H₂O, 36 mg/L CaCl₂·2H₂O, 40 mg/L Na₂SiO₃, 58 mg/L Na₂CO₃, 3 mg/L C₆H₈O₇·H₂O, 3 mg/L C₆H₅FeO₇·5H₂O, 10 mg/L C₁₀H₁₄N₂Na₂O₈·2H₂O, 114 ug/L H₃BO₃, 72 ug/L MnCl₂·4H₂O, 8.8 ug/L ZnSO₄·7H₂O, 3.2 ug/L CuSO₄·5H₂O, 0.96 ug/L Na₂MoO₄·2H₂O, 1.6 ug/L CoCl₂·6H₂O
 - Chemistry (Hardness: 0.55 mmol/L Ca+Mg; P: 0.34 mg/L; N: 4.1 mg/L; chelators 0.03 mmol/L)
 - pH 7.0

TEST SYSTEM

- Test type: static, daily shaken
 - Concentrations: 10 solutions with unspecified concentrations (stock solution is 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 times diluted)
 - Exposure vessel type: culture tubes containing 10 mL test solution
 - Number of replicates: 3 (test substance), 1 (control)
 - Photoperiod (intensity of irradiation): continuous

PHYSICAL MEASUREMENTS

- no data

DURATION OF TEST: 8 days

TEST PARAMETER: inhibition of turbidity after 8 days (extinction at 578 nm); more than 3% difference in extinction is considered as an effect

OBSERVATION TIMES: after 8 days

STATISTICAL METHOD: not specified

Result

: RESULTS:

Test substance

EC3 2900 mg/L

Conclusion

: CAS 56-81-5 (glycerine), purity not indicated

Reliability

: Most sensitive toxicity threshold value available.

: (4) not assignable

1. The test was not in accordance with OECD 201: test medium was different and extinction was only measured after 8 days. No quantitative relationship changes between extinction and growth inhibition was provided. Therefore it was not possible to deduce a 72-hours EC₅₀ value for the test substance. It is clear from this report, that glycerine is not very toxic for algae in the aquatic environment. It is not possible to confirm, however, whether algae were in the exponential growth phase for the duration of the test.
2. The turbidity of the test solution is expressed in "TE/F/578 nm". This refers to a measurement of the extinction of the test solution at 578 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027).
3. All publications refer to the same study.
4. Review articles containing multiple substances. Bringmann & Kuehn (GWF Wasser/abwasser, 117, 410-413, 1976) contained a comparison of

		the toxicity of <i>Pseudomonas putida</i> with <i>Microcystis aeruginosa</i> and in Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) the toxicity of <i>Microcystis aeruginosa</i> was compared to the toxicity of <i>Scenedesmus quadricauda</i> .
Flag	:	
25.01.2002		(46) (47) (48) (49)
Species	:	<i>Scenedesmus quadricauda</i> (Algae)
Endpoint	:	other: inhibition in cell growth after 8 days
Exposure period	:	8 day(s)
Unit	:	mg/l
EC3	:	> 10000
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	1978
GLP	:	no data
Test substance	:	other TS
Method	:	TEST ORGANISMS - Species: <i>Scenedesmus quadricauda</i> - Source/supplier: not specified - Method of cultivation: stemculture and preculture were maintained for 10 days in closed 100 mL erlenmeyer containing 20 mL medium at 27 C and relative humidity of 50% before a new culture was set up - Initial cell concentration: corresponding to an extinction value corresponding to a turbidity value of TE/F/578 nm = 20 DILUTION WATER - Source: dist. water GROWTH/TEST MEDIUM CHEMISTRY - Chemistry (Hardness: 0.55 mmol/L Ca+Mg; P: 0.34 mg/L; N: 4.1 mg/L; chelators 0-0.03 mmol/L) - pH 7.0 TEST SYSTEM - Test type: static, daily shaken - Concentrations: not specified - Exposure vessel type: culture tubes containing 10 mL of test solution - Number of replicates: 3 - Photoperiod (intensity of irradiation): continuous PHYSICAL MEASUREMENTS - no data DURATION OF TEST: 7-8 days TEST PARAMETER: inhibition of turbidity after 8 days (extinction at 578 nm); 3% reduction of extinction is considered as an inhibitory effect OBSERVATION TIMES: after 8 days
Result	:	STATISTICAL METHOD: not specified RESULTS: EC3 >10000 mg/L
Test substance	:	All publications refer to the same study.
Reliability	:	CAS 56-81-5 (glycerine), purity not indicated
	:	(4) not assignable

19.12.2001

1. The test was not in accordance with OECD 201: the test medium was different and extinction was only measured after 8 days. No quantitative relationship changes between extinction and growth inhibition were provided. Therefore it was not possible to deduce a 72-hours EC50 value for the test substance. It is clear from this report, that glycerine is not very toxic for algae in the aquatic environment.

2. The turbidity of the test solution is expressed in "TE/F/578 nm". This refers to a measurement of the extinction of the test solution at 578 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027).

3. All publications probably refer to the same study.

4. In Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) a comparison was made for the toxicity of several substances for *Microcystis aeruginosa* and *Scenedesmus quadricauda*.

(46) (50) (51) (48)

Species : other algae: Green Algae
Endpoint :
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 77712
Method : other: calculated
Year : 1999
GLP :
Test substance :

Remark : Calculated with EPIWIN, part ECOSAR v0.99f.
Reliability : (4) not assignable

19.12.2001 (10)

Species : other aquatic plant
Endpoint : growth rate
Exposure period : 28 day(s)
Unit :
Limit test :
Analytical monitoring : no data
Method : other: not mentioned
Year : 1970
GLP : no data
Test substance :

Method : TEST ORGANISMS
- Species: several species with their suppliers are included below.
CHLOROPHYCEAE:
1. *Dunaliella tertiolecta* Butcher (Wood Hole clone "Dun", Dr J. Strickland)
2. *Tetraselmis maculata* Butcher (Nanaimo strain "TMD", Dr T. Parsons)
3. *Nannochloris oculata* Droop (Millport strain no. 66, Dr M. Droop)
4. *Brachiomonas submarina* (Bohlin) Droop var. *pulsifera* 7/2a (Millport strain no. 44, Dr M. Droop)
CHRYSOPHYCEAE
5. *Monochrysis lutheri* Droop (Millport strain no. 60, Dr J. Strickland)
6. *Isochrysis galbana* Parke (Woods Hole clone "Iso", Dr J. Strickland)
7. *Coccolithus huxleyi* (Lohm) Kamptner (Woods Hole clone "BT-6", Dr T. Parsons)
8. *Prymnesium parvum* Carter (Reich's Israeli strain, Dr M. Rahat)
BACILLARIOPHYCEAE
9. *Phaedactylum tricornutum* Bohlin (Lewin strain 74-M, Dr J. Lewin)

10. *Skeletonema costatum* (Grev.) Cleve (Woods Hole clone "Skel", Dr R. Guillard)
11. *Cyclotella nana* Hustedt (Woods Hole clone "3H", Dr. R. Guillard)
- CRYPTOPHYCEAE
12. *Chroomonas salina* (Wislouch) Butcher (Woods Hole undetermined cryptomonad clone "3C", Dr F Haxo)
13. *Rhodomonas lens* Pascher and Ruttner (Lasker's Gulf-stream strain, Dr L. Provasoli)
14. *Hemiselmis virescens* Droop (Millport strain no. 64, Dr L. Provasoli)
- DINOPHYCEAE
15. *Amphidinium carteri* Hulburt (Woods Hole clone "Amphi 1" Dr L. Provasoli)
- RHODOPHYCEAE
16. *Porphyridium cruentum naegeli* (Vischer's strain no. 107, Dr F. Haxo)
- CYANOPHYCEAE
17. *Agmenellum quadruplicatum* (Menegh.) Brebisson (Van Baalen's strain "PR-6", Dr C. Van Baalen)
18. *Anacystis marina* (Hansg.) Drouet and Daily (Van Baalen's strain "6", Dr C. Van Baalen)

- Initial cell concentration: 2.5E4 cells/mL (test 1); 2.5E4-2.5E5 cells/mL (test 2)

DILUTION WATER

- Source: sea water, open ocean (salinity 33 ppt)

GROWTH/TEST MEDIUM CHEMISTRY

- Chemistry: Salinity 18 g Cl/L; pH 7.6-7.8; EDTA 21.8 µM. Further the medium contained anorganic salts (KNO₃, NaH₂PO₄ and Na₂SiO₂), vitamins, trace metals (chelated) and buffer (=tris HCl)

TEST SYSTEM-TEST 1

- Test type: static
- Concentrations: 0, 4.6 and 46 g/L
- Exposure vessel type: 125 mL screw capped erlenmeyer flasks containing 40 mL of test solution
- Number of replicates: not indicated
- Photoperiod (intensity of irradiation): continuously (2690-3228 lux) or dark
- Test temperature: 20±2 °C
- Shaken: mechanical agitation for a few minutes once every 24 hours

TEST SYSTEM-TEST 2

- Test type: static
- Concentrations: 0, 4.6, 46 and 92 g/L
- Exposure vessel type: 8 mL screw capped culture tubes containing 4 mL of test solution
- Number of replicates: not indicated
- Photoperiod (intensity of irradiation): continuously (2690-3228 lux) or dark

DURATION OF TEST: 28 days

TEST PARAMETER: growth (OD 600 nm (test 1 and 2) & haemocytometrically (test 1))

OBSERVATION TIMES: weekly (test 1); daily (test 2)

Result

: RESULTS:

Apart from *P. parvum* and *C. salina*, none of the species showed any significant growth on glycerol in the absence of light. However in the presence of light, glycerol enhanced the growth of 16 species, in particular members of the Chrysophyceae and Cryptophyceae, one diatom (*P. tricornutum*), one rhodophyte (*P. cruentum*), and one chlorophyte (*N.*

	oculata). A high concentration of glycerol was required for inducing or asserting growth enhancement of certain species, but was equally effective as the low concentrations or was inhibitory to other species. Some species showed obvious cytological and metabolic changes from growth on glycerol. The lowest effect concentration was a 28 day effect on growth at a concentration of 4600 mg/l for <i>Agmenellum quadruplicatum</i> and <i>Anacystis marina</i>.	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated	
Attached document	: attached document ref 100.xls	
Reliability	: (4) not assignable	
	Secondary literature, non-GLP and not a standard OECD-test.	
25.01.2002		(52)
Species	: other aquatic plant: duckweed	
Endpoint	:	
Exposure period	:	
Unit	:	
Method	:	
Year	: 1997	
GLP	:	
Test substance	:	
Remark	: Literature could not be retrieved.	
Result	: Glycerol produces the same effects as ethylene glycol on <i>Lemna gibba</i> . The effects described for ethylene glycol are a relatively high EC50 with regard to frond reproduction, no metabolism by duckweed, the fronds of duckweed are dark green, translucent and the growth medium contains gas bubbles which result from an enhanced uptake and subsequent respiration of sucrose. It is hypothesized that these effects are due to a disruption, of the pectin layer between cells as evidenced by the appearance of intercellular holes in the aerenchymatous tissues and a lifting of the cutin layer on the upper surface of the fronds. This disruption causes the plants to take up more water, lose their hydrophobicity and sink.	
Conclusion	: The result of the creation of intercellular holes by these compounds is to increase the uptake of soluble materials from the plant's aqueous growth medium. In case of a nutrient this can lead to stimulated growth; in case of a heavy metal ion or an organic toxicant, it can lead to enhanced toxicity.	
19.12.2001		(53)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	: aquatic
Species	: <i>Pseudomonas putida</i> (Bacteria)
Exposure period	: 16 hour(s)
Unit	: mg/l
EC0	: 10000
Analytical monitoring	: no
Method	: DIN 38412, part8
Year	: 1994
GLP	: yes
Test substance	: other TS

Method : TEST ORGANISMS
- Species: *Pseudomonas putida* MIGULA Stamm Berlin 33/2 (DSM 50026)
- Source/supplier: TTB-Mikrobiologie, Henkel KGaA

TEST DESCRIPTION
A stock solution of glycerine of 99.6 g/L (pH 5.3) was prepared. Test solutions (100 mL) were prepared by adding together the required volume of stock solution, nutrient medium (according to DIN 38412), water and inoculum of *Pseudomonas putida*. Test concentrations were 100, 300, 1000, 3000 and 10000 mg/l. One replicate from each treatment was shaken (100 rpm) for 16 h at 21-22°C. For the highest test concentration 3 replicates were included and also 3 replicates of a control treatment were included. At the end of the test, the extinction (436 nm) was measured.

Result : Based on the turbidity of the solution the growth of the bacteria can be estimated. For all test concentrations (100-10000 mg/L) no effects on the growth of bacteria were found. Compared to the control a positive effect of 0.5-3.4% was seen on the growth.

Test substance : CAS 56-81-5 (glycerine), purity 99.5%.
Reliability : (2) valid with restrictions
1. The EC₀ is set on the highest tested concentration of 10000 mg/L.
2. The guideline DIN 38412, part 8 contains the following validity criterium: the turbidity in the control treatment should be increased with a factor of 100 during the test. Because there is no information on the initial turbidity, it is not clear whether this validity criterium was met. If however the test was performed in accordance with the guideline mentioned in the report, the initial turbidity was "TE/F=5". From the report it was clear that at the end of the test the turbidity in the control was "TE/F=592", which suggests that the validity criterium was met.
3. The turbidity of the test solution is expressed in "TE/F/436 nm". This refers to a measurement of the extinction of the test solution at 436 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027).
4. The report was essentially confined to what is included in the current summary. According to the reviewer there is enough information to give this study a reliability of 2.

19.12.2001

(54)

Type : aquatic
Species : *Chilomonas paramecium* (Protozoa)
Exposure period : 48 hour(s)
Unit : mg/l
EC5 : > 10000
Method :
Year : 1980
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
- Species: *Chilomonas paramecium* Ehrenberg
- Laboratory culture: yes
- Initial cell concentration: 3.0E3 cells/ml

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a total test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water.

DILUTION WATER
- bidistilled water

GROWTH/TEST MEDIUM CHEMISTRY

- Medium (pre- and stem culture): 3190 mg/L glycine, 115 mg/L Ca(NO₃)₂·4H₂O, 28 mg/L Mg(NO₃)₂·7H₂O, 16 mg/L KNO₃, 10 mg/L K₂HPO₄, 0.8 mg/L biotin (as D-Biotin), 8.0 mg/L nicotinic acid, 4.0 mg/L thiamin.HCl, 4.0 mg/L p-aminobenzoic acid, 2.0 mg/L panthotenic acid (as D-Panthotenic acid, Na-salt), 20 mg/L pyridoxamine (as pyridoxaminedihydrochlorid), 8.0 mg/L cyanocobalamin (vitamine B12); sterilised and pasteurised.

- Test medium: mixture of solution 1 (sterilised) and solution 2 (pasteurised) containing 7940 mg/L glycine, 288 mg/L Ca(NO₃)₂·4H₂O, 69 mg/L Mg(NO₃)₂·7H₂O, 40 mg/L KNO₃, 26 mg/L K₂HPO₄, 15 µg/L biotin (as D-Biotin), 149 µg/L nicotinic acid, 74 µg/L thiamin.HCl, 74 µg/L p-aminobenzoic acid, 37 µg/L panthotenic acid (as D-Panthotenic acid, Na-salt), 372 µg/L pyridoxamine (as pyridoxaminedihydrochlorid), 149 µg/L cyanocobalamin (vitamine B12)

TEST SYSTEM

- Test cultures are prepared from pre-cultures (7 d, 20 C). Pre-cultures are prepared from stem cultures (3-4 d, 20 C).
- Test solution: 8 mL of test substance solution, 8 mL of test medium and 4 mL of pre-culture (15,000 cells/mL).
- Temperature: 20 C

DURATION OF TEST: 48 hours

TEST PARAMETER: cell growth using a Coulter counter

OBSERVATION TIMES: 48 hours

Result	:	At 10,000 mg/L the effect of glycerine was less than 5%.
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.
Conclusion	:	>10,000 mg/L
Reliability	:	(4) not assignable

The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (171 substances included).

19.12.2001

(55) (56)

Type	:	aquatic
Species	:	Clostridium sp. (Bacteria)
Exposure period	:	
Unit	:	
Analytical monitoring	:	no
Method	:	
Year	:	1992
GLP	:	no data
Test substance	:	other TS

Method : Clostridium pasteurianum is known as a classical acid producer and usually ferments carbohydrates to butyrate, acetate, carbon dioxide and molecular hydrogen. Recent reports indicate the ability of Clostridium pasteurianum to produce acetone, butanol and ethanol in media with high glucose concentrations.

Therefore this test was set up to study the ability of Clostridium pasteurianum to produce solvents from renewable biomass. In the test glucose and glycerol were included, but in this summary only the part related to glycerol is included.

	GROWTH MEDIUM - Components: 1.74 g/L K₂HPO₄, 0.66 g/L NH₄Cl, 0.251 g/L MgSO₄·7H₂O, 0.596 g/L KCl, 69 mg/L Fe-Na-EDTA, 6 g/L NaHCO₃, 4 mg p-aminobenzoic acid, 0.24 mg/L biotin, 0.5 g/L yeast extract, 1 mg/L resazurin, 0.5 g/L cysteine-HCl and 20-200 g/L glycerol - pH 7.0 - gas phase: 80% v/v N₂ and 20% v/v CO₂ TEST DESIGN - EXPERIMENT 1: The effect on different concentrations of glycerol in the growth medium is determined using concentrations of 20, 40, 60, 80, 100, 120, 170 and 200 g glycerol/L. - EXPERIMENT 2: The effect of iron limitation on glycerol fermentation is tested using an adapted growth medium (no yeast extract or Fe-Na-EDTA, supplemental FeSO₄·H₂O added (0, 3 or 20 µM) containing 40 g/L glycerol. ENDPOINT - Growth: measured spectrophotometrically by determination of the optical density at 578 nm. - Products (ethanol, acetate, butanol, butyrate and propanediol) are measured by a gas chromatographic method
Result	: EXPERIMENT 1: - Fermentation resulted in the production of mainly ethanol, propanediol and butanol. Acetate and butyrate were only produced in trace amounts. - The tolerance of <i>C. pasteurianum</i> to glycerol was rather high. Good growth was obtained at glycerol concentrations of up to 170 g/L. The optimum growth was found at glycerol concentrations of 100 g/L. EXPERIMENT 2: - Less iron in the medium resulted in lower amounts of reaction products with a changed pattern (ethanol/butanol decreased, lactate increased)
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.
Conclusion	: Good growth at glycerol concentrations up to 170 g/L.
Reliability	: (4) not assignable
	1. Secondary literature. 2. The test was not set up to determine the toxicity of glycerol, but can be used for this purpose. The sensitivity of the tested organism in comparison with other micro-organisms is not known.
19.12.2001	(57)
Type	: aquatic
Species	: <i>Entosiphon sulcatum</i> (Protozoa)
Exposure period	: 72 hour(s)
Unit	: mg/l
EC5	: 3200
Analytical monitoring	: no
Method	:
Year	: 1980
GLP	: no data
Test substance	: other TS
Method	: TEST ORGANISMS - Species: <i>Entosiphon sulcatum</i> - Laboratory culture: yes - Initial cell concentration: 1.5E3 cells/ml
	STOCK AND TEST SOLUTION AND THEIR PREPARATION - Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16,

32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a total test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water.

DILUTION WATER

- bidistilled water

GROWTH/TEST MEDIUM CHEMISTRY

- Medium (stem-, pre- and test culture): 290 mg/L Ca(NO₃)₂·4H₂O, 70 mg/L Mg(NO₃)₂·6H₂O, 40 mg/L KNO₃; pH 6.9.

TEST SYSTEM

- Test cultures are prepared from precultures (72 h, 25 C). Pre-cultures are prepared from stem cultures (72-96 h, 25 C).
 - Test solution: 8 mL of test substance solution, 8 mL of test medium, 2 mL bacteria suspension and 2 mL of preculture (15,000 cells/mL).
 - Temperature: 25 C

DURATION OF TEST: 72 hours

TEST PARAMETER: cell growth using a Coulter counter

OBSERVATION TIME: 72 hours

Result : EC₅ = 3200 mg/L
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (171 substances included).

19.12.2001

(50) (55) (58)

Type : aquatic
Species : *Pseudomonas putida* (Bacteria)
Exposure period : 16 hour(s)
Unit : mg/l
EC₃ : > 10000
Analytical monitoring : no data
Method : other: cell multiplication inhibition test
Year : 1980
GLP : no data
Test substance : other TS: glycerol. No indication about purity.

Method : TEST ORGANISMS
 - Species: *Pseudomonas putida*
 - Source/supplier: not specified
 - Method of cultivation: stemculture and preculture were renewed weekly (incubation at 25 C for 24 h)
 - Initial cell concentration: equivalent to an extinction value corresponding to a turbidity value of TE/F/436 nm = 10

DILUTION WATER

- Source: bidistilled water

GROWTH/TEST MEDIUM CHEMISTRY

- Chemistry: 1 g/L glucose, 0.21 g/L NaNO₃, 0.12 g/L K₂HPO₄, 60 mg/L KH₂PO₄, 10 mg/L FeSO₄·7H₂O, 0.2 g/L MgSO₄·7H₂O, 0.083 mg/L Al₂(SO₄)₃·18H₂O, 0.042 mg/L KI, 0.042 mg/L KBr, 0.083 mg/L TiO₂, 0.042 mg/L SnCl₂·2H₂O, 0.042 mg/L LiCl, 0.58 mg/L MnCl₂·4H₂O, 0.92 mg/L H₃BO₃, 0.083 mg/L ZnSO₄·7H₂O, 0.083 mg/L CuSO₄·5H₂O, 0.089 mg/L NiSO₄·6H₂O, 0.083 mg/L Co(NO₃)₂·6H₂O

	TEST SYSTEM - Test type: static - Concentrations: not specified, dilutions of stock in 1 to 16384 (2EXP0-2EXP14) parts of stock; controls containing saline instead of bacteria-suspension - Temperature: 25 C DURATION OF TEST: 16 h TEST PARAMETER: inhibition of turbidity after 16 hours (extinction at 436 nm); 3% reduction of extinction is considered as an inhibitory effect OBSERVATION TIMES: after 16 hours STATISTICAL METHOD: not specified
Result Test substance Reliability	: EC3 >10000 mg/L : CAS 56-81-5 (glycerine), purity not indicated : (4) not assignable 1. The test is not in accordance with the current accepted guidelines, but gives an indication of the toxicity of glycerine to <i>Pseudomonas putida</i>. 2. The turbidity of the test solution is expressed in "TE/F/436 nm". This refers to a measurement of the extinction of the test solution at 436 nm. The extinction is based on a calibration with test solutions containing different concentrations of formazine and therefore a relative turbidity value is given (see also DIN 38412 part 8, ISO 10712 and ISO 7027). 3. Secondary literature with information essentially confined to what is included in the current summary.
25.01.2002	(59) (50) (51)
Type Species Exposure period Unit Method Year GLP Test substance	: aquatic : <i>Pseudomonas putida</i> (Bacteria) : 75 minute(s) : mg/l : : 1998 : no data : other TS
Method	: Test solutions (6 mL) were prepared by adding together A. 5 mL distilled water, 0.5 mL imidazol buffer, 1 mL resazurin solution and 0.5 mL test culture (=reagent control) B. 5.5 mL borate buffer, 1 mL resazurin solution (=blank sample) C. solution of glycerol, 0.5 mL imidazol buffer, 1 mL resazurin solution and 0.5 mL test culture (= test sample) Test concentrations were not specified. Two replicates from each treatment were incubated for 75 minutes at 37 C. At the end of the test, the extinction (615 nm) was measured.
Remark Result Test substance	: Literature could not be retrieved. : Mean of 5 experiments: IC50 760000 mg/L : CAS 56-81-5 (glycerine), purity not indicated.
19.12.2001	(12)
Type Species Exposure period	: aquatic : <i>Uronema parduzci</i> (Protozoa) : 20 hour(s)

Unit : mg/l
EC5 : > 10000
Analytical monitoring : no
Method :
Year : 1981
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
 - Species: Uronema parduczi Chatton-Lwoff
 - Laboratory culture: yes
 - Initial cell concentration: 1.5E3 cells/ml
 - Feeding before test: Escherichia coli (alive)
 - Feeding during test: Escherichia coli (inactivated)

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Test substance is dissolved in sterile bidistilled water (pH 6.9). A series of dilutions is made using 1 part of test substance solution and 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192 and 16384 parts of water. Dilutions are prepared starting with a test substance solution of 16 mL and subsequently preparing the following solution by 50% dilution with bidistilled water.

DILUTION WATER

- bidistilled water

GROWTH/TEST MEDIUM CHEMISTRY

- medium used for stem-culture: 228 mg/L CaCl₂·2H₂O, 86 mg/L MgSO₄·7H₂O, 29 mg/L KH₂PO₄, 57 mg/L K₂HPO₄
 - medium used for pre-and test culture: 290 Ca(NO₃)₂·4H₂O, 70 mg/L Mg(NO₃)₂·6H₂O, 40 mg/L KNO₃

TEST SYSTEM

- Test cultures are prepared from pre-cultures (22 h, 25 C). Precultures are prepared from stem-cultures (max 72 h, 25 C).
 - Test solution: 8 mL of test substance solution, 8 mL of test medium, 2 mL bacteria suspension and 2 mL of micro-organisms (15,000 cells/mL).
 - Temperature: 25 C

DURATION OF TEST: 20 hours

TEST PARAMETER: cell growth using a Coulter counter

OBSERVATION TIMES: 20 hours

Result : At 10,000 mg/L the effect of glycerine was less than 5%.
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable

The test design was clearly described, but there was no information on the (actual) tested concentrations. Individual data are also not included in this review article (169 substances included).

19.12.2001

(60) (55)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS**4.6.2 TOXICITY TO TERRESTRIAL PLANTS****4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS****4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES****4.7 BIOLOGICAL EFFECTS MONITORING**

Remark : A validated method for biological monitoring of glycerol is not available.

Source : Unichema Chemie B.V. Gouda
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

24.09.2001 (5)

4.8 BIOTRANSFORMATION AND KINETICS

Remark : Glycerol is readily absorbed into the gastrointestinal tract, metabolised by standard pathways in mammals and its products used to produce glucose, glycogen and fats. Literature could not be retrieved.

Source : Croda Universal Ltd Goole, North Humberside
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

15.11.2001 (61) (62)

4.9 ADDITIONAL REMARKS

Memo : Frog Embryo Teratogenesis Assay - Xenopus (FETAX)

Method : FETAX is a 96-h whole-embryo developmental toxicity screening assay according to test guideline ASTM E1439-91 (1991). The test was performed in triplo at three different laboratories.

Groups of 20 embryos were exposed to 10 concentrations of glycerol in presence and absence of metabolic activation (microsomes from Aroclor 1254 induced rats and an NADPH- generating system) during 96 hours. Exposure medium was FETAX-AB (reconstituted water which includes 100 U/mL penicillin and streptomycin). Controls included were treated with FETAX-AB (4 dishes), FETAX (4 dishes), metabolic activation system (2 dishes) and activated cyclophosphamide (2 dishes with microsomes, 2 with NADPH-generating system and 2 without any activation).

Statistical method: Probit analysis (Litchfield-Wilcoxon), trimmed Spearman Karber, Steel and Torrie

Result

Criteria for evaluating results: TI (teratogenic index i.e. 96-h LC50/96-h EC50(malformations)) > 1.5 and inhibition of growth > 30% compared to tests with microsomal fraction only.

: Laboratory 1 without rat liver microsomes (Aroclor treated)
96-h LC50 14.84 mg/ml (95% CI 10.41-19.26 mg/ml)
96-h EC50 (malformations) 10.89 mg/ml (95% CI 10.21-11.56 mg/ml)
LOEC (growth) 12.67 mg/ml (95% CI 8.09-17.24 mg/ml)
TI 1.35 (95% CI 1.02-1.68); TI = teratogenic index (LC50/EC50 malformations)

Laboratory 1 with rat liver microsomes (Aroclor treated)
96-h LC50 10.33 mg/ml (95% CI 6.18-14.48 mg/ml)
96-h EC50 (malformations) 10.76 mg/ml (95% CI 10.26-11.25 mg/ml)
LOEC (growth) 8.67 mg/ml (95% CI 6.05-11.28 mg/ml)
TI 0.97 (95% CI 0.54-1.40); TI = teratogenic index (LC50/EC50 malformations)

Laboratory 2 without rat liver microsomes (Aroclor treated)
96-h LC50 19.10 mg/ml (95% CI 17.52-20.69 mg/ml)
96-h EC50 (malformations) 11.48 mg/ml (95% CI 10.81-12.15 mg/ml)
LOEC (growth) 10.83 mg/ml (95% CI 10.02-11.65 mg/ml)
TI 1.67 (95% CI 1.44-1.90); TI = teratogenic index (LC50/EC50 malformations)

Laboratory 2 with rat liver microsomes (Aroclor treated)
96-h LC50 18.85 mg/ml (95% CI 16.72-20.98 mg/ml)
96-h EC50 (malformations) 11.26 mg/ml (95% CI 10.92-11.60 mg/ml)
LOEC (growth) 10.83 mg/ml (95% CI 9.20-12.47 mg/ml)
TI 1.67 (95% CI 1.52-1.83); TI = teratogenic index (LC50/EC50 malformations)

Laboratory 3 without rat liver microsomes (Aroclor treated)
96-h LC50 9.42 mg/ml (95% CI 8.27-10.56 mg/ml)
96-h EC50 (malformations) 6.67 mg/ml (95% CI 5.67-7.66 mg/ml)
LOEC (growth) 3.63 mg/ml
TI 1.41 (95% CI 1.33-1.50); TI = teratogenic index (LC50/EC50 malformations)

Conclusion

Laboratory 3 with rat liver microsomes (Aroclor treated)
96-h LC50 13.46 mg/ml (95% CI 11.05-15.87 mg/ml)
96-h EC50 (malformations) 5.84 mg/ml (95% CI 4.79-6.88 mg/ml)
LOEC (growth) 2.13 mg/ml (95% CI 0.66-3.60 mg/ml)
TI 2.33 (95% CI 1.82-2.85); TI = teratogenic index (LC50/EC50 malformations)

: With metabolic activation:
96-h LC50 14.2 mg/ml (RSD 31%)
96-h EC50 (malformations) 9.29 mg/ml (RSD 29%)
LOEC (growth) 7.21 mg/ml (RSD 58%)
TI 1.66 (RSD 40%); TI = teratogenic index (LC50/EC50 malformations)

Without metabolic activation:
96-h LC50 14.5 mg/ml (RSD 33%)
96-h EC50 (malformations) 9.68 mg/ml (RSD 24%)
LOEC (growth) 9.04 mg/ml (RSD 51%)

TI 1.48 (RSD 16%); TI = teratogenic index (LC50/EC50 malformations)

According to the criteria set, glycerol gave an ambiguous result. Certain criteria suggested that glycerol is non-teratogenic, but others suggested it is. Severe malformations were observed at concentrations approaching the 96h LC50 with metabolic activation.

25.01.2002

(63)

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION**5.1.1 ACUTE ORAL TOXICITY**

Type : LD50
Value : = 27200 mg/kg bw
Species : rat
Strain : Long-Evans
Sex : female
Number of animals : 12
Vehicle :
Doses :
Method : other: not indicated
Year : 1953
GLP :
Test substance :

Method : TEST ORGANISMS:
 - Number: 12
 - Mean weight at study initiation: 114 g

ADMINISTRATION:
 - Dose: 27.26 mg/kg bw
 - Route: oral (gavage)
 - Vehicle: none (undiluted)
 - Post dose observation period: 10 days

EXAMINATIONS: mortality, clinical signs, body weight (frequency not indicated), macroscopy in animals that died and selected survivors, histopathology of brain, heart, liver, spleen, stomach, intestine and kidney.

STATISTICAL METHOD: LD50 was calculated using logarithmic-probit graph paper

Result : MORTALITY: not indicated

CLINICAL SIGNS: muscle spasms and clonic convulsions prior to death. Survivors appeared normal within 2.5 hours after administration.

NECROPSY FINDINGS: hyperaemia of pylores and small intestine; congestion of the lungs; pale spleen; 3 animals showed hyperaemia of the cerebral meninges

Test substance : CAS 56-81-5 (glycerine)
 Natural glycerine, achieved from market stock, purity not indicated (impurities were fatty acids and esters)
 Synthetic glycerol, purity 99.5% (rest mainly water with very small amounts of glycerol polymers and glyceraldehyde)

Conclusion : Results for natural and synthetic glycerine were comparable
 Most reliable and elaborate study available.

Reliability : (2) valid with restrictions
 The report was limited to the above mentioned.

Flag : Critical study for SIDS endpoint

17.12.2001

(64) (65)

Type : LD50

Value	:	= 23000 mg/kg bw
Species	:	mouse
Strain	:	Swiss
Sex	:	male
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: not indicated
Year	:	1953
GLP	:	
Test substance	:	
Method	:	TEST ORGANISMS: - Number: 91 in total (not further specified) - Mean weight at study initiation: 20 g ADMINISTRATION: - Doses: 15000 - 31500 mg/kg bw - Route: oral (gavage) - Vehicle: none (undiluted) - Post dose observation period: 10 days EXAMINATIONS: mortality, clinical signs, body weight (frequency not indicated), macroscopy in animals that died and selected survivors, histopathology of brain, heart, liver, spleen, stomach, intestine and kidney. STATISTICAL METHOD: LD50 was calculated using logarithmic-probit graph paper
Result	:	MORTALITY: not indicated CLINICAL SIGNS: body tremor, Straub tail and clonic convulsions prior to death NECROPSY FINDINGS: hyperaemia of small intestine and lungs at the two highest dose levels; hyperaemia of the kidneys and mucosa of the small intestine (not further specified)
Test substance	:	CAS 56-81-5 (glycerine) Natural glycerine, achieved from market stock, purity not indicated (impurities were fatty acids and esters) Synthetic glycerin, purity 99.5% (rest mainly water with very small amounts of glycerin polymers and glyceraldehyde)
Conclusion	:	Results for natural and synthetic glycerine were comparable Most reliable and elaborate study available.
Reliability	:	(2) valid with restrictions The report was limited to the above mentioned
Flag	:	Critical study for SIDS endpoint
17.12.2001		(64)
Type	:	LD50
Value	:	= 10000 mg/kg bw
Species	:	guinea pig
Strain	:	
Sex	:	male
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: not indicated
Year	:	1953
GLP	:	

Test substance	:	
Method	:	TEST ORGANISMS: - Number: 29 in total (probably 9-10/treatment) - Mean weight at study initiation: 325 g ADMINISTRATION: - Dose: 7250 mg/kg bw (middle dose) - Route: oral (gavage) - Vehicle: none (undiluted) - Post dose observation period: 10 days EXAMINATIONS: mortality, clinical signs, body weight (frequency not indicated), macroscopy in animals that died and selected survivors, histopathology of brain, heart, liver, spleen, stomach, intestine and kidney. STATISTICAL METHOD: LD50 was calculated using logarithmic-probit graph paper
Result	:	MORTALITY: not indicated CLINICAL SIGNS: tremor of head and body after auditory stimuli immediately after administration, tremor prior to death NECROPSY FINDINGS: hyperaemia of pylores and small intestine; congestion of the lungs; pale spleen
Test substance	:	CAS 56-81-5 (glycerine) Natural glycerine, achieved from market stock, purity not indicated (impurities were fatty acids and esters) Synthetic glycerin, purity 99.5% (rest mainly water with very small amounts of glycerin polymers and glyceraldehyde)
Conclusion	:	LD50 10000 mg/kg bw for natural glycerin LD50 11500 mg/kg bw for synthetic glycerin
Reliability	:	(2) valid with restrictions The report was limited to the above mentioned.
Flag	:	Critical study for SIDS endpoint
17.12.2001		
Type	:	LD50
Value	:	> 25300 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	
Method	:	other: not indicated
Year	:	1976
GLP	:	
Test substance	:	
Method	:	TEST ORGANISMS: - Source: Gassner of Sulzfeld - Age: not indicated - Number: 5/sex/treatment - Weight at study initiation: 170-230 g ADMINISTRATION: - Doses: not indicated

(64)

- Post dose observation period: 7 days

EXAMINATIONS: LD50 calculation

STATISTICAL METHOD: Probit analysis (Finney)

Test substance : CAS 56-81-5 (glycerine), purity not indicated (DAB 7 purity).
Reliability : (4) not assignable
1 The report is limited to the above mentioned
2 Only an LD50 value was mentioned

17.12.2001 (66)

Type : LD50
Value : = 27500 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Reliability : (4) not assignable
The information was confined to the above.

16.11.2001 (65)

Type : LD50
Value : > 25000 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1983
GLP :
Test substance : other TS

Test substance : CAS 56-81-5 (Glycerine), purity not indicated.
Reliability : (4) not assignable
The information was confined to the above.

17.12.2001 (67)

Type : LD50
Value : = 26250 - 28750 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Reliability : (4) not assignable
The information was confined to the above.

16.11.2001 (65)

Type : LD50
Value : > 5000 mg/kg bw
Species : rat
Strain :
Sex :

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Number of animals :
 Vehicle :
 Doses :

Reliability : (4) not assignable
 The information was confined to the above.

16.11.2001

(65)

Type : LD50
 Value : = 15750 mg/kg bw
 Species : rat
 Strain :
 Sex :
 Number of animals :
 Vehicle :
 Doses :
 Method : other
 Year :
 GLP : no data
 Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
 Reliability : (4) not assignable
 The information in the report was confined to the above.

17.12.2001

(65)

Type : LD50
 Value : = 58400 mg/kg bw
 Species : rat
 Strain :
 Sex :
 Number of animals :
 Vehicle :
 Doses :
 Method :
 Year : 1954
 GLP :
 Test substance :

Test substance : CAS 56-81-5 (glycerine), purity 83-87%.
 Reliability : (4) not assignable
 The information was confined to the above.

17.12.2001

(68) (65) (69)

Type : LD50
 Value : = 27500 mg/kg bw
 Species : rat
 Strain : Wistar
 Sex : male
 Number of animals : 10
 Vehicle : water
 Doses :
 Method :
 Year : 1941
 GLP :
 Test substance :

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
 Reliability : (4) not assignable
 The information was confined to the above.

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

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(70)

Type : LD50
Value : > 10000 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP :
Test substance :

Remark : Literature could not be retrieved.
Source : UNION DERIVAN S.A. VILADECANS
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable

25.01.2002

Type : LD50
Value : = 12600 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1987
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable

25.01.2002

(71)

Type : LD50
Value : > 24000 mg/kg bw
Species : Rat
Strain : Fischer 344
Sex : Female
Number of animals : 5
Vehicle :
Doses : several doses up to 24 g/kg
Method : other: not indicated
Year : 1979
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS:
 - Age: 12-14 weeks
 - Number: 5/treatment
 - Weight at study initiation: 150-200 g

 ADMINISTRATION:
 - Doses: several doses up to 24 g/kg

	- Doses per time period: single dose - Volume administered: undiluted test substance - Post dose observation period: 14 days
	EXAMINATIONS: mortality and clinical signs daily; necropsy on half of animals that died and selection of surviving animals in highest dose group at day 14
Result	: MORTALITY: - Number of deaths at each dose: none after 48 h
	CLINICAL SIGNS: not reported
Test substance	: NECROPSY FINDINGS: not reported CAS 56-81-5 (glycerine), purity not indicated (glycerine/water mixture of unknown composition).
Reliability	: (4) not assignable 1. Secondary literature; the information in the report is confined to the above. 2. The LD50 is based on mortality at 24 h post treatment. No information after a 48 h observation period is given.
17.12.2001	(72)
Type	: LD50
Value	: ca. 26000 mg/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Doses	:
Method	: other
Year	: 1950
GLP	: no data
Test substance	:
Remark	: Comparative acute toxicity of synthetic and natural glycerin. LD50 (natural glycerin) : 20.65 cc/kg LD50 (synthetic glycerin): 20.81 cc/kg
Test substance	: CAS 56-81-5 (glycerine), purity 99.8% for synthetic glycerine and for natural not indicated.
Reliability	: (4) not assignable The information was confined to the above.
17.12.2001	(73) (65)
Type	: LD50
Value	: = 38000 mg/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Doses	:
Method	:
Year	: 1976
GLP	: no data
Test substance	: as prescribed by 1.1 - 1.4
Remark	: Literature could not be retrieved.
Source	: Unichema Chemie B.V. Gouda

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ID: 56-81-5

DATE: 29.01.2002

EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Test substance	: CAS 56-81-5 (glycerine), DAB 7 purity.
Reliability	: (4) not assignable
25.01.2002	(74)
Type	: LD50
Value	: = 22400 ml/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Doses	:
Reliability	: (4) not assignable
16.11.2001	The information was confined to the above. (65)
Type	: LD50
Value	: = 37736 mg/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Doses	:
Method	:
Year	:
GLP	:
Test substance	: other TS
Test substance	: CAS 56-81-5 (glycerine), purity 83-87%.
Reliability	: (4) not assignable
16.11.2001	The information was confined to the above. (65)
Type	: LD50
Value	: = 31250 mg/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Doses	:
Method	:
Year	:
GLP	:
Test substance	: other TS
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.
Reliability	: (4) not assignable
16.11.2001	The information was confined to the above. (65)
Type	: LD50
Value	: = 28000 mg/kg bw
Species	: Mouse
Strain	:
Sex	:
Number of animals	:

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Vehicle	:		
Doses	:		
Method	:		
Year	:		
GLP	:		
Test substance	:	other TS	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
		The information was confined to the above.	
16.11.2001			(65)
Type	:	LD50	
Value	:	= 25888 mg/kg bw	
Species	:	mouse	
Strain	:		
Sex	:		
Number of animals	:		
Vehicle	:		
Doses	:		
Method	:		
Year	:		
GLP	:		
Test substance	:	other TS	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
		The information was confined to the above.	
16.11.2001			(65)
Type	:	LD50	
Value	:	= 37950 mg/kg bw	
Species	:	mouse	
Strain	:	NMRI	
Sex	:	male/female	
Number of animals	:	10	
Vehicle	:		
Doses	:		
Method	:	other: not indicated	
Year	:		
GLP	:		
Test substance	:		
Method	:	TEST ORGANISMS:	
		- Source: Gassner of Sulzfeld	
		- Age: not indicated	
		- Number: 5/sex/treatment	
		- Weight at study initiation: 15-25 g	
		ADMINISTRATION:	
		- Doses: not indicated	
		- Post dose observation period: 7 days	
		EXAMINATIONS: LD50 calculation	
		STATISTICAL METHOD: Probit analysis (Finney)	
Test substance	:	CAS 56-81-5 (glycerine), DAB 7, purity not specified	
Reliability	:	(4) not assignable	
		1 The report is limited to the above mentioned	
		2 Only an LD50 value was mentioned	

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

20.11.2001

(66)

Type : LD50
Value : > 38000 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year :
GLP :
Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.

20.11.2001

(67)

Type : LD50
Value : = 37763 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Test substance : CAS 56-81-5 (glycerine), purity 83-87%.
Reliability : (4) not assignable
 The information was confined to the above.

15.11.2001

(68) (69)

Type : LD50
Value : = 25888 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.

15.11.2001

(68) (69)

Type : LD50
Value : = 12500 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.

15.11.2001

(75)

Type : LD50
Value : ca. 26000 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance :

Remark : Comparative acute toxicity of synthetic and natural glycerin.
 LD50 (natural glycerin) : 20.65 cc/kg
 LD50 (synthetic glycerin): 20.81 cc/kg
Test substance : CAS 56-81-5 (glycerine), purity 99.8% for synthetic glycerine and not indicated for natural glycerine.
Reliability : (4) not assignable
 The information was confined to the above.

16.11.2001

(73)

Type : LD50
Value : = 4250 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1977
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable

25.01.2002

(76)

Type : LD50
Value : = 27000 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Reliability : (4) not assignable
 25.01.2002 (77)

Type : LD50
Value : = 7750 mg/kg bw
Species : guinea pig
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : other TS

Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.
 15.11.2001 (70) (78)

Type : LDLo
Value : = 1428 mg/kg bw
Species : human
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
 16.11.2001 (79)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD0
Value : > 18700 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals : 6
Vehicle :
Doses : 6200-18700 mg/kg bw
Method : other
Year : 1953
GLP : no data
Test substance : other TS

Remark : No. of animals: 6.

	Initial body weight of the tested animals: 3 kg. Treatment: 8 hours under occlusion.	
	Comparative acute toxicity of synthetic and natural glycerin.	
Result	: No signs of clinical toxicity were observed for either synthetic or natural glycerol.	
Test substance	: CAS 56-81-5 (glycerine), purity 99.5%	
Conclusion	: Most reliable study available.	
Reliability	: (4) not assignable The information in the report was confined to the above.	
Flag 17.12.2001	: Critical study for SIDS endpoint	(64) (65)
Type	: other	
Value	:	
Species	: rat	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	: 1960	
GLP	: no data	
Test substance	: other TS	
Method	: TESTORGANISMS - Weight at study initiation: 105-125 g - Number of animals: 5 males/treatment	
	ADMINISTRATION: - Type of exposure: dermal (animals dipped in test substance) - Exposure duration: 20 and 40 min (after 24 hours animals were dipped in chloroform for 2 min) - Size of application area: 4.5 X 5.5 cm - Concentrations: 2 mL test substance	
	EXAMINATIONS: Amount of urine excreted and colour of urine (excreted spontaneously or after squeezing) 60, 120 and 180 min after application; kidney histopathology	
Result	: Total urine excreted after 20 minutes exposure ~12.5 mL, after 40 minutes exposure ~ 9 mL. Haemoglobinuria was observed in 1/5 rats after 20 min and in 3/5 rats after 40 min exposure. Subsequent exposure to chloroform gave haemoglobinuria in all animals.	
Test substance	: CAS 56-81-5 (glycerine), DAB 6, purity not specified	
Conclusion	: Treatment with glycerine induced haemoglobinuria in rats. From the effect of chloroform, it was concluded that the capillaries of the skin area were not destroyed.	
Reliability	: (4) not assignable 1. The experimental design was poorly described. The report was limited to the above. The study was not conducted to current regulatory test guidelines. 2. It cannot be excluded that the result is influenced by the way of application (dipping into the test substance), the size of the application area and the squeezing of the animals to obtain urine.	
21.01.2002		(80)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : 7500 - 10100 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : i.p.
Exposure time :
Method :
Year : 1976
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Test substance : CAS 56-81-5 (glycerine), purity not retrievable (DAB 7 purity).
Reliability : (4) not assignable
 The information in the report is confined to the above.
 14.11.2001 (66) (67)

Type : LD50
Value : = 4420 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : i.p.
Exposure time :
Method :
Year :
GLP : no data
Test substance : no data
Remark : Literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (81)

Type : LD50
Value : 8600 - 9500 mg/kg bw
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : i.p.
Exposure time :
Method :
Year : 1976
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Test substance : CAS 56-81-5 (glycerine), purity not retrievable (DAB 7 purity).
Reliability : (4) not assignable
 The information in the report is confined to the above.
 14.11.2001 (66) (67)

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Type	:	LD50
Value	:	= 8700 mg/kg bw
Species	:	Mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1978
GLP	:	no data
Test substance	:	no data
Remark	:	Literature could not be retrieved.
Source	:	Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(82)

Type	:	LD50
Value	:	= 100 mg/kg bw
Species	:	Rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	s.c.
Exposure time	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	Literature could not be retrieved.
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(83)

Type	:	LD50
Value	:	= 91 mg/kg bw
Species	:	Mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	s.c.
Exposure time	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	Literature could not be retrieved.
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(83)

Type	:	LD50

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Value	:	5200 - 6600 mg/kg bw	
Species	:	Rat	
Strain	:		
Sex	:		
Number of animals	:		
Vehicle	:		
Doses	:		
Route of admin.	:	i.v.	
Exposure time	:		
Method	:		
Year	:	1976	
GLP	:	no data	
Test substance	:	as prescribed by 1.1 - 1.4	
Test substance	:	CAS 56-81-5 (glycerine), purity not retrievable (DAB 7 purity).	
Reliability	:	(4) not assignable	
		The information in the report is confined to the above.	
14.11.2001			(66) (67)
Type	:	LD50	
Value	:	5700 - 6700 mg/kg bw	
Species	:	mouse	
Strain	:		
Sex	:		
Number of animals	:		
Vehicle	:		
Doses	:		
Route of admin.	:	i.v.	
Exposure time	:		
Method	:		
Year	:	1976	
GLP	:	no data	
Test substance	:	as prescribed by 1.1 - 1.4	
Test substance	:	CAS 56-81-5 (glycerine), purity not retrievable (DAB 7 purity).	
Reliability	:	(4) not assignable	
		The information in the report is confined to the above.	
14.11.2001			(66) (67)
Type	:	LD50	
Value	:	4250 - 4370 mg/kg bw	
Species	:	mouse	
Strain	:		
Sex	:		
Number of animals	:		
Vehicle	:		
Doses	:		
Route of admin.	:	i.v.	
Exposure time	:		
Method	:		
Year	:		
GLP	:	no data	
Test substance	:	other TS	
Remark	:	Comparative acute toxicity of synthetic and natural glycerin. LD50 (natural glycerin) : 4.37 g/kg LD50 (synthetic glycerin): 4.25 g/kg	
Test substance	:	CAS 56-81-5 (glycerine), purity 99.8% for synthetic and no data for natural glycerine.	
Reliability	:	(4) not assignable	
		The information in the report is confined to the above.	
16.11.2001			(73)

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Type : LD50
Value : = 4250 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : i.v.
Exposure time :
Method :
Year : 1950
GLP : no data
Test substance : no data
Remark : Literature could not be retrieved.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (84)

Type : LD50
Value : = 53000 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : i.v.
Exposure time :
Method :
Year :
GLP : no data
Test substance : no data
Remark : Literature could not be retrieved.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (83)

Type : LD50
Value : > 25000 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. :
Exposure time :
Method :
Year :
GLP :
Test substance : other TS
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.
 20.11.2001 (67)

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : no data
Exposure time : 24 hour(s)
Number of animals : 8
Vehicle :
PDII :
Result : not irritating
Classification :
Method : other: not indicated
Year : 1971
GLP : no
Test substance : other TS

Method : TEST ANIMALS:
 - Sex: male
 - Weight at study initiation: ≥ 2 kg
 - Number of animals: 8
 ADMINISTRATION/EXPOSURE
 - Area of exposure: 6.25 cm²
 - Total volume applied: 0.5 ml
 - Exposure period: 24 h
 EXAMINATIONS
 - Scoring system: Draize
 - Examination time points: 24 and 72 h after application

This method was used by 19 different laboratories.
Result : AVERAGE SCORE
 - Overall irritation score per laboratory (24 and 72 h reading were added): 0.0-0.4 (max. 30 for each time point)
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (2) valid with restrictions
 The information in the report is confined to the above.

17.12.2001

(85)

Species : rabbit
Concentration :
Exposure : no data
Exposure time : no data
Number of animals : 6
Vehicle :
PDII :
Result : not irritating
Classification :
Method : other: Draize
Year : 1979
GLP : no data
Test substance : other TS

Method : TEST ANIMALS:
 - Strain: New Zealand White
 - Sex: female
 - Source: Keene Ridge Farms, Moriarty, NM
 - Weight at study initiation: 2-6 kg
 - Number of animals: 6
 ADMINISTRATION/EXPOSURE
 - Preparation of test substance: undiluted

- Total volume applied: 0.5 ml
EXAMINATIONS
- Scoring system: Draize
- Examination time points: 24 and 72 h after application
Result : AVERAGE SCORE (score represents the mean of application to intact and abraded skin): 0.1
Test substance : CAS 56-81-5 (glycerine), purity not indicated (Glycerol/Water mixture of unknown composition).
Conclusion : Not irritating.
Reliability : (4) not assignable
1. Secondary literature; the information in the report is confined to the above.
2. The exposure time is not indicated.

25.01.2002

(86)

Species : rabbit
Concentration :
Exposure :
Exposure time :
Number of animals :
Vehicle :
PDII :
Result : not irritating
Classification :
Method : Draize Test
Year : 1953
GLP : no data
Test substance : other TS

Method : TEST ANIMALS:
- Weight at study initiation: 2.0-3.8 kg
- Number of animals: 6/treatment
ADMINISTRATION/EXPOSURE
- Doses: 0.5-4.0 mL/kg bw
- Area of exposure: 30% of body surface
- Occlusion: none at the two lower dose levels, occlusion at the two higher dose levels
- Exposure period: 8 hours/day, 5 days/week, 45 weeks
- Vehicle: none
- Total volume applied: 0.5-4.0 mL
EXAMINATIONS
- Scoring system: Draize (1944)
- Examination time points: not indicated
- Other: body weight and urinalysis, macroscopy and microscopy of thyroid, heart, lung, stomach, liver, spleen, adrenal gland, kidney, small intestine, bladder and treated skin.
Remark : Comparative acute toxicity of synthetic and natural glycerin.
No signs of local irritation after 90 days of application.
Result : AVERAGE SCORE
No signs of irritation (no specifications)
OTHER EFFECTS: no treatment related effects on the other parameters measured.
Test substance : CAS 56-81-5 (glycerine), purity 99.5% for synthetic and natural glycerine.
Reliability : (2) valid with restrictions
The information in the report is confined to the above.

25.01.2002

(64)

Species : rabbit
Concentration :

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ID: 56-81-5

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Exposure :
Exposure time :
Number of animals :
Vehicle :
PDII :
Result : slightly irritating
Classification :
Method : other
Year : 1986
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002

(87)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : .1 ml
Exposure time :
Comment :
Number of animals : 6
Vehicle :
Result : not irritating
Classification :
Method : other: Draize (1944)
Year : 1971
GLP : no data
Test substance : other TS

Method : TEST ANIMALS:
 - Sex: male
 - Weight at study initiation: ≥ 2 kg
 - Number of animals: 6

ADMINISTRATION/EXPOSURE

- Preparation of test substance: undiluted
 - Amount of substance instilled: 0.1 ml

EXAMINATIONS

- Scoring system: Draize
 - Observation at: 1, 24, 72 h and 7 days
 - Tool used to assess score: at 24, 72 h and 7 days fluorescein examination if no effects were seen at the previous examination

Result : The same method was used in 20 different laboratoria.
 : AVERAGE SCORE (24-h median)
 - Overall irritation score per laboratory: 0-2 (110 is maximum score)
Test substance : CAS 56-81-5 (glycerine), purity not indicated.
Reliability : (2) valid with restrictions
 The information in the report is confined to the above.

17.12.2001

(85)

Species : rabbit
Concentration : undiluted
Dose : .1 ml

Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other: Draize
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Method	:	TEST ANIMALS: - Strain: New Zealand White - Sex: female - Source: Keene Ridge Farms, Moriarty, NM - Weight at study initiation: 2-6 kg - Number of animals: 6 ADMINISTRATION/EXPOSURE - Preparation of test substance: undiluted - Amount of substance instilled: 0.1 ml EXAMINATIONS - Scoring system: Draize - Observation at: 1, 24, 48, 72 and 96 h - Tool used to assess score: fluorescein
Result	:	AVERAGE SCORE - Overall irritation score: 0.4 at 1 h, 0 at 24-96 h
Test substance	:	REVERSIBILITY: yes, within 24 h CAS 56-81-5 (glycerine), purity not indicated (Glycerol/Water mixture of unknown composition).
Conclusion	:	Not irritating.
Reliability	:	(4) not assignable
25.01.2002		Secondary literature; the information in the report is confined to the above. (72)
Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year	:	
GLP	:	yes
Test substance	:	no data
Remark	:	Rabbit strain: New Zealand White; sex: male. The conjunctiva was slightly to moderately irritated in all rabbits one hour after treatment. Thereafter the irritation diminished and had disappeared at 48 hours after treatment.
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.
Reliability	:	(4) not assignable
25.01.2002		The information in the report is confined to the above. (65)

Species : rabbit
Concentration :
Dose : .1 ml
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : not irritating
Classification :
Method : other
Year : 1953
GLP : no data
Test substance : other TS

Method : TEST ANIMALS:
 - Number of animals: 4

 ADMINISTRATION/EXPOSURE:
 - Amount of substance instilled: 0.1 mL
 - Vehicle: none
 - Postexposure period: 48 hours

EXAMINATIONS

- Scoring system: Draize (1944)
 - Observation times: 1, 24 and 48 hours
 - Tool used to assess score: fluorescein staining

Remark : Comparative acute toxicity of synthetic and natural glycerin.

Result : Irritation was observed with both synthetic and natural glycerol, but was absent at 24 and 48 hours.

Test substance : CAS 56-81-5 (glycerine), purity 99.5% for synthetic and natural glycerine.

Reliability : (2) valid with restrictions
 The information in the report is confined to the above.

17.12.2001

(64)

Species : rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : slightly irritating
Classification :
Method : other
Year : 1986
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.

Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable

25.01.2002

(87)

Species : rabbit
Concentration :
Dose :
Exposure time :

Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	no data
Remark	:	Literature could not be retrieved.
Source	:	Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(88)
Species	:	human
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	A strong burning and stinging sensation, with tear production but no injury apparently from contact with the neat chemical. Literature could not be retrieved.
Source	:	Unichema Chemie B.V. Gouda
Reliability	:	(4) not assignable
25.01.2002		(89)

5.3 SENSITIZATION

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	other TS: glycerol. No indication about purity.
Remark	:	Medicolegal aspects of occupational dermatitis survey in a foam rubber factory. Skin patch tests for 48 hours.
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated (unknown mixture of glycerine and water).
Reliability	:	(4) not assignable The information was confined to the above.

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ID: 56-81-5

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(90)

Type : other
Species : guinea pig
Number of animals : 12
Vehicle :
Result : not sensitizing
Classification :
Method : other
Year : 1953
GLP : no data
Test substance : other TS

Method : TEST ANIMALS:
 - Weight at study initiation: 350-429 g
 - Number of animals: 12/treatment
 ADMINISTRATION/EXPOSURE
 - Induction schedule: 10 times on alternate days
 - Concentrations used for induction: 0.1 mL of 0.1% solution of natural or synthetic glycerol in isotonic sodium chloride solution.
 - Challenge schedule: 2 weeks after last induction
 - Concentrations used for challenge: 0.05 mL of (presumably) 0.1% solution

Remark : EXAMINATIONS
 - Grading system: diameter, height and colour of the reaction compared to the reaction at the first sensitizing injection
 : Comparative acute toxicity of synthetic and natural glycerin.
 Following 10 injections of 0.1 ml of a 0.1% solution of natural or synthetic glycerin, the investigators noted that a further injection of 0.05 ml of the solutions gave no indications of sensitization to glycerin in any of the 24 adult white male guinea pigs.
Result : No response was observed to challenge for either synthetic or natural glycerol.
Test substance : CAS 56-81-5 (glycerine), purity 99.5% for natural and synthetic glycerine.
Conclusion : Most reliable study available.
Reliability : (4) not assignable
 The information in the report is confined to the above.

17.12.2001

(64)

Type : Patch-Test
Species : human
Number of animals :
Vehicle :
Result : not sensitizing
Classification :
Method : other
Year :
GLP : no data
Test substance : other TS: glycerol. No indication about purity.

Remark :
 Out of "several thousand" dermatitis patients who were tested, only two showed reactions in 20-24-hr covered patch tests with 50% glycerol. However, because the purity of the glycerol tested was not given, it is not excluded that the observed effects in two patients could have been caused by an impurity or a contamination of the sample (e.g. propylene glycol or butanetriol). With respect to the very high number of individuals tested and

5. TOXICITY

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	the concentration of the test compound (50%) one would expect to see more incidences, if glycerol had a relevant allergenic potential (Lit. Henkel, 1992).
	Literature could not be retrieved.
Source	: Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability 25.01.2002	: (4) not assignable (91)
Type	: Patch-Test
Species	: human
Number of animals	:
Vehicle	:
Result	: ambiguous
Classification	:
Method	: other
Year	: 1979
GLP	: no data
Test substance	: no data
Remark	: Literature could not be retrieved. Out of "several thousand" dermatitis patients tested, two showed skin reactions in 20 to 24-hr covered patch tests with 50% glycerol and were thus diagnosed as glycerol sensitized. One also reacted with 1% glycerol. Both had regularly used a skin cream containing 10% glycerol.
Source	: Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability 25.01.2002	: (4) not assignable (92) (93)

5.4 REPEATED DOSE TOXICITY

Type	:
Species	: rat
Sex	: male/female
Strain	: Sprague-Dawley
Route of admin.	: inhalation
Exposure period	: 14 days
Frequency of treatm.	: 5 days/week, 6 hours/day
Post exposure period	:
Doses	: 1000, 2000 and 4000 mg/m ³
Control group	: yes, concurrent no treatment
LOAEL	: = 1000 mg/m ³
Method	: other: not indicated
Year	: 1992
GLP	: no data
Test substance	:
Method	: TEST ORGANISMS - Age: not indicated - Weight at study initiation: not indicated - Number of animals: 10/sex/treatment - Source: Charles River Breeding Laboratories ADMINISTRATION / EXPOSURE - Exposure period: 14 days, 5 days/week, 6 hours/day (total 10 exposures) - Route of administration: nose only

- Doses: 0, 1000, 2000 and 4000 mg/m³ (calculated to be equivalent to oral doses 339, 678, 1355 mg/kg bw based on average body weight of 0.425 kg and 6 L/h respiratory volume)
- Particle size: MMAD <1.5 µm (respirable)
- Preparation of particles: viscous-liquid aerosol generator
- Air changes: not indicated

CLINICAL OBSERVATIONS AND FREQUENCY:

- Mortality/clinical signs: twice daily
- Body weight: at 2 or 3 days intervals
- Food consumption: weekly
- Haematology: not specified (complete blood count included)
- Biochemistry: blood urea nitrogen, creatinine, glucose, protein, albumin, albumin/globulin, ASAT, ALAT, LDH, gamma glutamyl transferase, cholesterol, triglycerides and phospholipids
- Urinalysis: not conducted

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

- Organ weights: lungs, liver, kidneys, brain and heart
- Macroscopic: not specified (complete necropsy)
- Microscopic: respiratory tract (with associated lymphnodes) and gross lesions (all animals); liver, kidneys, heart of control and high dose animals; lungs trachea and anterior nasal cavity were stained with hematoxylin and eosin and duplicate slides with Alcian blue/periodic acid Schiff (Goblet cell changes)

ANALYSES: target concentration and homogeneity of aerosol

- Method: sampling with aerosol monitor and gravimetric and GC analyses
- Sampling times: actual concentration 2 samples per exposure chamber; homogeneity and uniformity in mock exposure before start of the experiment (6 samples) and during animal exposure (10 samples/concentration)

Result

STATISTICAL METHODS: ANOVA, least significant difference

: ANALYSES:

- Actual dose level: 1000, 1930 and 3910 mg/m³ (98-100% of target)
- Stability: not reported
- Homogeneity (uniformity): relative standard deviation 1.6-2.5% of mean value

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Mortality: 2 males at 1000 mg/m³ and 1 male and 1 female at 2000 mg/m³
- Clinical signs: no treatment related effects
- Body weight gain: decreased in males and females at all concentrations (58-28% in females)
- Food consumption: no treatment related effects
- Clinical chemistry: glucose decreased in females at all concentrations (28-19%)
- Haematology: no treatment related effects
- Organ weights: no treatment related effects
- Gross pathology: no treatment related effects
- Histopathology: minimal to midl squamous metaplasia of the epiglottis in males and females at 0, 1000, 1930 and 3910 mg/m³ (1/10, 13/18, 16/19 and 13/14, respectively). Although a dose-related increase in the frequency of squamous metaplasia was not apparent, the frequency of mild metaplasia was greatest at the top dose (7 animals with minimal and 6 with

	mild).	
	STATISTICAL RESULTS: all effects mentioned showed statistical significance, except the decreased weight gain in males	
Test substance	: CAS 56-81-5 (glycerine), purity >99.8%	
Conclusion	: LOAEL 1000 mg/m ³ based on local effects on the epithelium of the upper respiratory tract. Most reliable study. Most reliable study available.	
Reliability	: (2) valid with restrictions 1 The report is limited to the above mentioned. No individual values were included. 2 The effect on body weight can be attributed to stress due to the nose only exposure and is therefore considered not related to exposure to the test substance 3 The effects on glucose were seen in females only and showed no relationship with concentration. Although a relationship between glycerol exposure and glucose in serum can not be fully excluded, the biological relevance of this effect is considered of minor importance.	
Flag 25.01.2002	: Critical study for SIDS endpoint	(73) (94)
Type	:	
Species	: rat	
Sex	: male/female	
Strain	: Sprague-Dawley	
Route of admin.	: inhalation	
Exposure period	: 13 weeks	
Frequency of treatm.	: 5 days/week, 6 hours/day	
Post exposure period	:	
Doses	: 33, 165 and 660mg/m ³	
Control group	: yes, concurrent no treatment	
NOAEL	: = 167 mg/m ³	
Method	: other: not indicated	
Year	: 1992	
GLP	: no data	
Test substance	:	
Method	: TEST ORGANISMS - Age: not indicated - Weight at study initiation: not indicated - Number of animals: 15/sex/treatment - Source: Charles River Breeding Laboratories	
	ADMINISTRATION / EXPOSURE - Exposure period: 13 weeks, 5 days/week, 6 hours/day - Route of administration: nose only - Doses: 0, 33, 165, 660 mg/m ³ (calculated to be equivalent to oral doses of 11.2, 55.9 and 224 mg/kg bw based on average body weight of 0.425 kg and 6 L/h respiratory volume) - Particle size: MMAD <2.0 µm (respirable) - Preparation of particles: viscous-liquid aerosol generator - Air changes: not indicated	
	CLINICAL OBSERVATIONS AND FREQUENCY: - Mortality/clinical signs: twice daily - Body weight: weekly - Food consumption: weekly	

Result

- Haematology: not specified (complete blood count included)
- Biochemistry: blood urea nitrogen, creatinine, glucose, protein, albumin, albumin/globulin, ASAT, ALAT, LDH, gamma glutamyl transferase, cholesterol, triglycerides and phospholipids

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

- Organ weights: lungs, liver, kidneys, brain and heart
- Macroscopic: not specified (complete necropsy)
- Microscopic: total of 40 tissues of high dose and control animals; lungs trachea and anterior nasal cavity were stained with hematoxylin and eosin and duplicate slides with Alcian blue/periodic acid Schiff (Goblet cell changes)

Three rats/sex of control and high dose group were killed during week 10, lung lobes were excised and 2 samples/rat were examined by transmission electron microscopy for abnormalities associated with the Clara cells. The same procedure was followed for 3 rats/sex of all groups during terminal necropsy

ANALYSES: target concentration and homogeneity of aerosol

- Method: sampling with aerosol monitor and gravimetric and GC analyses
- Sampling times: actual concentration 2 samples per exposure chamber; homogeneity and uniformity in mock exposure before start of the experiment (6 samples) and during animal exposure (20 samples/concentration)

STATISTICAL METHODS: ANOVA, least significant difference

: ANALYSES:

- Actual dose level: 33, 167 and 662 mg/m³ (100-101% of target)
- Stability: not reported
- Homogeneity (uniformity): relative standard deviation <1% of mean value

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Mortality: none
- Clinical signs: no treatment related effects
- Body weight gain/food consumption: no treatment related effects
- Clinical chemistry: triglycerides decreased in males at 33 (34%) and 167 mg/m³ (22%) only.
- Haematology: no treatment related effects
- Organ weights: no treatment related effects
- Gross pathology: no treatment related effects
- Histopathology: minimal squamous metaplasia of the epiglottis in 2/25, 1/19, 4/20 and 10/21 rats at 0, 33, 167 and 662 mg/m³; 1 male at 662 mg/m³ showed mild squamous metaplasia.

No differences in morphology of the Clara cells in control and high dose rats

STATISTICAL RESULTS: all effects mentioned showed statistical significance (squamous metaplasia only significant at high concentration)

**Test substance
Conclusion**

: CAS 56-81-5 (glycerine), purity >99.8%
: NOAEL 167 mg/m³ based on local irritant effects on the upper respiratory tract.
Most reliable study.

Reliability	: Most reliable study available. (2) valid with restrictions 1 The report is limited to the above mentioned. No individual values were included. 2 The effects on triglycerides were seen in males only and showed no relationship with concentration. Although a relationship between glycerol exposure and triglycerides in serum can not be fully excluded, the biological relevance of this effect is considered of minor importance.
Flag 25.01.2002	: Critical study for SIDS endpoint (73) (94)
Type	:
Species	: rat
Sex	: male/female
Strain	: Long-Evans
Route of admin.	: oral feed
Exposure period	: 2 year
Frequency of treatm.	:
Post exposure period	:
Doses	: 5, 10 and 20% in diet calculated to be equivalent to doses of: males 2000, 4000 and 8000 mg/kg bw, females 2500, 5000 and 10000 mg/kg bw
Control group	:
Method	: other: not indicated
Year	:
GLP	:
Test substance	:
Method	: TEST ORGANISMS - Age: not indicated - Weight at study initiation: 96-109 g (males), 92-108 g (females) - Number of animals: 22/sex/treatment, 26/sex for controls - Source: Institute of Experimental Biology of University of California ADMINISTRATION / EXPOSURE - Exposure period: 2 year (1 year for the high dose group) - Route of administration: oral in diet - Doses: 5, 10 and 20% in diet; males 2000, 4000 and 8000 mg/kg bw, females 2500, 5000 and 10000 mg/kg bw CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: daily in cage and weekly examination outside the home cage - Mortality: daily - Body weight: weekly - Food consumption: weekly - Haematology: erythrocyte and leucocyte count and haemoglobin after 3, 6, 12, 18 and 24 months - Urinalysis: albumin, glucose, casts and red and white blood cells after 3, 6, 12, 18 and 24 months (24-48 urine collection) ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weights: liver, kidneys, heart, spleen and lungs - Macroscopic: no details provided - Microscopic: liver, spleen, adrenals, kidney, small intestine, gonads and urinary bladder OTHER EXAMINATIONS: glycogen and lipid content of the liver of

	surviving rats at 0 and 20% glycerol.
	ANALYSES: not performed
	STATISTICAL METHODS: Chi-square test, student t-test, ANOVA (Fisher)
Result	: TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: not indicated - Clinical signs: not reported - Body weight gain: no statistically significant differences between treated and control animals - Food consumption: slightly increased (significant) in males at 5 and 10% natural glycerin - Haematology: no treatment related effects - Urinalysis: albumin: no significant treatment related effects (92% incidence in females at 20% natural glycerin compared to 54-64% in controls); glucose, casts, red and white blood cells: no treatment related effects - Organ weights: incidental increases and decreases were reported without apparent relationship to treatment - Gross pathology: no lesions related to treatment. - Histopathology: Incidental bronchiectasis, pneumonia, pulmonary abscesses, taenia infestation of the liver, hydronephrosis and pyelonephritis (total 27 rats were affected). - Other: liver glycogen and lipid did not significantly differ between 0 and 20% glycerin (liver glycogen natural glycerin 4.2-4.3% and synthetic glycerin 3.7-4.2%)
Test substance	: CAS 56-81-5 (glycerine), Natural glycerine, achieved from market stock, purity not indicated (impurities were fatty acids and esters) Synthetic glycerin, purity 99.5% (rest mainly water with very small amounts of glycerin polymers and glyceraldehyde)
Conclusion	: NOAEL 10000 mg/kg bw based on the absence of treatment related effects in high dose animals. Most reliable study. Most reliable study available.
Reliability	: (2) valid with restrictions 1 The report was confined to the above. 2 No individual data were included and microscopy was performed on distinct organs only.
Flag 25.01.2002	: Critical study for SIDS endpoint (64)
Type	:
Species	: rat
Sex	: male
Strain	: other: Carworth
Route of admin.	: oral feed
Exposure period	: 4 weeks
Frequency of treatm.	:
Post exposure period	:
Doses	: 20% glycerine in diet (calculated to be equivalent to oral doses of 8824 mg/kg bw/day based on average bodyweight of 0.425 kg and food intake of 18.75 g/d)
Control group	: no data specified
Method	: other: not indicated
Year	:
GLP	: no data
Test substance	: other TS

Method : At the end of 4 weeks, 5 rats of each treatment were killed, liver, lipids and cholesterol were determined and epididymus fat pads were quantitatively excised and weighed.

Result : Mentioned criteria, as well as weight gain and food intake (efficiency) revealed no adverse effects attributable to glycerin consumption.

Test substance : CAS 56-81-5 (glycerine), purity not indicated.

Reliability : (4) not assignable
1. Secondary literature, the information is confined to the above.
2. In comparison with the control diet less glucose monohydrate, sucrose and dextrin and more cellulose was present in the 20% glycerol diet.

16.11.2001 (95)

Type :
Species : rat
Sex : no data
Strain :
Route of admin. : oral feed
Exposure period : 8 weeks
Frequency of treatm. :
Post exposure period :
Doses : 12 g/10 g diet (calculated to be equivalent to 52941 mg/kg bw based on 18.75 g/d food intake and average bodyweight of 0.425 kg)

Control group :
Method :
Year :
GLP :
Test substance : other TS

Method : Before the start of the experiment 10 rats received a minimum diet (sufficient to maintain their weight) during 5 weeks. Thereafter a supplement of 12 g glycerine/10 g diet was added to the diet of 5 of the rats during 8 weeks, while the other 5 rats remained on the original diet. After these 8 weeks groups were switched.

Result : Untreated animals (receiving no glycerine) showed weight loss, while animals receiving glycerine exhibited normal growth.

Test substance : CAS 56-81-5 (glycerine), purity not indicated.

Reliability : (4) not assignable
The information was confined to the above.

11.12.2001 (65)

Type :
Species : rat
Sex : no data
Strain :
Route of admin. : oral feed
Exposure period : 25 weeks
Frequency of treatm. :
Post exposure period :
Doses : 20 and 41% in diet (calculated to be equivalent to 8824 and 18088 mg/kg bw /day based on 18.75 g/d food intake and average bodyweight of 0.425 kg)

Control group : yes, concurrent no treatment
Method :
Year :
GLP :
Test substance : other TS

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Method	:	30 rats received a glycerine-supplemented diet (20 or 41%) during 25 weeks. After this period rats were necropsied. Kidneys, liver and intestines were examined.	
Result	:	No abnormalities, normal growth rate.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
		The information was confined to the above.	
11.12.2001			(65)
Type	:		
Species	:	rat	
Sex	:	male/female	
Strain	:		
Route of admin.	:	oral feed	
Exposure period	:	20 weeks	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	1, 3, 6, 10, 15, 20, 30, 40, 50 and 60% in diet (calculated to be equivalent to oral doses of 441, 1324, 2647, 4412, 6618, 8824, 13235, 17647, 22059 and 26471 mg/kg bw/day based on 18.75 g/d food intake and average bodyweight of 0.425 kg)	
Control group	:	yes, concurrent no treatment	
Method	:		
Year	:		
GLP	:		
Test substance	:	other TS	
Method	:	5 rats/sex/treatment received glycerine in their diet during 20 weeks. Animals were observed daily and body weight was recorded weekly. Hb was determined at regular intervals. Selected animals from each group were tested for effects on exercise, water intake and urinary output. All animals that died and a selection of the survivors were necropsied. Another selection was sacrificed and investigated for effects on dry matter, fat and liver glycogen.	
Result	:	Decreased growth rate at 40% and above. Pathological effects at 10% and above (dose related) consisting mainly of hydropic and fatty degeneration of liver parenchymous cells.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
		1 The information was confined to the above.	
		2 During the first weeks dead animals were replaced by additional animals.	
11.12.2001			(65)
Type	:		
Species	:	rat	
Sex	:	no data	
Strain	:		
Route of admin.	:	oral feed	
Exposure period	:	40 weeks	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	20, 41 and 61% in diet (calculated to be equivalent to oral doses of 8824, 18088 and 26912 mg/kg bw/day based on 18.75 g/d food intake and average bodyweight of 0.425 kg)	
Control group	:	yes, concurrent no treatment	
Method	:		
Year	:		

GLP	:		
Test substance	:	other TS	
Method	:	Glycerine replaced the starch in the diet.	
Result	:	Decreased activity, unkept coat and impaired growth at 61%. Increased water consumption at 41% (30%) and 61% (230%)-> dose related	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable The information was confined to the above.	
16.11.2001			(65)
Type	:		
Species	:	rat	
Sex	:	male/female	
Strain	:		
Route of admin.	:	oral feed	
Exposure period	:	1 year	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	5, 10, 20% in diet (calculated to be equivalent to oral doses of 2206, 4412 and 8824 mg/kg bw day based on 18.75 g/d food intake and average bodyweight of 0.425 kg)	
Control group	:	yes, concurrent no treatment	
Method	:		
Year	:		
GLP	:		
Test substance	:	other TS	
Method	:	63 rats/sex/treatment received the test substance for 1 year. Data were collected on growth, food intake and caloric efficiency. Clinical investigations on blood and urine were performed. Macroscopic and microscopic examination of all animals at 20% was performed. For the other treatment groups liver, kidneys, spleen, gonads and adrenals were investigated.	
Result	:	No adverse effects.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable The information was confined to the above.	
11.12.2001			(65)
Type	:		
Species	:	rat	
Sex	:		
Strain	:	Wistar	
Route of admin.	:	oral feed	
Exposure period	:	20 days	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:		
Control group	:		
Remark	:	Literature could not be retrieved.	
Result	:	Rats were fed a glycerol containing diet over a period of 20 days. Two control groups were included one receiving normal laboratory diet ad libitum and a pair-fed control (on normal diet). Body weight gain was reduced in glycerol fed animals. Liver weight was slightly increased compared to controls and kidney weight was strongly increased. Liver and kidney enzymatic activity was high in glycerol fed rats. At the end of the	

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	experiment 95% of the glycerol was retained and blood and liver glucose were above normal levels. Water intake and urine production were about 5 times higher in animals treated with glycerol compared to controls.	
Test substance	: CAS 56-81-5 (glycerine), incorporated in diet replacing the carbohydrate of the standard diet	
Reliability 25.01.2002	: (4) not assignable	(96)
Type	:	
Species	: rat	
Sex	: male/female	
Strain	:	
Route of admin.	: oral feed	
Exposure period	: 2 years	
Frequency of treatm.	:	
Post exposure period	:	
Doses	: diets containing 5, 10, or 20% glycerin (=calculated to be equivalent to 2206, 4412 and 8824 mg/kg bw/day based on 18.75 g/d food intake and average bodyweight of 0.425 kg)	
Control group	: yes	
Method	: other	
Year	:	
GLP	: no data	
Test substance	: other TS	
Remark	: Literature could not be retrieved.	
Result	: No deleterious or toxicological effects were noted among the groups of rats fed for two years on diets containing 5, 10 or 20% of either Atlas synthetic glycerin or natural glycerin, or among either of these experimental groups and the controls.	
Source	: Simel S.p.A. Industria Chimica Cremona EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Test substance	: CAS 56-81-5 (glycerine), ATLAS synthetic glycerin and natural glycerin.	
Reliability 25.01.2002	: (4) not assignable	(97)
Type	:	
Species	: rat	
Sex	: male/female	
Strain	:	
Route of admin.	: drinking water	
Exposure period	: 3 months	
Frequency of treatm.	:	
Post exposure period	:	
Doses	: 1, 2, 5, 10 or 20% solution of glycerin (calculated to be equivalent to 667, 1334, 3335, 6670 and 13340 mg/kg bw/day based on density of 1.26 g/cm ³ , water intake of 22.5 ml/d and average bodyweight of 0.425 kg)	
Control group	: yes	
Method	: other	
Year	:	
GLP	: no data	
Test substance	: no data	
Result	: Two of the 12 rats receiving 20% glycerin died during the 6th week of the test; the others showed, at this point, disturbance of the development and growth but recovered and grew normally in the further course of the experiment such that at the end of the test period no indication of injury attributable to glycerin ingestion was perceivable.	

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Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
	:	The information in the report is confined to the above.	
16.11.2001			(68) (65) (69)
Type	:		
Species	:	Rat	
Sex	:	no data	
Strain	:		
Route of admin.	:	drinking water	
Exposure period	:	10 and 17 days	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	2.5 % in drinking water (calculated to be equivalent to oral doses of 1668 mg/kg bw /day based on density of 1.26 g/cm ³ , water intake of 22.5 ml/d and average bodyweight of 0.425 kg)	
Control group	:	other: see method	
Method	:	Control animals received 2.5% glucose solution as drinking water.	
Result	:	No effects on body and liver weight. Significant decrease of cholesterol synthesis and serum cholesterol.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Conclusion	:	Absence of effects on cholesterol synthesis are attributed to an extraordinarily wide within group variance by the author of the report.	
Reliability	:	(4) not assignable	
	:	The information in the report is confined to the above.	
12.12.2001			(65)
Type	:		
Species	:	Rat	
Sex	:	no data	
Strain	:		
Route of admin.	:	drinking water	
Exposure period	:	10 days	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	1.5 and 3% in drinking water (calculated to be equivalent to oral doses of 1000 and 2000 mg/kg bw/day based on density of 1.26 g/cm ³ , water intake of 22.5 ml/d and average bodyweight of 0.425 kg)	
Control group	:	no data specified	
Result	:	Decreased serum cholesterol levels compared with controls.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	:	(4) not assignable	
	:	The information in the report is confined to the above.	
16.11.2001			(65)
Type	:		
Species	:	Rat	
Sex	:	Female	
Strain	:		
Route of admin.	:	drinking water	
Exposure period	:	6 months	
Frequency of treatm.	:		
Post exposure period	:		
Doses	:	5% in drinking water (calculated to be equivalent to oral doses of 3335 mg/kg bw/day based on density of 1.26 g/cm ³ , water intake of 22.5 ml/d and average bodyweight of 0.425 kg)	

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Control group	:	other: tap water
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS
Method	:	5 females (6-8 weeks old) were given natural or synthetic glycerin during 6 months. Animals were weighed weekly. Haematological parameters and Hb were determined monthly. Macroscopic and microscopic investigations were done on heart, lungs, liver, spleen, stomach, intestines, kidneys, thymus, thyroid and adrenals.
Result	:	No effects on growth, red blood cells and haemoglobin. White blood cell counts differed within groups during the study. This was attributed to regular blood sampling procedures. Macroscopic incidental findings were a small thymus in 2 animals and slight interstitial pneumonia in one on natural glycerine and small spleen (with small lymphnodes and moderate hemosiderin deposits) and thymus atrophy in one animal that died on synthetic glycerol. Calcified masses in kidney tubulus between cortex and medulla in 3/5 rats on natural glycerin and 3/5 rats on synthetic glycerin.
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated.
Reliability	:	(4) not assignable The information in the report is confined to the above.
11.12.2001		(73) (65)
Type	:	
Species	:	Rat
Sex	:	male/female
Strain	:	
Route of admin.	:	drinking water
Exposure period	:	96 days
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	0, 1, 2, 5, 10 or 20% aqueous glycerin solution (calculated to be equivalent to oral doses of 667, 1334, 3335, 6670 and 13340 mg/kg bw/day based on density of 1.26 g/cm ³ , water intake of 22.5 ml/d and average bodyweight of 0.425 kg)
Control group	:	Yes
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	Literature could not be retrieved.
Result	:	Growth of the rats receiving 10% or less of glycerin was normal. The animals receiving 20% glycerin exhibited normal growth for the first few weeks, followed by a temporary impairment. Growth of these rats then returned to normal.
Source	:	Simel S.p.A. Industria Chimica Cremona EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(98)
Type	:	
Species	:	Rat
Sex	:	male/female
Strain	:	

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Route of admin. : Gavage
Exposure period : approx. 1/2 year
Frequency of treatm. :
Post exposure period :
Doses : 1.0 ml of a 50% aqueous glycerine solution/100 g bw (equivalent to 6300 mg/kg bw/day based on density of 1.26 g/cm³)
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : other TS

Result : All of the rats developed normally and the mortality of all groups was comparable.
 After 6, 12 and 18 weeks, rats from each group were sacrificed, dissected and their organs removed for histological examination. No pathological changes were disclosed and determination of liver glycogen revealed normal levels. X-ray examinations carried out on animals of each group at the beginning as well as in intervals of 6 weeks, remained without abnormal findings.

Test substance : CAS 56-81-5 (glycerine), purity not indicated.

Reliability : (4) not assignable
 The information in the report is confined to the above.

11.12.2001

(65) (69)

Type :
Species : Rat
Sex : male/female
Strain :
Route of admin. : gavage
Exposure period : 50 days
Frequency of treatm. : on weekdays
Post exposure period :
Doses : 10 ml/kg bw (= 2520 mg/kg bw based on density of 1.26 g/cm³)
Control group : no data specified
Method :
Year :
GLP :
Test substance : other TS

Method : Observations consisted of mortality, body weight gain, behaviour, liver glycogen.
 Post mortem examination was done on the endocrine glands, pituitary, adrenal and ovaries. The skeleton was investigated by X-rays.

Result : No abnormal effects were found.

Test substance : CAS 56-81-5 (glycerine), 20% aqueous glycerine solution.

Reliability : (4) not assignable
 The information in the report is confined to the above.

11.12.2001

(65) (69)

Type :
Species : Rat
Sex : male
Strain :
Route of admin. : gavage
Exposure period : 44 days
Frequency of treatm. : daily
Post exposure period :

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Doses : 1, 5, 10 and 20% in water (equivalent to 115, 575, 1150 and 2300 mg/kg bw based on density 1.26 and mean animal weight of 110 g)

Control group : yes, concurrent vehicle

Method : other: not indicated

Year :

GLP :

Test substance :

Method : TEST ORGANISMS
 - Age: not indicated
 - Weight at study initiation: 75-110 g
 - Number of animals: 20/treatment

ADMINISTRATION / EXPOSURE

- Exposure period: 44 days
 - Route of administration: oral (gavage)
 - Doses: 1, 5, 10 and 20% in water (dosing volume 1 mL); about 115, 575, 1150 and 2300 mg/kg bw (based on density 1.26 and mean animal weight of 110 g)

CLINICAL OBSERVATIONS AND FREQUENCY:

Not indicated

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

- Macroscopic: no details provided
 - Microscopic: liver, kidney and urinary bladder

ANALYSES: not performed

Result : STATISTICAL METHODS: not indicated
 : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
 - Mortality: 15% in treatment and control groups
 - Clinical signs: no treatment related effects
 - Body weight gain: no differences between treated and control animals
 - Histopathology: no findings

Test substance : CAS 56-81-5 (glycerine), in water, purity not indicated

Conclusion : The data are insufficient to draw a sound conclusion on a NOAEL. No effects were found at doses between 115 and 2300 mg/kg.

Reliability : (4) not assignable
 1 The report is limited to the above mentioned
 2 It is not clear whether or not no histopathological findings were observed in the glycerol treated animals, since the report is in fact a study on 1,3-butyleneglycol, using glycerol as controls.

11.12.2001

(99)

Type :

Species : Rat

Sex : male

Strain :

Route of admin. : gavage

Exposure period : 21 days

Frequency of treatm. : daily

Post exposure period :

Doses : 20% in water (equivalent to 1525 mg/kg bw based on density 1.26 and mean animal weight of 165 g)

Control group : yes, concurrent vehicle

Method : other: not indicated
Year :
GLP :
Test substance :

Method : TEST ORGANISMS
 - Age: not indicated
 - Weight at study initiation: 140-160g
 - Number of animals: 8/treatment

ADMINISTRATION / EXPOSURE

- Exposure period: 21 days
 - Route of administration: oral (gavage)
 - Doses: 20% in water (dosing volume 1 mL); ~1525 mg/kg bw (based on density 1.26 and mean animal weight of 165 g)

OBSERVATIONS:

- Mortality: frequency not indicated
 - Body weight on day 0, 3, 6, 9, 12, 15, 18 and 21
 - O₂-consumption on day 0, 3, 6, 9, 12, 15, 18 and 21 (by method of Loeser (1938))

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
 - Mortality: 0/8 controls, 5/8 treated
 - Body weight gain: no differences between treated and control animals

Test substance : CAS 56-81-5 (glycerine), in water, purity not indicated
Reliability : (4) not assignable

1 The report is limited to the above mentioned. The test was set up for the determination of O₂-consumption
 2 The decreased O₂-consumption on day 18 and 21 is considered less reliable, since measurements were performed on only 3 survivors.

11.12.2001

(99)

Type :
Species : rat
Sex : male
Strain :
Route of admin. : gavage
Exposure period : 44 days
Frequency of treatm. : daily
Post exposure period :
Doses : 10% in water (1260 mg/kg bw based on density 1.26)
Control group : yes, concurrent vehicle
Method : other: not indicated
Year :
GLP :
Test substance :

Method : TEST ORGANISMS
 - Age: not indicated
 - Weight at study initiation: 75-100 g
 - Number of animals: 10/treatment

ADMINISTRATION / EXPOSURE

- Exposure period: 44 days
 - Route of administration: oral (gavage)
 - Doses: 10% in water (dosing volume 1 cm³/100 g bw); 1260 mg/kg bw (based on density 1.26)

	OBSERVATIONS: - Haematology on day 2 and weekly thereafter (Hb, erythrocytes, total and differential leucocytes)	
Result	: TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Eosinophils increased during week 3 to 6 (attributed to an infection with worms)	
Test substance	: CAS 56-81-5 (glycerine), in water, purity not indicated	
Reliability	: (4) not assignable The report is limited to the above mentioned. The test was set up to determine blood parameters.	
11.12.2001		(99)
Type	:	
Species	: rat	
Sex	: female	
Strain	:	
Route of admin.	: gavage	
Exposure period	: 3 days	
Frequency of treatm.	: 3 times/day	
Post exposure period	:	
Doses	: 950, 1900 and 3800 mg/kg bw	
Control group	: yes	
LOAEL	: = 950 mg/kg bw	
Method	: other: not indicated	
Year	:	
GLP	:	
Test substance	:	
Method	: TEST ORGANISMS: - Source: Charles River - Age: not indicated - Number: 10/treatment (20 for controls) - Weight at study initiation: 150-210 g	
	ADMINISTRATION: - Doses: 950, 1900 and 3800 mg/kg bw (100, 80, 60, 40, 20%)- Vehicle water - Controls: water - Dosing schedule: 3 times daily (sacrificed after second dose on day 3)	
Result	: OBSERVATIONS: gastro-intestinal irritation, macroscopic and microscopic investigation of stomach and intestinal mucosa In animals treated with undiluted test substance a dose related increase in the number of animals showing hyperaemia, petechial haemorrhage and erosions was seen. Dilution of the applied test substance lead to reduction of the effects.	
Test substance	: CAS 56-81-5 (glycerine)(concentration in water 100%, 80%, 60%, 40% and 20%), purity not specified	
Conclusion	: LOAEL for local irritant effects 950 mg/kg bw	
Reliability	: (4) not assignable 1 The report is limited to the above mentioned. 2 Only effects on the gastrointestinal tract were reported.	
11.12.2001		(100)
Type	:	
Species	: rat	
Sex	: no data	

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Strain : no data
Route of admin. : oral unspecified
Exposure period : 25 Weeks
Frequency of treatm. : with each meal
Post exposure period : no data
Doses : Approximately 20000 mg/kg bw/d
Control group : no data specified
NOAEL : ca. 2000 mg/kg bw
Method : other
Year : 1933
GLP : no data
Test substance : no data

Remark : Literature could not be retrieved.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002

Type :
Species : rabbit
Sex :
Strain :
Route of admin. : other: see remark
Exposure period : 30-40 days
Frequency of treatm. :
Post exposure period :
Doses :
Control group :
Method : other
Year :
GLP : no data
Test substance : other TS

Method : 4 Rabbits were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.

Remark : Literature could not be retrieved.
Result : Doses of 10 ml of the glycerin solution daily were well tolerated by the rabbits. Those animals which were autopsied at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell cholesterol levels showed any consistent changes which could be attributed to the intake of glycerin.

Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable

(101)

Type :
Species : dog
Sex : male/female
Strain :
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. :
Post exposure period :
Doses : diets containing 5, 10 and 20% glycerin (no reference values available for conversion to mg/kg)
Control group : yes

Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	These studies were conducted to investigate the oral toxicity of Atlas synthetic glycerin compared to natural glycerin. Literature could not be retrieved.
Result	:	The observations failed to reveal any treatment effects from either the Atlas synthetic or the natural glycerin.
Source	:	Unichema Chemie B.V. Gouda
Test substance	:	Atlas synthetic glycerin and natural glycerin.
Reliability	:	(4) not assignable
25.01.2002		(97) (65)
Type	:	
Species	:	dog
Sex	:	no data
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	50 weeks
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	35% in diet (no reference values available for conversion to mg/kg)
Control group	:	
Method	:	other: not indicated
Year	:	
GLP	:	
Test substance	:	
Method	:	TEST ORGANISMS - Age: 5 weeks - Weight at study initiation: ~1.5 kg - Number of animals: 3/treatment (controls were litter mates) ADMINISTRATION / EXPOSURE - Exposure period: 50 weeks - Route of administration: in diet - Doses: 35% in diet (after week 36 intake was reduced to 50-80% of previous) OBSERVATIONS: - Body weight (frequency not indicated) - Erythrocyte counts (frequency not indicated)
Result	:	- Body weight: until week 36 no differences between animals on glycerol rich diet and controls; after week 36 weight loss (16%, 1.8 kg) in dogs on glycerol rich diet (not in controls) - Erythrocyte counts did not reveal any differences between dogs of both groups.
Test substance	:	CAS 56-81-5 (glycerine), purity not specified The test diet contained 35% glycerol and 17% carbohydrate; controls received a diet containing 52% carbohydrate
Conclusion	:	No conclusion can be drawn, because of the limited data available
Reliability	:	(3) invalid 1 The report is limited to the above mentioned 2 The actual dose received by the dogs could not be

	calculated by the reviewer, because no information on the dog's body weight and the amount of food provided was included.	
11.12.2001		(65) (102)
Type	:	
Species	:	dog
Sex	:	male/female
Strain	:	other: Mongrel
Route of admin.	:	gavage
Exposure period	:	3 days
Frequency of treatm.	:	3 times/day
Post exposure period	:	
Doses	:	950, 1900 and 3800 mg/kg bw
Control group	:	
NOAEL	:	= 950 mg/kg bw
Method	:	other: not indicated
Year	:	
GLP	:	no
Test substance	:	
Method	:	TEST ORGANISMS: - Age: not indicated - Number: 1/treatment (2 at highest dose) - Weight at study initiation: 8.9-16 kg ADMINISTRATION: - Doses: 950, 1900 and 3800 mg/kg bw (undiluted) - Controls: water - Dosing schedule: 3 times daily (sacrificed after second dose on day 3) OBSERVATIONS: gastro-intestinal irritation, macroscopic and microscopic investigation of stomach and intestinal mucosa
Result	:	At 950 mg/kg bw: no abnormalities. At 1900 mg/kg bw: the mucosa was severely hyperaemic with petechial haemorrhages At 3800 mg/kg bw: the stomach mucosa was (slightly to) severely hyperaemic with areas with petechial haemorrhages or erosions; duodenum appeared normal or with hyperaemic areas.
Test substance	:	CAS 56-81-5 (glycerine) undiluted, purity not specified
Conclusion	:	NOAEL for local irritant effects 950 mg/kg bw
Reliability	:	(4) not assignable 1 The report is limited to the above mentioned. 2 Only effects on the gastrointestinal tract were reported.
11.12.2001		(103)
Type	:	
Species	:	dog
Sex	:	male/female
Strain	:	
Route of admin.	:	oral unspecified
Exposure period	:	4 months
Frequency of treatm.	:	twice weekly
Post exposure period	:	
Doses	:	2.0 ml/kg (= 2520 mg/kg based on density of 1.26 g/cm ³)
Control group	:	
Method	:	One animal/sex/treatment received glycerine during 4 months (after 1 months there was a two-week interruption).

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Examinations included growth, weekly urine tests and histological examination of the liver, kidney, spleen, bladder, stomach, intestines, spleen, adrenal, heart, muscle and lungs.

Remark : No information on the formulation administered was provided.

Result : No abnormalities were observed.

Test substance : CAS 56-81-5 (glycerine), purity not indicated.

Reliability : (4) not assignable

The information in the report is confined to the above.

11.12.2001 (65)

Type :
Species : guinea pig
Sex : no data
Strain :
Route of admin. : other: see method
Exposure period : 30-40 days
Frequency of treatm. :
Post exposure period :
Doses :
Control group :
Method : other
Year :
GLP : no data
Test substance : other TS

Method : 10 Guinea pigs were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.

Remark : Literature could not be retrieved.

Result : Guinea pigs receiving more than 5 ml of a 50% glycerin solution (= 6300 mg/kg bw/day based on density of 1.26 g/cm³ and average bodyweight of 500g) daily by stomach tube died with acute symptoms.

Autopsies performed on animals at termination of the experiment revealed no pathological changes. The plasma cholesterol level showed no consistent changes attributable to the intake of glycerin while the red blood count of the guinea pigs (2 exposed via stomach tube, 1 exposed via drinking water) receiving glycerin dropped during the experiment indicating a probable anemic effect of glycerin in the guinea pig.

Source : Unichema Chemie B.V. Gouda

Test substance : CAS 56-81-5 (glycerine), purity not indicated.

11.12.2001 (104)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test

System of testing : TA1535, TA1537, TA98 and TA100

Test concentration : 100, 333.3, 1000, 3333, 10000 µg/plate

Cycotoxic concentr. : >= 10000 µg/plate

Metabolic activation : with and without

Result : negative

Method : other: not indicated

Year : 1983

GLP : no data

Test substance :

Method : SYSTEM OF TESTING

	- Species/cell type: TA1535, TA1537, TA98 and TA100 - Metabolic activation system: liver S9 fraction from rats and hamsters treated with Aroclor 1254 - Deficiency: histidine
	ADMINISTRATION: - Dosing: 100, 333.3, 1000, 3333, 10000 µg/plate - Number of replicates: 3 - Application: preincubation assay - Positive controls: 2-aminoanthracene (all strains with S9); 4-nitro-o-phenylenediamine (TA98 without S9); sodium azide (TA100 and TA1535 without S9); 9-aminoacridine (TA1537 without S9) - Negative control: water - Pre-incubation time: 20 min
	CRITERIA FOR EVALUATING RESULTS: - Statistical method: Margolin (1981) if result is positive
Remark	: 1. The test was performed by three different labs. These are the results of the first lab.
	: 2. The test was performed twice and gave exactly the same results.
Result	: GENOTOXIC EFFECTS:
	- With metabolic activation(rat): negative
	- With metabolic activation(hamster): negative
	- Without metabolic activation: negative
	CYTOTOXIC CONCENTRATION: - With or without metabolic activation: >= 10000 µg/plate
Test substance	: CAS 56-81-5 (Glycerine), purity >99%.
Conclusion	: Most reliable study available
Reliability	: (2) valid with restrictions
	Non-GLP study.
Flag	: Critical study for SIDS endpoint
17.12.2001	(105)
Type	: Ames test
System of testing	: TA1535, TA1537, TA98 and TA100
Test concentration	: 100, 333, 1000, 3333, 10000 µg/plate
Cycotoxic concentr.	: >= 10000 µg/plate
Metabolic activation	: with and without
Result	: negative
Method	: other: not indicated
Year	: 1983
GLP	: no data
Test substance	:
Method	: SYSTEM OF TESTING
	- Species/cell type: TA1535, TA1537, TA98 and TA100
	- Metabolic activation system: liver S9 fraction from rats and hamsters treated with Aroclor 1254
	- Deficiency: histidine
	ADMINISTRATION: - Dosing: 100, 333, 1000, 3333, 10000 µg/plate - Number of replicates: 3 - Application: preincubation assay - Positive controls: 2-aminoanthracene (all strains with S9); 4-nitro-o-phenylenediamine (TA98 without S9); sodium azide (TA100 and TA1535 without S9); 9-aminoacridine (TA1537 without S9) - Negative control: water - Pre-incubation time: 20 min

	CRITERIA FOR EVALUATING RESULTS: - Statistical method: Margolin (1981) if result is positive
Remark	: 1. The test was performed by three different labs. These are the results of the second lab.
Result	: GENOTOXIC EFFECTS: - With metabolic activation(rat): negative - With metabolic activation(hamster): negative - Without metabolic activation: negative
	CYTOTOXIC CONCENTRATION: - With or without metabolic activation: ≥ 10000 $\mu\text{g}/\text{plate}$
Test substance	: CAS 56-81-5 (Glycerine), purity $>99\%$.
Conclusion	: Most reliable study available.
Reliability	: (2) valid with restrictions Non-GLP study.
Flag	: Critical study for SIDS endpoint
21.01.2002	(105)
Type	: Ames test
System of testing	: TA1535, TA1537, TA98 and TA100
Test concentration	: 100, 333, 1000, 3333, 10000 $\mu\text{g}/\text{plate}$
Cycotoxic concentr.	: ≥ 10000 $\mu\text{g}/\text{plate}$
Metabolic activation	: with and without
Result	: negative
Method	: other: not indicated
Year	: 1983
GLP	: no data
Test substance	:
Method	: SYSTEM OF TESTING - Species/cell type: TA1535, TA1537, TA98 and TA100 - Metabolic activation system: liver S9 fraction from rats and hamsters treated with Aroclor 1254 - Deficiency: histidine
	ADMINISTRATION: - Dosing: 100, 333, 1000, 3333, 10000 $\mu\text{g}/\text{plate}$ - Number of replicates: 3 - Application: preincubation assay - Positive controls: 2-aminoanthracene (all strains with S9); 4-nitro-o-phenylenediamine (TA98 without S9); sodium azide (TA100 and TA1535 without S9); 9-aminoacridine (TA1537 without S9) - Negative control: DMSO - Pre-incubation time: 20 min
	CRITERIA FOR EVALUATING RESULTS: - Statistical method: Margolin (1981) if result is positive
Remark	: 1. The test was performed by three different labs. These are the results of the third lab.
Result	: GENOTOXIC EFFECTS: - With metabolic activation(rat): negative - With metabolic activation(hamster): negative - Without metabolic activation: negative
	CYTOTOXIC CONCENTRATION: - With or without metabolic activation: ≥ 10000 $\mu\text{g}/\text{plate}$
Test substance	: CAS 56-81-5 (Glycerine), purity $>99\%$.
Conclusion	: Most reliable study available.
Reliability	: (2) valid with restrictions

Flag 21.01.2002	: Non-GLP study. : Critical study for SIDS endpoint	(105)
Type	: Ames test	
System of testing	: TA 98, TA 100, TA 1535, TA 1537, TA 1538	
Test concentration	: 200-1000 ug/plate	
Cycotoxic concentr.	: no cytotoxicity observed	
Metabolic activation	: with and without	
Result	: Negative	
Method	:	
Year	: 1988	
GLP	: no data	
Test substance	:	
Method	: SYSTEM OF TESTING - Species/cell type: Salmonella typhimurium TA98, TA100, TA1535, TA 1537 and TA1538 - Deficiencies: histidine - Metabolic activation system: rat S-9 ADMINISTRATION: - Dosing: 200, 400, 600, 800 and 1000 ug/plate - Number of replicates: 3 - Application: preincubation assay - Positive and negative control groups and treatment: without S-9: 2-nitrofluorene (TA98, TA1538), sodium azide (TA100, TA1535) and 9-aminoanthracene (TA1537) with S-9: 2-aminoanthracene - Pre-incubation time: not indicated DESCRIPTION OF FOLLOW UP REPEAT STUDY: indepent repeat with TA100 (not reported) CRITERIA FOR EVALUATING RESULTS: reproducible, dose-related increase in the number of revertants	
Result	: - With metabolic activation: negative - Without metabolic activation: negative In TA100 the number of revertants was increased compared to solvent controls, without relationship with the applied concentration. Therefore the test was repeated with TA100 at slightly higher glycerol concentrations with a negative result (no data available) PRECIPITATION CONCENTRATION: not indicated	
Test substance	: CYTOTOXIC CONCENTRATION: no cytotoxicity up to 1000 ug/plate	
Conclusion	: CAS 56-81-5 (glycerine), purity >99.5%	
Reliability	: Most reliable study available. : (2) valid with restrictions 1 The report is limited to the above mentioned. No individual values were included. 2 It is not clear from the report, why 5000 ug/plate was not included as the highest concentration tested. OECD 471 states that in absence of precipitate and/or cytotoxicity the highest test concentration should be 5000 ug/plate. 3 In TA 100 the number of revertants was increased in the initial experiment compared to solvent controls, without relationship with the applied concentration. It is reported that the results of the repeated	

experimnt with TA100 confirmed that glycerol is not mutagenic in TA100 (data not shown).	
Flag 17.12.2001	: Critical study for SIDS endpoint (106)
Type	: HGPRT assay
System of testing	: CHO-cells
Test concentration	: 100-1000 ug/mL
Cycotoxic concentr.	: no cytotoxicity observed
Metabolic activation	: with and without
Result	: Negative
Method	:
Year	: 1988
GLP	: no data
Test substance	:
Method	: SYSTEM OF TESTING - Cell type: CHO-K1-BH4 - Proficiencies: HGPRT gene - Metabolic activation system: rat S-9 ADMINISTRATION: - Dosing: 100, 200, 400, 600, 800 and 1000 ug/mL - Number of replicates: not indicated - Negative control: water (solvent) - Positive control groups: without S-9 ethyl methanesulphonate; with S-9 dimethylbenzanthracene - Treatment: 5 hours CRITERIA FOR EVALUATING RESULTS: At least a three-fold increase in mutation frequency above controls in a dose dependent manner. Result : - With metabolic activation: negative - Without metabolic activation: negative At 800 and 1000 ug/mL the mutation frequency was increased ≥ 3 fold (24E-06 and 6E-06, respectively) compared to controls (2E-06). PRECIPITATION CONCENTRATION: not indiated CYTOTOXIC CONCENTRATION: Not observed Test substance : CAS 56-81-5 (glycerine), purity >99.5% Conclusion : Most reliable study available. Reliability : (2) valid with restrictions 1 The report is limited to the above mentioned. No individual values were included. 2 The increased mutation frequency seen at the two highest concentrations (800 and 1000 ug/mL) in absence of a concentration response relationship does not meet the criteria set for a positive response set by the author of the report. 3 It is not clear from the report, why 5000 ug/mL was not included as the highest concentration tested. OECD 476 states that in absence of precipitate and/or cytotoxicity the highest test concentration should be 5000 ug/mL. Flag 17.12.2001: Critical study for SIDS endpoint (106) Type : Sister chromatid exchange assay System of testing : CHO cells

Test concentration	:	200-1000 ug/ml
Cycotoxic concentr.	:	no cytotoxicity observed
Metabolic activation	:	with and without
Result	:	Negative
Method	:	
Year	:	1988
GLP	:	no data
Test substance	:	
Method	:	SYSTEM OF TESTING - Species/cell type: CHO-cells WBL - Metabolic activation system: rat S-9 - No. of metaphases analyzed: 50/concentration ADMINISTRATION: - Dosing: 200, 400, 600, 800 and 1000 ug/mL - Number of replicates: 2 - Treatment: 2 hours with S-9; 25.5 hours without S-9 - Negative control: water (solvent) - Positive control groups: triethylenemelamine(-S9); cyclophosphamide (+S9) CRITERIA FOR EVALUATING RESULTS: A reproducible, dose-dependent increase in frequency of SCE's compared to solvent control
Result	:	GENOTOXIC EFFECTS: - With metabolic activation: negative - Without metabolic activation: negative CYTOTOXIC CONCENTRATION: Not cytotoxicity at any of the concentrations tested.
Test substance	:	CAS 56-81-5 (glycerine), purity >99.5%
Conclusion	:	Most reliable study available.
Reliability	:	(2) valid with restrictions The report is limited to the above mentioned. No individual values were included.
Flag	:	Critical study for SIDS endpoint
25.01.2002		
Type	:	Unscheduled DNA synthesis
System of testing	:	Rat hepatocytes
Test concentration	:	100-1000 ug/mL
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	:	Negative
Method	:	
Year	:	1988
GLP	:	no data
Test substance	:	
Method	:	SYSTEM OF TESTING - Radiographic UDS assay in rat hepatocytes ADMINISTRATION: - Dosing: 100, 250, 500, 750 and 1000 ug/mL - Test was performed twice (doses second exp 750 and 1000 ug/mL) - Negative control: not applicable - Positive control: methylaminofluorene

(106)

	CRITERIA FOR EVALUATING RESULTS: - reproducible dose dependent increase of number of nuclear grains - Statistical method: ANOVA, followed by Dunnett's test
Result	: The number of nuclear grains in controls and treated hepatocytes did not differ significantly. Positive controls were within expected ranges.
Test substance	: CAS 56-81-5 (glycerine), purity >99.5%
Conclusion	: Most reliable study available.
Reliability	: (2) valid with restrictions The report is limited to the above mentioned. No individual values were included.
Flag 25.01.2002	: Critical study for SIDS endpoint (106)
Type	: Chromosomal aberration test
System of testing	: CHO cells
Test concentration	: 100-1000 ug/mL
Cycotoxic concentr.	: no cytotoxicity observed
Metabolic activation	: with and without
Result	: Negative
Method	:
Year	: 1988
GLP	: no data
Test substance	:
Method	: SYSTEM OF TESTING - Species/cell type: CHO-cells WBL - Metabolic activation system: rat S-9 - No. of cells scored: 100/concentration (50 for positive controls)
	ADMINISTRATION: - Dosing: 100, 200, 400, 600, 800 and 1000 ug/mL - Treatment: 10 and 14 hours (with S-9) without recovery; 2 hours (without S-9) with 10 and 14 hr recovery - Negative control: water (solvent) - Positive control groups: triethylenemelamine(-S9); cyclophosphamide (+S9)
Result	: CRITERIA FOR EVALUATING RESULTS: A statistically significant, reproducible and dose-dependent increase in frequency of cells with aberrations compared to solvent control. GENOTOXIC EFFECTS: - With metabolic activation: negative - Without metabolic activation: negative In the initial assay with metabolic activation a statistically significant increase in the number of aberrations compared to controls was seen only at 200 ug/mL (recovery period 10 hr) PRECIPITATION CONCENTRATION: not indicated MITOTIC INDEX: without S-9 (10 hr): 84-97% of control without S-9 (14 hr): 78-101% of control with S-9 (10 hr rec.): 59-92% of control (no relationship with concentration) with S-9 (14 hr rec): no decrease observed

	CYTOTOXIC CONCENTRATION: No cytotoxicity observed with concentrations tested.	
Test substance	: CAS 56-81-5 (glycerine), purity >99.5%	
Conclusion	: Most reliable study available.	
Reliability	: (2) valid with restrictions 1 The report is limited to the above mentioned. No individual values were included. 2 The isolated increase in number of aberrations seen at 200 ug/mL (+S-9) is considered of no biological relevance, since there was no relationship with the concentration tested. 3 It is not clear from the report, why 5000 ug/mL was not included as the highest concentration tested. OECD 473 states that in absence of precipitate and/or cytotoxicity the highest test concentration should be 5000 ug/mL.	
Flag 25.01.2002	: Critical study for SIDS endpoint	(106)
Type	: Ames test	
System of testing	: Salmonella typhimurium strain TA-100	
Test concentration	: 0.1 and 1 mmol per plate	
Cycotoxic concentr.	:	
Metabolic activation	: with and without	
Result	: Negative	
Method	: Other	
Year	: 1979	
GLP	: no data	
Test substance	: no data	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (4) not assignable The information in the report is confined to the above.	
16.11.2001		(107)
Type	: Ames test	
System of testing	: Salmonella typhimurium strain TA100	
Test concentration	: 0.5 mg/plate	
Cycotoxic concentr.	:	
Metabolic activation	: with and without	
Result	: Negative	
Method	:	
Year	:	
GLP	: no data	
Test substance	: other TS	
Remark	: The liver microsomal fraction was from PCB-induced rats.	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Reliability	: (4) not assignable Secondary literature with information essentially confined to what is included in the current summary.	
16.11.2001		(108)
Type	: Bacillus subtilis recombination assay	
System of testing	: Bacillus subtilis H17 and M45	
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	: Positive	
Method	:	
Year	:	

GLP	:		
Test substance	:		
Method	:	Cells were incubated for 30 min. in presence of the test substance. After treatment viable cells were counted.	
Test substance	:	CAS 56-81-5 (glycerine), purity not indicated	
Reliability	:	(4) not assignable Only a short abstract is available. It can not be excluded that interference of the test substance with the cell surface may have influenced the study outcome.	
16.11.2001			(109)
Type	:	Escherichia coli reverse mutation assay	
System of testing	:	various	
Test concentration	:		
Cycotoxic concentr.	:		
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	other	
Year	:	1985	
GLP	:	no data	
Test substance	:	no data	
Remark	:	Literature could not be retrieved.	
Source	:	Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Reliability	:	(4) not assignable	
25.01.2002			(110) (111)
Type	:	Ames test	
System of testing	:	Salmonella typhimurium strain TA-100	
Test concentration	:	0.1 and 1 mmol per plate	
Cycotoxic concentr.	:		
Metabolic activation	:	with and without	
Result	:	negative	
Method	:		
Year	:		
GLP	:		
Test substance	:		
Remark	:	Literature could not be retrieved.	
Source	:	Wolff Walsrode AG Walsrode EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Reliability	:	(4) not assignable	
25.01.2002			
Type	:	Ames test	
System of testing	:	TA92, TA94, TA98, TA100, TA1535 and TA1537	
Test concentration	:	<= 50 mg/plate	
Cycotoxic concentr.	:		
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	other: not indicated	
Year	:		
GLP	:	no data	
Test substance	:	other TS	
Method	:	SYSTEM OF TESTING - Species/cell type: TA92, TA94, TA98, TA100, TA1535 and TA1537	

	- Deficiency: histidine - Metabolic activation system: Rat liver S9 mix (polychlorinated biphenyls induced)
	ADMINISTRATION: - Dosing: 6 concentrations $\leq$ 50 mg/plate - Number of replicates: 2 - Application: preincubation assay - Negative control: phosphate buffer - Pre-incubation time: 20 min
	CRITERIA FOR EVALUATING RESULTS: The result was considered positive if the number of revertant colonies found was twice or more that of the control.
Result	: GENOTOXIC EFFECTS: - With metabolic activation: negative - Without metabolic activation: negative
Test substance	: CAS 56-81-5 (glycerine), purity 99.4%
Reliability	: (4) not assignable 1. The information in the report is confined to the above. The article is a review article of more than 200 investigated substances. 2. The strains used are no standard strains as recommended by the OECD. 3. No standard positive controls were included. It is not clear whether the positive results obtained with some of the tested compounds were achieved with the same batch of bacteria as glycerine.
15.11.2001	(112)
Type	: Chromosomal aberration test
System of testing	: CHO cells
Test concentration	: 1 mg/ml
Cycotoxic concentr.	:
Metabolic activation	: without
Result	: negative
Method	: other: not indicated
Year	:
GLP	: no data
Test substance	: other TS
Method	: SYSTEM OF TESTING - Species/cell type: CHO cells - No. of metaphases analyzed: 100
	ADMINISTRATION: - Dosing: 1 mg/ml and two doses at lower concentrations (maximum dose based on 50% growth reduction at 1.0 mg/ml) - Negative control: untreated and solvent-treated (physiol. saline) cells - Incubation time: 24 and 48 h (last 2 h in presence of colcemid)
	CRITERIA FOR EVALUATING RESULTS: negative if incidence was less than 4.9%, equivocal if it was between 5.0 and 9.9%, and positive if it was more than 10.0%
Result	: GENOTOXIC EFFECTS: - Without metabolic activation: negative (only 48 h result reported)
	FREQUENCY OF EFFECTS: 2.0% of polyploid cells and 1.0% of cells with structural aberrations at 1.0 mg/ml
Test substance	: CAS 56-81-5 (glycerine), purity 99.4%
Reliability	: (4) not assignable 1. The information in the report is confined to the above. The article is a

	review article of more than 200 investigated substances. 2. No standard positive controls were included. It is not clear whether the positive results obtained with some of the tested compounds were achieved with the same batch of bacteria as glycerine.	
15.11.2001		(112)
Type	: Ames test	
System of testing	: TA98, TA100, TA1535, TA1537 and TA1538	
Test concentration	: 1 - 10000 µg/plate	
Cycotoxic concentr.	:	
Metabolic activation	: no data	
Result	: negative	
Method	: other: Ames	
Year	: 1975	
GLP	: no data	
Test substance	: other TS	
Method	: SYSTEM OF TESTING - Species/cell type: TA98, TA100, TA1535, TA1537 and TA1538 - Deficiency: histidine - Metabolic activation system: rat liver homogenate	
	ADMINISTRATION: - Dosing: 1 - 10000 µg/plate - Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (TA100 and TA1535); 2-aminofluorene (TA98, TA100 and TA1538); 9-aminoacridine (TA1537)	
Result	: GENOTOXIC EFFECTS: - negative	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated (glycerine/water mixture of unknown composition).	
Reliability	: (4) not assignable 1 Secondary literature; the information in the report is confined to the above mentioned. 2 Although it was indicated that a test with metabolic activation was included, it could not be established whether a test without metabolic activation was included also.	
11.12.2001		(86)
Type	:	
System of testing	: Human lymphocytes	
Test concentration	: 200 mmol/l = 18.4 g/l	
Cycotoxic concentr.	: no cytotoxicity observed	
Metabolic activation	:	
Result	:	
Method	:	
Year	: 1982	
GLP	:	
Test substance	: other TS	
Method	: Lymphocyte proliferative growth was stimulated with PMA during 3 days. Cells were incubated in presence of [3H]thymidine for 20 hours. The effect of a number hydroxyl radical scavengers on [3H]thymidine incorporation was determined.	
Result	: Glycerine was found to inhibit [3]thymidine incorporation (16.4% of control).	
Test substance	: CAS 56-81-5 (Glycerine), purity not indicated.	
03.01.2002		(113)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	: Dominant lethal assay
Species	: rat
Sex	: male/female
Strain	:
Route of admin.	:
Exposure period	:
Doses	: 10, 100 and 1000 mg/kg bw
Result	: ambiguous
Method	:
Year	: 1985
GLP	: no data
Test substance	:
Method	: Male rats (number not indicated) were treated with glycerine (most probably injected in the abdomen). Thereafter the animals were mated with 11-12 untreated females/treatment. Two weeks after mating females were sacrificed and the number of implantation sites, foetal loss, live foetuses and anomalous foetuses was established.
Result	: Implantation sites: 101, 104 and 91 at 10, 100 and 1000 mg/kg bw respectively (controls 116) Foetal loss: 11, 20 and 59% at 10, 100 and 1000 mg/kg bw respectively (controls 8%) Live foetuses: 90, 83 and 37 at 10, 100 and 1000 mg/kg bw respectively (controls 107) Anomalies: none in glycerine treated and control animals
Test substance	: CAS 56-81-5 (Glycerine), purity not indicated.
Conclusion	: Glycerine may have a potential mutagenic effect on gender cells, which results in post-implantation deaths. The effect however, did not reach statistical significance.
Reliability	: (4) not assignable 1 The report is limited to the above. 2 No positive control group was included. The study did not use an adequate number of animals. Group sizes of 30-50 are recommended in the OECD guideline (478). 3 Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. In a two generation study no effects on pregnancy were found at doses upto 2000 mg/kg bw.
24.01.2002	(114)
Type	: other: chromosome aberration test
Species	: rat
Sex	: male
Strain	:
Route of admin.	: other: injection in the abdomen
Exposure period	:
Doses	: 1000 mg/kg bw
Result	: negative
Method	:
Year	: 1985
GLP	: no data
Test substance	:
Method	: 10 male rats received 1000 mg/kg bw glycerine in water or isotonic salt solution (dosing volume 2 mL). After 50 hours animals were killed and chromosome preparations were prepared from marrow cells from the femoral bone. Cytogenic analysis was performed in 50 metaphases.

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Result	:	Number of cells with aberrations 2.2% (0% in concurrent controls) Number of cells with gaps 1.6% (0% in concurrent controls) Polyploid cells 3.2% (0% in concurrent controls)
Test substance	:	CAS 56-81-5 (Glycerine), purity not indicated.
Conclusion	:	Glycerine did not induce a statistically significant increase in chromosomal aberrations when compared to control values.
Reliability	:	(4) not assignable The report is limited to the above. No positive control group was included.
24.01.2002		(114)

5.7 CARCINOGENICITY

Species	:	mouse
Sex	:	
Strain	:	other: ddY
Route of admin.	:	drinking water
Exposure period	:	
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	5% solution
Result	:	
Control group	:	yes
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse= about 10 mg/kg bw; s.c.). Literature could not be retrieved.
Result	:	The incidence of pulmonary tumor-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.
Reliability	:	(4) not assignable
25.01.2002		(115)
Species	:	mouse
Sex	:	male
Strain	:	other: ddY
Route of admin.	:	drinking water
Exposure period	:	1-4 weeks
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	5% in drinking water
Result	:	
Control group	:	other: see method
Method	:	
Year	:	1987
GLP	:	no data
Test substance	:	other TS
Method	:	TEST ORGANISMS - Age: 6 weeks - Number of animals: 20 males/treatment

ADMINISTRATION / EXPOSURE

	- Single s.c. injection of 4NQO (10 mg/kg bw) on day 1 followed by treatment with glycerol during, 1, 2, 3 or 4 weeks - Dose: 5% in drinking water (~8350 mg/kg bw) - Vehicle (4NQO): olive oil and cholesterol (20:1) - Controls: untreated, 4NQO treated (single) and glycerol treated (4 weeks) - Observation period: 25 weeks												
	Examinations: - Mortality - Body weight - No./size of pulmonary tumours - Tumour histopathology												
Result	STATISTICAL METHODS: Student's t-test, chi-square test : Mortality: 2 animals in the group that was treated with glycerine for 3 weeks and 1 animal in the group that was treated with glycerine for 4 weeks. These animals showed rapidly growing subcutaneous sarcomas at the injection site from week 16-20 (invading into pleural and peritoneal cavities and liver). Body weight: tendency to increase with increasing exposure time to glycerine (not significant) Pulmonary tumours: - No. of tumour bearing mice: <table> <tr> <td>controls (not receiving 4NQO)</td><td>1/20</td></tr> <tr> <td>controls (receiving 4NQO)</td><td>8/20</td></tr> <tr> <td>treatment (1 week glycerine)</td><td>11/20</td></tr> <tr> <td>treatment (2 weeks glycerine)</td><td>11/19</td></tr> <tr> <td>treatment (3 weeks glycerine)</td><td>7/18</td></tr> <tr> <td>treatment (4 weeks glycerine)</td><td>15/19</td></tr> </table> - Mean number of tumours/mouse: significantly increased after 4 weeks of glycerine - The number of tumour-bearing mice was identical between untreated controls (1/20) and animals receiving glycerine only for 4 weeks. - Tumour volume: tendency to increase with increasing exposure time to glycerine (not significant) Other tumours: 3 control mice receiving 4NQO developed sarcomas at the injection site in week 22. Necropsy revealed metastases to the lungs and liver. Histopathology: Most tumours were papillary or solid ones. In some papillary tumours atypical cells with polygonal nuclei (hyperchromasia) were observed.	controls (not receiving 4NQO)	1/20	controls (receiving 4NQO)	8/20	treatment (1 week glycerine)	11/20	treatment (2 weeks glycerine)	11/19	treatment (3 weeks glycerine)	7/18	treatment (4 weeks glycerine)	15/19
controls (not receiving 4NQO)	1/20												
controls (receiving 4NQO)	8/20												
treatment (1 week glycerine)	11/20												
treatment (2 weeks glycerine)	11/19												
treatment (3 weeks glycerine)	7/18												
treatment (4 weeks glycerine)	15/19												
Test substance	: CAS 56-81-5 (Glycerine), purity not indicated.												
Conclusion	: The data suggest that glycerine modulates the initial process of tumourgenesis by 4NQO.												
Reliability	: (4) not assignable												
	The information was confined to the above.												
25.01.2002	(116)												
Species	: mouse												
Sex	: male												
Strain	: other: ddY												
Route of admin.	: drinking water												
Exposure period	: 25 weeks												
Frequency of treatm.	:												
Post exposure period	:												
Doses	: 5% in drinking water												
Result	:												

Control group : other: see method
Method :
Year : 1986
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
 - Age: 6 weeks
 - Number of animals: 20 males/treatment

ADMINISTRATION / EXPOSURE

- Single s.c. injection of 4NQO (10 mg/kg bw) on day 1 followed by treatment with glycerol from week 5-30.
- Dose: 5% in drinking water (~8350 mg/kg bw)
- Vehicle (4NQO): olive oil and cholesterol (20:1)
- Controls: untreated, 4NQO treated (single) and glycerol treated (25 weeks)
- Observation period: 30 weeks

Examinations:

- Mortality
- Body weight
- No. of pulmonary tumours
- Tumour histopathology

Result : STATISTICAL METHODS: not specified
 : Mortality: 2 animals in the group receiving 4NQO only showing fibrosarcomas at the injection site (week 25-28).

Body weight: no treatment related effects

Pulmonary tumours:

- No. of tumour bearing mice:
 controls 2/20
 controls (receiving glycerine) 2/20
 treatment (receiving 4NQO only) 51/20
 treatment (NQO + glycerine) 17/20
- Mean number of tumours/mouse: significantly increased after NQO + glycerine (2.9/mouse versus 0.1-0.45/mouse in the other groups). Treatment with glycerine alone did not result in an increase in number of tumour-bearing mice above that observed in untreated controls.

Other tumours: Additional 2 mice receiving 4NQO only developed fibrosarcomas at the injection site (no lung tumours).

Histopathology:

In NQO treated mice all tumours were identified as type II adenomas.
 In NQO + glycerine treated animals 52 tumours were identified as type II adenomas and 6 as Clara cell adenomas.

Test substance : CAS 56-81-5 (Glycerine), purity not indicated.
Reliability : (4) not assignable
 The information was confined to the above.

25.01.2002

(117)

Species : mouse
Sex : male
Strain : other: ddY
Route of admin. : drinking water
Exposure period : 4-25 weeks
Frequency of treatm. :

Post exposure period	:	
Doses	:	5% in drinking water
Result	:	
Control group	:	other: see method
Method	:	
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Method	:	TEST ORGANISMS - Age: 6 weeks - Number of animals: 10 males/treatment ADMINISTRATION / EXPOSURE - Single s.c. injection of 4NQO (10 mg/kg bw) on day 1 followed by treatment with glycerine for 4-25 weeks. - Dose: 5% in drinking water (~8350 mg/kg bw) - Vehicle (4NQO): olive oil and cholesterol (20:1) - Controls: untreated, 4NQO treated (single) and glycerol treated (25 weeks) - Observation period: 25 weeks Examinations: - No. of pulmonary tumours - Tumour histopathology STATISTICAL METHODS: not specified
Result	:	Pulmonary tumours: - No. of tumour bearing mice: controls 0/10 controls (glycerine 25 weeks) 0/10 controls (receiving 4NQO) 1/10 treatment (4 weeks glycerine) 8/10 treatment (25 weeks glycerine) 8/9 treatment (glycerine week 4-25) 7/10 - Mean number of tumours/mouse (significant increase after NOQ + glycerine): controls 0 controls (glycerine 25 weeks) 0 controls (receiving 4NQO) 0.1 treatment (4 weeks glycerine) 3.5 treatment (25 weeks glycerine) 2.3 treatment (glycerine week 4-25) 1.9 Histopathology: All tumours were adenomas.
Test substance	:	CAS 56-81-5 (Glycerine), purity not indicated.
Reliability	:	(4) not assignable The information was confined to the above.
08.01.2002		
Species	:	rat
Sex	:	male/female
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	2 yr
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	5 or 10 g/kg (24 males and 24 females)

(118)

Result	:	
Control group	:	
Method	:	
Year	:	1953
GLP	:	no data
Test substance	:	no data
Remark	:	Not all literature could be retrieved.
Result	:	No increase in tumour incidence.
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable
25.01.2002		(119) (64)
Species	:	rat
Sex	:	male/female
Strain	:	Long-Evans
Route of admin.	:	oral feed
Exposure period	:	
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	5, 10 and 20% in diet
Result	:	
Control group	:	
Method	:	
Year	:	1953
GLP	:	
Test substance	:	
Method	:	TEST ORGANISMS - Age: not indicated - Weight at study initiation: 96-109 g (males), 92-108 g (females) - Number of animals: 22/sex/treatment, 26/sex for controls - Source: Institute of Experimental Biology of University of California
		ADMINISTRATION / EXPOSURE - Exposure period: 2 year (1 year for the high dose group) - Route of administration: oral in diet - Doses: 5, 10 and 20% in diet; males 2000, 4000 and 8000 mg/kg bw, females 2500, 5000 and 10000 mg/kg bw
		CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: daily in cage and weekly examination outside the home cage - Mortality: daily - Body weight: weekly
		ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weights: liver, kidneys, heart, spleen and lungs - Macroscopic: no details provided - Microscopic: liver, spleen, adrenals, kidney, small intestine, gonads and urinary bladder
		ANALYSES: not performed
		STATISTICAL METHODS: Chi-square test, student t-test, ANOVA (Fisher)
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

	- Mortality and time to death: not indicated - Clinical signs: not reported - Body weight gain: no statistically significant differences between treated and control animals - Histopathology: Malignant neoplasms in 5/26, 1/22, 5/22, 0/22, 0/21, 5/22 and 0/22 animals in control and at 5%, 10%, 20% natural glycerol and at 5%, 10%, 20% synthetic glycerol, respectively. - Benign neoplasms in 0/26, 2/22, 1/22, 0/22, 4/21, 4/22 and 1/22 animals in controls and at 5%, 10%, 20% natural glycerol and at 5%, 10%, 20% synthetic glycerol, respectively. - Among the benign tumours 3 rats were found with pheochromacytomas and 2 with granulosa cell tumours.
Test substance	: CAS 56-81-5 (glycerine), Natural glycerine, achieved from market stock, purity not indicated (impurities were fatty acids and esters) Synthetic glycerol, purity 99.5% (rest mainly water with very small amounts of glycerol polymers and glyceraldehyde).
Conclusion	: No increased incidence in tumour incidence following treatment with glycerol.
Reliability	: (2) valid with restrictions The report was confined to the above.
Flag 29.01.2002	: Critical study for SIDS endpoint (64)

5.8.1 TOXICITY TO FERTILITY

Type	: Two generation study
Species	: rat
Sex	: male/female
Strain	:
Route of admin.	: gavage
Exposure period	: 8-12 weeks (starting before mating and continuing, in females, until weaning).
Frequency of treatm.	: daily
Premating exposure period	:
Male	: 8 weeks
Female	: 8 weeks
Duration of test	:
No. of generation studies	:
Doses	: 20% in water, about 2 g/kg/day
Control group	: yes, concurrent vehicle
Method	:
Year	: 1953
GLP	: no data
Test substance	:
Method	: TEST ORGANISMS - Age: not indicated - Weight at study initiation: not indicated - Number of animals: 10/sex/treatment for Parent and F1 ADMINISTRATION / EXPOSURE - Test durations: until F2-generation was 100 days of age - Premating period: 8 weeks - Exposure period: 12 weeks (until weaning of F1) - Route of administration: oral (gavage, dose volume 10 mL/kg) - Doses: 20% solution in water, ~2000 mg/kg bw

	MATING PROCEDURES: not indicated (starting when females were between 170 and 215 g)
	STANDARDIZATION OF LITTERS: not performed
	PARAMETERS ASSESSED: - Clinical observations: frequency not indicated - Estrous cycle: in F1 and F2 between 60 and 100 days - Body weight: in F1 and F2 during day 15 and 60 at 2-day intervals - Sperm examination: not performed
	ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weights F1 and F2: pituitary, thyroid, adrenals, ovaries, testicles - Histopathology F1 and F2: endocrine system organs on 26 animals
Result	: No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility and reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation. Onset of oestrus cycle and weight and microscopy of the endocrine organs were comparable to control values for both F1 and F2 animals. In the parent generation all 10 females became pregnant (litter size 9.0, controls 8.1) and in the F1 9/10 females became pregnant (litter size 8.7, controls 8.1).
Test substance	: CAS 56-81-5 (glycerine), 20% solution in water
Conclusion	: Most reliable study available.
Reliability	: (2) valid with restrictions 1 The report was essentially confined to the above mentioned. 2 Although the study used a significantly high dose level (~2 g/kg bw/day), the significance attached to this study is somewhat limited by the use of 10 females per dose level.
Flag 18.12.2001	: Critical study for SIDS endpoint (120)
Type	: Fertility
Species	: rat
Sex	: male
Strain	: Sprague-Dawley
Route of admin.	: other: intratesticular
Exposure period	:
Frequency of treatm.	: on day 1 and 7
Premating exposure period	:
Male	:
Female	:
Duration of test	: 14 days
No. of generation studies	:
Doses	: 50 uL glycerol solution
Control group	: yes, concurrent vehicle
Method	: other: not indicated
Year	: 1984
GLP	: no
Test substance	:

Method : TEST ORGANISMS
- Age: 48 days, 69 days and 90-95 days (3 separate experiments)
- Weight at study initiation: not indicated
- Number of animals: 14, 8 and 8

ADMINISTRATION / EXPOSURE
- Exposure: twice (7 days apart)
- Route of administration: intratesticular injection (right testis treated, left testis control)
- Doses: not indicated (50 uL)

OBSERVATIONS:
- Testes weight (14 days after first injection)
- Spermatogenesis (14 days after first injection)

STATISTICAL METHOD:
Student's t-test

Result : Testis weight (treated side) significantly decreased compared to controls for all ages
Spermatogenesis: treatment resulted in complete loss of spermatogenic cells

Test substance : CAS 56-81-5 (glycerine), solution in water and ethanol (amounts not specified), purity not specified

Reliability : (4) not assignable
1 The information is confined to the above mentioned.
2 The relevance of the exposure route is considered to be low. After oral exposure no effects were observed according to the author of the report.

25.01.2002

(121)

Type : Fertility
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : other: intratesticular
Exposure period :
Frequency of treatm. : single dose
Premating exposure period
 Male :
 Female :
Duration of test : 73 days
No. of generation studies :
Doses : 200 uL
Control group : yes, concurrent vehicle
Method : other: not indicated
Year : 1984
GLP : no
Test substance :

Method : TEST ORGANISMS
- Age: 48-101 days
- Weight at study initiation: 342-372 g
- Number of animals: 12/treatment

Animals were allowed to mate three times between day 15 and 73

	ADMINISTRATION / EXPOSURE
	- Exposure: single
	- Route of administration: intratesticular injection (left testis treated, right testis control)
	- Doses: 200 uL
	OBSERVATIONS: interim kills of 3-5 animals on day 7, 15 and 73:
	- Histopathology/weight of testis, seminal vesicles, prostate and epididymides
	- No. of sperm/epididymis
	STATISTICAL METHOD:
	Student's t-test
Result	: WEIGHTS: Weight of testis significantly decreased at all sampling times; no effect on weight of prostate and seminal vesicles; weight of the epididymides significantly decreased after 73 days. SPERM: No. of sperm cells significantly decreased after 15 days and almost no sperm cells after 73 days HISTOPATHOLOGY: Day 7: nucleated bodies in seminiferous tubules (almost no spermatogenic cells), normal germ cells and Leydig cells Day 15 and 73: few nucleated bodies in seminiferous tubules (almost no spermatogenic cells), normal Leydig cells. No resumption of spermatogenesis
Test substance	: CAS 56-81-5 (glycerine), solution in water (7:3), purity not specified
Conclusion	: Intratesticular injection of glycerine inhibits spermatogenesis strongly over a prolonged period of time.
Reliability	: (4) not assignable 1 The information is confined to the above mentioned. 2 The relevance of the exposure route is considered to be low. After oral exposure no effects were observed according to the author of the report 3 Additional in vitro testing showed no decreased ability to metabolise progesterone compared to controls.
25.01.2002	(121)
Type	: Fertility
Species	: rat
Sex	: male
Strain	: Sprague-Dawley
Route of admin.	: other: intratesticular
Exposure period	:
Frequency of treatm.	: single
Premating exposure period	:
Male	:
Female	:
Duration of test	: 20-21 weeks
No. of generation studies	:
Doses	: 200 uL/testis
Control group	: yes, concurrent vehicle
Method	: other: not indicated
Year	: 1984
GLP	: no
Test substance	:
Method	: TEST ORGANISMS - Age: 86-90 days - Weight at study initiation: not indicated

	- Number of animals: 8/treatment, 8 for water treated controls
	ADMINISTRATION / EXPOSURE
	- Exposure: single
	- Route of administration: intratesticular injection
	- Doses: 200 uL
	PROCEDURE:
	Starting at 14 days after injection animals were allowed to mate with a virgin female for a period of 5 days (females were added in week 2, 3, 4, 5 and 6). 3 treated and 4 control animals were kept until week 21 and were allowed to mate during week 20 and 21.
	OBSERVATIONS:
	- Mating behaviour
	- After each mating period: no. mated, no. pregnant and no. of foetuses by examination of the uterus contents of the females 10 days after cohabitation
Result	: Mating behaviour and the number of matings did not differ between treated and control animals. Pregnancy rate decreased markedly by the third mating period (week 4) (2/8 females pregnant compared to 7/8 in controls). From week 5 onwards no pregnancies were observed in females mated with treated males. The mean number of foetuses/mated female in controls was 10-13. In treated animals this number was 11 (week 2), 8 (week 3) and 2 (week 4).
Test substance	: CAS 56-81-5 (glycerine), solution in water and ethanol (amounts not specified),purity not specified
Conclusion	: Treatment with glycerine did not affect sexual behaviour. However, fertility was affected strongly and prolonged.
Reliability	: (4) not assignable 1 The information is confined to the above mentioned. 2 The relevance of the exposure route is considered to be low. After oral exposure no effects were observed according to the author of the report.
25.01.2002	(121)
Type	: Fertility
Species	: rat
Sex	: male
Strain	:
Route of admin.	: other: intratesticular
Exposure period	:
Frequency of treatm.	:
Premating exposure period	:
Male	:
Female	:
Duration of test	:
No. of generation studies	:
Doses	: 862 mg/kg (1 day prior to mating)
Control group	:
Method	:
Year	: 1984
GLP	: no data
Test substance	: no data

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Remark	:	Literature could not be retrieved. Study type: TDLO
Result	:	Paternal effects (Spermatogenesis). Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source	:	Simel S.p.A. Industria Chimica Cremona EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability 25.01.2002	:	(4) not assignable
(122)		
Type	:	Fertility
Species	:	monkey
Sex	:	male
Strain	:	
Route of admin.	:	other: intratesticular
Exposure period	:	
Frequency of treatm.	:	
Premating exposure period	:	
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	119 mg/kg (1 day prior to mating)
Control group	:	
Method	:	
Year	:	1989
GLP	:	no data
Test substance	:	no data
Remark	:	Study type: TDLO Paternal effects (Spermatogenesis; Testes, epididymus, sperm duct). Literature could not be retrieved.
Result	:	Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without evidence of toxic or endocrine side effects.
Source	:	Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability 25.01.2002	:	(4) not assignable
(123)		

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	day 6 to day 15 of gestation inclusive
Frequency of treatm.	:	daily
Duration of test	:	20 days
Doses	:	13.1-1310 mg/kg bw
Control group	:	other: sham treated
NOAEL maternal tox.	:	= 1310 mg/kg bw
NOAEL teratogen.	:	= 1310 mg/kg bw
Method	:	other: not indicated
Year	:	1974
GLP	:	no
Test substance	:	

Method	: TEST ORGANISMS - Age: adult - Mean weight at study initiation: 214-230 g - Number of animals: 25-28 females/treatment ADMINISTRATION / EXPOSURE - Test duration: 20 days - Exposure period: day 6-15 of gestation inclusive - Definition of day 0: observation of vaginal sperm plug - Route of administration: oral (gavage) - Doses: 13.1, 60.8, 282 and 1310 mg/kg bw (dosing volume <6 mL/kg) - Vehicle: none MATING PROCEDURES: most probably 1 male/1 femalePARAMETERS ASSESSED DURING STUDY: - Mortality/clinical observations: daily - Body weight: on day 0, 6, 11, 15 and 20 - Food consumption: daily - Examination of uterine content: no. of implantation sites, resorptions and live and dead fetuses - Examination of fetuses: body weight, sex, external abnormalities, visceral (1/3 of fetuses) and skeletal (2/3 of fetuses) examination ORGANS EXAMINED AT NECROPSY: urogenital tract
Result	: STATISTICAL METHODS: not indicated MATERNAL TOXIC EFFECTS BY DOSE LEVEL: - Mortality: none - Body weight: no treatment related effects - Food consumption: no data - Clinical signs: not reported - Number pregnant per dose level: 23/25, 24/25, 22/28, 22/25 and 21/25 for controls and at 13.1, 60.8, 282 and 1310 mg/kg bw - Number aborting: none - Number of implantations: 11.3, 10.8, 12.3, 11.8 and 11.1 for controls and at 13.1, 60.8, 282 and 1310 mg/kg bw - Number of resorptions (no of dams involved): 2, 1, 1, 2 and 2 for controls and at 13.1, 60.8, 282 and 1310 mg/kg bw FETAL DATA: - Litter size and weights: No treatment related effects - Number viable: 10.8, 11.1, 11.2, 11.0 and 10.2 per litter for controls and at 13.1, 60.8, 282 and 1310 mg/kg bw - Sex ratio: no treatment related effects - External abnormalities: none reported - Visceral abnormalities: none - Skeletal abnormalities: delayed ossification in all treatment groups and controls without relationship to treatment
Test substance	: CAS 56-81-5 (glycerine) (syrup), purity not specified
Conclusion	: Most reliable study available.
Reliability	: (2) valid with restrictions 1 No data on uterus weights, no of corpora lutea and food consumption were included in the report. 2 No analyses of the test substance concentration were included. 3 For foetal external, visceral and skeletal examinations only summary tables were included.

5. TOXICITY

ID: 56-81-5

DATE: 29.01.2002

Flag : Critical study for SIDS endpoint
25.01.2002 (124)

Species : Mouse
Sex : Female
Strain : CD-1
Route of admin. : Gavage
Exposure period : day 6 to day 15 of gestation inclusive
Frequency of treatm. : Daily
Duration of test : 17 days
Doses : 12.8-1280 mg/kg bw
Control group : other: sham treatment
NOAEL maternal tox. : = 1280 mg/kg bw
NOAEL teratogen. : = 1280 mg/kg bw
Method : other: not indicated
Year : 1974
GLP : No
Test substance :

Method : TEST ORGANISMS
- Age: adult
- Mean weight at study initiation: 28.1-33.2 g
- Number of animals: 25 females/treatment

ADMINISTRATION / EXPOSURE

- Test duration: 17 days
- Exposure period: day 6-15 of gestation inclusive
- Definition of day 0: observation of vaginal sperm plug
- Route of administration: oral (gavage)
- Doses: 12.8, 59.4, 276 and 1280 mg/kg bw
- Vehicle: none

MATING PROCEDURES: most probably 1 male/1 female

PARAMETERS ASSESSED DURING STUDY:

- Mortality/clinical observations: daily
- Body weight: on day 0, 6, 11, 15 and 17
- Food consumption: daily
- Examination of uterine content: no. of implantation sites, resorptions and live and dead fetuses
- Examination of fetuses: body weight, sex, external abnormalities, visceral (1/3 of fetuses) and skeletal (2/3 of fetuses) examination

ORGANS EXAMINED AT NECROPSY: urogenital tract

Result : STATISTICAL METHODS: not indicated
: MATERNAL TOXIC EFFECTS BY DOSE LEVEL:
- Mortality: none
- Body weight: no treatment related effects
- Food consumption: no data
- Clinical signs: not reported
- Number pregnant per dose level: 22/25, 23/25, 20/25, 22/25 and 21/25 for controls and at 12.8, 59.4, 276 and 1280 mg/kg bw
- Number aborting: none
- Number of implantations: 11.1, 11.6, 12.0, 11.5 and 10.9 for controls and at 12.8, 59.4, 276 and 1280 mg/kg bw
- Number of resorptions (no of dams involved): 5, 6, 9, 4 and 9 for controls and at 12.8, 59.4, 276 and 1280 mg/kg bw

	FETAL DATA: - Litter size and weights: No treatment related effects - Number viable: 10.8, 11.1, 11.2, 11.0 and 10.2 per litter for controls and at 12.8, 59.4, 276 and 1280 mg/kg bw - Sex ratio: no treatment related effects - External abnormalities: none reported - Visceral abnormalities: none - Skeletal abnormalities: delayed ossification in all treatment groups and controls without relationship to treatment	
Test substance	: CAS 56-81-5 (glycerine) (syrup), purity not specified	
Conclusion	: Most reliable study available.	
Reliability	: (2) valid with restrictions	
	1 No data on uterus weights, no of corpora lutea and food consumption were included in the report.	
	2 No analyses of the test substance concentration were included.	
	3 For foetal external, visceral and skeletal examinations only summary tables were included.	
Flag	: Critical study for SIDS endpoint	
25.01.2002		(124)
Species	: Rabbit	
Sex	: Female	
Strain	: other: Dutch-belted	
Route of admin.	: Gavage	
Exposure period	: day 6 to day 18 of gestation inclusive	
Frequency of treatm.	: Daily	
Duration of test	: 29 days	
Doses	: 11.8-1180 mg/kg bw	
Control group	: other: sham treated	
NOAEL maternal tox.	: = 1180 mg/kg bw	
NOAEL teratogen.	: = 1180 mg/kg bw	
Method	: other: not indicated	
Year	: 1974	
GLP	: No	
Test substance	:	
Method	: TEST ORGANISMS	
	- Age: adult	
	- Mean weight at study initiation: 2.09-2.38 kg	
	- Number of animals: 15-20 females/treatment	
	ADMINISTRATION / EXPOSURE	
	- Test duration: 29 days	
	- Exposure period: day 6-18 of gestation inclusive	
	- Route of administration: oral (gavage)	
	- Doses: 11.8, 54.8, 254.5 and 1180 mg/kg bw (dosing volume <6 mL/kg)	
	- Vehicle: none	
	MATING PROCEDURES: artificial insemination	
	PARAMETERS ASSESSED DURING STUDY:	
	- Mortality/clinical observations: daily	
	- Body weight: on day 0, 6, 12, 18 and 29	
	- Food consumption: daily	
	- Examination of uterine content: no. of corpora lutea, implantation sites, resorptions and live and dead fetuses	
	- Examination of fetuses: body weight, sex, external	

	abnormalities on day 29, neonatal survival, visceral and skeletal examination on day 30	
	ORGANS EXAMINED AT NECROPSY: urogenital tract	
Result	STATISTICAL METHODS: not indicated : MATERNAL TOXIC EFFECTS BY DOSE LEVEL: - Mortality: 1 animal at 54.8 mg/kg bw, 2 at 254.5 mg/kg bw and 1 at 1180 mg/kg bw - Body weight: no treatment related effects, significant decrease only at 254.5 mg/kg bw (14%) compared to controls. - Food consumption: no data - Clinical signs: not reported - Number pregnant per dose level: 14/15, 12/15, 10/18, 13/20 and 13/15 for controls and at 11.8, 54.8, 254.5 and 1180 mg/kg bw - Number aborting: 2 at 254.5 mg/kg bw - Number of corpora lutea: 9.7, 11.7, 5.6, 8.2 and 11.2 for controls and at 11.8, 54.8, 254.5 and 1180 mg/kg bw - Number of implantations: 6.1, 5.1, 5.4, 7.3 and 6.4 for controls and at 11.8, 54.8, 254.5 and 1180 mg/kg bw - Number of resorptions (no of dams involved): 5, 2, 4, 2 and 6 for controls and at 11.8, 54.8, 254.5 and 1180 mg/kg bw FETAL DATA: - Litter size: No treatment related effects - Fetal weight: decreased(14%) at 254.5 mg/kg bw compared to controls. - Number viable: 5.1, 4.7, 4.8, 5.9 and 5.5 per litter for controls and at 11.8, 54.8, 254.5 and 1180 mg/kg bw mg/kg bw - Sex ratio: no treatment related effects - External abnormalities: none reported - Visceral abnormalities: no treatment related effects - Skeletal abnormalities: delayed ossification increased at 254.5 mg/kg bw (without relationship to treatment)	
Test substance	: CAS 56-81-5 (glycerine), purity not indicated.	
Conclusion	: Most reliable study available.	
Reliability	: (2) valid with restrictions 1 No data on uterus weights and food consumption were included in the report. 2 The number of pregnant females at 54.8 mg/kg bw is lower than required by OECD 414 (1981). 3 No analyses of the test substance concentration were included. 4 For foetal external, visceral and skeletal examinations only summary tables were included.	
Flag 25.01.2002	: Critical study for SIDS endpoint	(124)
Species	: other: FETAX	
Sex	:	
Strain	:	
Route of admin.	:	
Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	
Method	: other: ASTM E1439-91	
Year	: 1999	
GLP	:	
Test substance	:	

- Method** : In this report an interlaboratory validation study of the FETAX-assay was included. This validation is part of the ASTM process to evaluate the repeatability and reliability of the FETAX assay. In this test three laboratories tested 12 coded chemicals (one of which was glycerol) with and without Aroclor 1254 induced microsomes.
- All tests were performed in accordance with the ASTM standard guide. For each test duplicate test concentrations (10x2) were included. In each laboratory each test was performed three times, so 9 tests were performed in total.
- To judge the developmental hazard the teratogenic index was calculated (TI=96h LC50/96 h EC50 malformation) and it was checked whether significant growth inhibition was found at concentrations <30% of the 96-h LC50 (with microsomes).
- Developmental hazard for mammalian species is predicted when the mean TI (with microsomes) exceeds 1.5 and the minimal concentration that inhibits growth (MCIG with microsomes) exceeds 30% of the LC50.
- Statistical method: Probit analysis (Litchfield-Wilcoxon), trimmed Spearman Karber, Steel and Torrie.
- Result** : All results included here are related to the tests with the inclusion of microsomes (for detailed results see section 4.9).
- Mean TI: 1.66; individual laboratories showed mean values of 0.97, 1.67 and 2.33.
For all tests the MCIG > 30% of the LC50.
- Additionally results of mammalian developmental toxicity tests were obtained from the National Toxicology Program, the available scientific literature, two general papers on developmental toxicity screening tests and/or two reference source books. Only references to oral administration in mouse, rabbit and rat were employed. Based on these references glycerol was indicated as non-teratogen and therefore the FETAX assay was falsely positive.
- Conclusion** : In the FETAX test glycerol gave an ambiguous result.
In mammalian experiments (literature) glycerol was not teratogenic.
- Reliability** : (4) not assignable
1. FETAX (Frog Embryo Teratogenesis Assay-Xenopus) is a 96 hours whole-embryo developmental assay that can be used in detecting mammalian toxicants when an in vitro metabolic activation system is employed.
 2. The report is actual a publication on the validation of the FETAX-assay and shows that the assay is falsely positive in the case of glycerol. The literature that is checked for information on the mammalian teratogenicity of glycerol is the following:
A. Shepard T.H., Catalog of Teratogenic Agents, 4th edition, 1983.
B. Lewis R.J., Reproductive Active Chemicals- a reference guide, 1991
C. Hardin B.D. et al., Evaluatuion of 60 chemicals in preliminary developmental toxicity test, Teratogen. Carcinog. Mutagen. 7: 29-48, 1987
D. Kavlock et al., Further evaluation of an in vivo teratology screen. teratogen. carcinog. Mutagen. 7: 7-16, 1987.
Since the reliability of the previous mentioned publications cannot be checked, the reliability is set at 4.

20.12.2001

(63)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience : other: Consumer exposure

Result : Dermal consumer exposure

Cosmetics

Consumers can be exposed to numerous cosmetic products containing glycerol such as soaps, bath and shower foams, creams, lotions and deodorants. Creams, lotions and deodorants are expected to remain on the skin for a longer time than the other products mentioned.

Paints, printing inks and resins

Other products containing glycerol, which can result in dermal contact, are the paints, printing inks and resins (like gums). It is anticipated that the maximum concentration of glycerol in these products is 20%.

Migration from Paper and Plastic Articles

Glycerol is also used in papers as softener or flexibiliser. Since the substance is incorporated in the paper matrix migration of the substance is expected to be low (no migration studies are available). Glycerol is also used in cellulose films. From this product glycerol may also migrate. However, the uptake is expected to be low. Both scenarios have not been considered further.

Oral intake by consumers

The following product groups are identified containing glycerol: pharmaceuticals, cosmetics (i.e. toothpaste), cellulose films (edible - meat casings, sausage skins) and food and drinks.

For sausages, the skin is probably 1% of the sausage (in weight) and the skin consists of maximal 5% of glycerol.

Food and drinks

Glycerol is present in the following food and drink products: bakery products, beverages, sweets and candies, flavours, soft drinks concentrates, humectants for edible purposes. Substantial reviews on the safety of glycerol have been prepared by international organisations like the WHO, JECFA and the European SCF.

Inhalation by consumers

Due to the fact that consumer products containing glycerol are almost exclusively liquids, creams or the substance is contained in a matrix, dust formation and hence inhalation is not considered as a relevant exposure path for the public.

The only identified exposure is due to inhalation of cigarette smoke. Cigarettes (1 gram each) contain maximally 5% glycerol, which is probably inhaled by the smoker (uptake 100%).

05.12.2001

Type of experience : other: Worker exposure

Result : Occupational exposure

Occupational exposure to glycerol can occur during production of this substance, during formulation or transformation into other product or during use of product where glycerol is present. The dermal route is considered to be the most relevant exposure route.

Dermal exposure of workers

Paints

To assess the exposure of workers to paint it is assumed that 8 hours a day a worker is painting. The exposure is estimated with Derwin v1.42 to be 0.19 mg cm⁻² day⁻¹. Since both hands are exposed of the painter, the exposure is 440 cm² * 0.19 mg cm⁻² day⁻¹ = 84 mg day⁻¹

Using the mass of the painter (70 kg), the uptake is 1.2 mg kg⁻¹ day⁻¹.

25.01.2002

Remark : Literature could not be retrieved.

Result : Fertility study of 64 male employees engaged in the manufacture of glycerol. Compared with a control group of 63 workers, no significant differences were found in several sperm quality parameters of which sperm counts/mL and percent normal forms are considered to be most reliable.

Source : Unichema Chemie B.V. Gouda
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable

25.01.2002

(125)

Remark : Literature could not be retrieved.

Result : A total of 179 cases with various intracranial disorders was treated by intravenous administration of 10-15% solution in an attempt to control cerebral edema. There was no evidence of significant adverse findings attributable to the administration of glycerol.

Source : Unichema Chemie B.V. Gouda
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable

25.01.2002

(126)

Result : Two cases of adverse effects after oral administration of glycerine in patients.
A 82-year old female (hypertensive, mentally senile) received 200 ml 50% glycerine orally for primary angle closure glaucoma. This woman developed headache, shaking of the arm, quivering of the eyes and nausea.
A 68-year old female (diabetic) received 280 ml 50% glycerine orally within a period of 3 days. This glycerine was felt responsible for the ensuing severe diabetic acidosis.

24.09.2001

(127)

Method	: 14 volunteers (10 men, 4 women) drank orange juice mixed with 30 mL of 95% glycerol after each of the 3 daily meals.	
Result 24.09.2001	: No overt signs of toxicity or effect on food consumption	(128) (102)
Method	: 37 Cases (human) of cerebral edema caused by acute cerebral infarction, were treated daily with 1.2 g/kg of glycerin i.v. or 1.5 g/kg orally. Seventeen other patient with cases of edema of the central nervous system were similarly treated.	
Result 14.11.2001	: During treatment, mortality of the group with cerebral infarction was 11%, however these deaths were not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of 4 days of treatment. No toxic effects were observed which were attributable to glycerin administration.	(127) (129)
Method	: Skin patch test in 15 workers with glycerol diluted 500 to 1000 times with water.	
Result 24.09.2001	: Negative	(90)
Result 24.09.2001	: 1. Acute ingestion of glycerol in male subjects led to an increase in plasma glycerides, the same procedure in women led to no significant change in the glyceride concentration. 2. When glycerol was ingested chronically (42 days), both men and women showed increased serum glyceride concentration, the increase was significantly greater in men, however.	(130)
Remark 24.09.2001	: In case control studies a possible association between exposure to hydrocarbons (one example mentioned is glycerol) and tubular necrosis, chronic tubulointerstitial damage and glomerulonephritis is found. The development of glomerulonephritis appears to mainly a immune mediated disease. No study with glycerol exposure was reported, only general hydrocarbon exposure or other specific substances were mentioned.	(131) (132)
Result 24.09.2001	: A case of acute colonic ischemia following a glycerin enema in preparation for coronary artery bypass surgery was reported.	(133)
Result Source 16.05.1994	: Free glycerol is present in human plasma, and its excretion in the urine stops if levels fall below 1 mg/ml plasma. Croda Universal Ltd Goole, North Humberside EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	(134) (135)

Remark : Literature could not be retrieved.
Result : The Joint FAO/WHO Expert Committee on Food Additives considered that glycerol was of such low toxicity that it was acceptable for use without a formal acceptable daily intake figure being specified.
Source : Croda Universal Ltd Goole, North Humberside
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
 16.11.2001 (136)

Type of experience : other: human; skin irritation

Remark : Literature could not be retrieved.
Result : In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (137)

Remark : Literature could not be retrieved.
Result : Slightly irritating after 48 hours application of 0.05 ml on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (138)

Remark : Literature could not be retrieved.
Result : A strong burning and stinging sensation, with tear production but no injury apparently from contact with the neat chemical.
Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
 25.01.2002 (89)

05.12.2001

5.11 ADDITIONAL REMARKS

Type : adsorption

Remark : Glycerol is readily absorbed after ingestion.
 Literature could not be retrieved.

Source : Unichema Chemie B.V. Gouda
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
 25.01.2002 (139)

Type : Biochemical or cellular interactions

Remark : Because glycerol in mouse studies was found to enhance

24.09.2001	pulmonary tumorigenesis, it was tested for its ability to induce active oxygen formation in lung macrophages treated with 4-nitroquinoline. No effect on active oxygen species formation was found.	(140)
Type	: Biochemical or cellular interactions	
Remark	: Glycerol-related lung tumorigenesis enhancing effects found in tests with mice were inhibited by vitamin E treatment and promoted by treatment with iron. This suggests a mechanism of oxidative stress in the nuclei.	
16.11.2001		(141)
Type	: Biochemical or cellular interactions	
Method	: Male mice were treated with a single s.c. injection of 4NQO alone (n=9) or followed by glycerine (5% solution in drinking water) during 1, 7 or 28 days (3 mice each). 30 minutes before animals were killed they were injected with a BrdU solution in their tail vein. BrdU uptake in epithelial cell of the distal airways was determined using anti-BrdU antibodies.	
Result	: No significant difference in number of BrdU positive cells between 4NQO and 4NQO/glycerol treated mice.	
Conclusion	: Any tumour promoting effect of glycerol may occur independently from pulmonary cell kinetics.	
07.01.2002		(116)
Type	: Cytotoxicity	
Remark	: In concentrations exceeding 20 % glycerol is toxic to bacteria due to dehydration. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
14.11.2001		(142)
Type	: Distribution	
Remark	: Glycerol is distributed over the extracellular space.	
24.09.2001		(143)
Type	: Excretion	
Remark	: Glycerol is removed from the body by the liver (80-90%) and the kidneys (10-20%).	
24.09.2001		(143)
Type	: Metabolism	
Remark	: Rapid transformation takes place either to carbon dioxide or to esters with free fatty acids. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
Reliability	: (4) not assignable	
25.01.2002		(139)
Type	: Metabolism	

Remark	: Glycerine constitutes approx. 10% of the amount of fat present in human food. Aside from furnishing energy by contributing to the general pool of oxidizable organic compounds it has no special role in nutrition. Glycerine is well absorbed from the intestinal tract of rats and dogs. Phosphorylation of glycerine to alpha-glycerophosphate takes primarily place in liver and kidneys. Once glycerine is incorporated into the carbohydrate metabolic pathway it can form glucose and glycogen in addition to being oxidized for energy via the tricarboxylic acid cycle. Further exogenous glycerine also participates in lipogenesis. Literature could not be retrieved.	
Source	: Unichema Chemie B.V. Gouda	
Reliability	: (4) not assignable	(144) (145) (67)
Type	: Metabolism	
Remark	: 1. Glycerol metabolism is regulated by the enzymes glycerol kinase, (cytosolic) NAD ⁺ -dependent G3P dehydrogenase and (mitochondrial) FAD-linked G3P dehydrogenase. 2. Glycerol is readily absorbed in the intestine, slower absorption also occurs in the stomach. Max. serum level is attained within 15 min. following an oral dose of 5 g. 3. A safe oral dose in humans is 1 g/kg bw every 6 hours. A single intravenous dose of 50 g in a 5% solution can be administered without adverse symptoms.	
24.09.2001		(146)
Type	: Metabolism	
Remark	: In lean persons (short fasting) 38% of plasma glycerol (0.054 umol/ml) was turned into glucose and 2.2% into CO ₂ ; In lean persons (long fasting) 76% of plasma glycerol (0.142 umol/ml) was turned into glucose and 5.4% into CO ₂ ; In obese persons (short fasting) 56% of plasma glycerol (0.105 umol/ml) was turned into glucose and 3.1% into CO ₂ ; In obese persons (long fasting) 96% of plasma glycerol (0.180) was turned into glucose and 4.4% into CO ₂ ; Turnover rates of glycerol are directly proportional to plasma glycerol. The major role of glycerol in the body is as a precursor of glucose.	
24.09.2001		(147)
Type	: Metabolism	
Remark	: 1. Continuous intravenous administration of glycerol at 1g/kg bw did not result in increased serum insulin. 2. Glycerol turnover rate is fairly constant at 0.74 g/kg/hour.	
24.09.2001		(67)
Type	: Metabolism	
Remark	: In vitro experiments with rat liver homogenates showed that glycerol inhibits the incorporation of acetate into cholesterol. In vivo experiments with rats showed a significant effect of glycerol on cholesterol synthesis and level. In four out of five experiments cholesterol	

24.09.2001	levels were reduced; it cannot be stated unequivocally however, that glycerol is hypocholesteremic.	(148)
Remark	: Glycerol has a hemolytical potency. With 0.002 ml Glycerol/ml blood hemolysis in a 10% extent was observed for bovine erythrocytes. Pharmacodynamics: glycerol acts as a contact laxative. Literature could not be retrieved.	
Source Reliability 25.01.2002	: Unichema Chemie B.V. Gouda : (4) not assignable	(149) (139)
Remark	: Glycerol may have protective effects against tobacco smoke induced cancer. When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced.	
16.11.2001		(150)
Remark	: 1. The route of administration is of influence on the toxicity of glycerol in humans. Toxic effects, apart from nausea and vomiting, do not occur after oral administration. Toxic effects reported after intraperitoneal and subcutaneous administration are albuminuria, hemoglobinuria, anemia and renal damage. 2. Glycerol has a dehydration effect on the central nervous system. Intraocular pressure begins to fall at plasmaconcentrations of 10 mmoles per liter. 3. Concentration and dilutant used are also of influence on toxicity. Use of saline as dilutant diminishes the toxic effects of glycerol.	
16.11.2001		(143)
Remark	: 1. Subcutaneous injection of glycerol solutions leads to rapid haemoglobinaemia (detectable with the naked eye), followed by haemoglobinuria, in the rat and rabbit. The severity of haemoglobinaemia depends on the total dose of glycerol, not on its concentration. 0.2 cc/kg bw was the lowest effective dose in rats. Guinea-pig and mouse are less sensitive to subcutaneous glycerol injection. 2. Intravenous injection of up to 20 times the lowest effective subcutaneous dose produces no haemolysis in rats.	
14.11.2001		(151)
Remark	: Subcutaneous injection of 1.75 mL of 50% glycerol/100g bw in rats caused severe haemolysis followed by necrosis of the tubular portions of the nephrons, but no apparent damage to the glomeruli. Effects were reversible within 6-12 weeks.	
11.12.2001		(152)
Remark	: Intraperitoneal administration	

	Rats injected intraperitoneally with 1 ml 100% glycerol/100g bw had severe convulsions and died within 2 hours after injection. Rats injected with 1 ml 50% glycerol/100g bw also had severe convulsions and most of them died within 4 hours. Other signs of toxicity were haemoglobinuria, fluid in the peritoneal cavity, dehydrated tissues and renal damage (necrosis of epithelium of the proximal tubules, presence of eosinophilic casts in the loops of Henle, distal convoluted tubules and collecting tubules). Subcutaneous administration of 1 ml 100% glycerol or 50%/100 g bw to rats produced haemoglobinuria, severe oedema at the site of injection, extremely hydrated tissues and renal tubular necrosis; in some animals at 100% glycerol mild convulsions were reported. Intravenous injection of both 100% glycerol and 50% glycerol at 1 ml/100g bw to rats led to severe convulsions and death in all animals (unless kept alive by dextrose-saline injection). Other signs included haemoglobinuria and occasionally renal damage.	
11.12.2001		(153)
Remark	: Intramuscular injection of 50% glycerol in the hind limb of rats at 10 ml/kg bw led to decreased renal bloodflow and oliguria, associated with a reduction of glomerular filtration.	
11.12.2001		(154)
Remark	: Glycerol injected intraperitoneally or taken per os causes hyperglycemia in fasted rabbits.	
16.11.2001		(155)
Remark	: Subcutaneous injections of strong solutions of glycerol in rats produce haemolysis and renal tubular necrosis. ' Similar doses given intravenously produce neither of these effects. Similar doses given intraperitoneally produce renal tubular necrosis but no haemolysis.	
16.11.2001		(156)
Remark	: A preload of glycerol (30 kcal) was given to 12 healthy, non-obese subjects. Effects on food intake were recorded, blood glucose, glycerol and free fatty acids (FFA) were measured. Plasma glycerol was increased before the meal and food intake was lowered when a glycerol preload was taken. No effects on other parameters.	
11.12.2001		(157)
Remark	: 1. The acute ingestion of glycerol (1 ml/kg bw) in male subjects led to an increase in plasma glycerides after 4 hours, the same procedure in women led to no significant change in the glyceride concentration. 2. When glycerol was ingested chronically (1 ml/kg/day, 42	

11.12.2001	days), both men and women showed increased serum glyceride concentration. The increase was significantly greater in men, however.	(130)
Remark	: Glycerol has a dehydrating action on cerebral edema when blood glycerol level reaches 10 mM (920 mg/l).	
11.12.2001		(67)
Remark	: Patients with acute cerebral ischemia may benefit from an infusion with 10% glycerol; this may improve cerebral oxidative phosphorylation or provide the brain with an additional energy source.	
11.12.2001		(158)
Remark	: A three year old boy showed a unique intolerance to glycerol: 1-5 hrs after oral administration of glycerol in doses of 0.5-1.0 g/kg he had euphoria, mental confusion, drowsiness, nausea and vomiting, on one occasion the glycerol also provoked hypoglycemia; intravenously administered glycerol induced an immediate loss of consciousness with spontaneously recovery after 30 min., there were no changes in blood glucose values.	
11.12.2001		(159)
Remark	: Intravenous administration of 20% glycerol lowers acute elevations of intracranial pressure in children with intracranial hypertension. In two cases (of 152) transient hematuria and hemoglobinemia were seen.	
11.12.2001		(160)
Remark	: Female rats received a single i.p. injection of 3.5 mL glycerol (12.5% solution)/ kg bw. Urinary output, creatinine clearance , blood pressure, intrarenal blood flow and kidney histopathology were investigated during a 6 hour observation period. Urinary output and creatinine clearance were decreased. No effects on blood pressure and blood flow through the kidney became apparent. Incidental slight renal damage (necrosis of proximal tubular cells) was seen.	
11.12.2001		(161)
Remark	: Generally recognised as safe as a miscellaneous and/or general food additive under US FDA CFR 21 SS 182.1320 (Glycerin). Literature could not be retrieved.	
Source	: Croda Universal Ltd Goole, North Humberside	
Reliability	: EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
25.01.2002	: (4) not assignable	

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EVALUATION OF CERTAIN FOOD ADDITIVES

Fifty-ninth report of the
Joint FAO / WHO Expert Committee on
Food Additives



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Geneva, 4–13 June 2002

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Toxicological evaluation of certain food additives. WHO Food Additives Series No. 50, in press

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 10. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International programme on Chemical Safety (IPCS).

The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 4 to 13 June 2002. The meeting was opened by Mr D.G. Aitken, Chef de Cabinet, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Aitken noted that a number of general issues were on the agenda, including consideration of papers on risk analysis and intake that had been referred by the Codex Committee on Food Additives and Contaminants (2) to the present Committee for comment. These two papers outline the specific roles of the Expert and Codex Committees in the development of Codex standards. The iterative process by which the two committees consider these and other issues highlights the importance of communication between them and for ensuring that the Expert Committee adequately responds to the needs of the Codex and that the roles of the two committees are properly defined.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (2), there have been fifty-eight previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the fifty-seventh meeting (Annex 1, reference 154).

The tasks before the Committee were:

- to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
- to evaluate certain food additives and flavouring agents (sections 3 and 4 and Annex 2); and
- to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

Annatto extracts, ethyl carbamate, methylmercury and sodium dichloroisocyanurate were removed from the agenda because data necessary for their evaluation were not available. The evaluation of curcumin was deferred until 2003 because the Committee at its fifty-seventh meeting extended the temporary acceptable daily intake (ADI) to that time. Gum arabic was removed from the agenda because new information was not provided on differences in the

origin, manufacture, quality and use patterns of gum arabic from *Acacia senegal* and from *Acacia seyal*.

Nitrite was added to the agenda because the pivotal observed toxic effects of nitrate, which was evaluated at the present meeting, are consequent on its conversion to nitrite in vivo.

The Committee also considered the safety of the secondary components of a large number of flavouring agents for which the minimum assay values were below 95% and confirmation of use as flavours of a number of flavouring agents.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of the food additives on the agenda, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), as well as the principles elaborated at subsequent meetings of the Committee (Annex 1, references 77, 83, 88, 94, 101, 107, 116, 122, 131, 137, 143, 149, 152 and 154), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference 76) embraces the major observations, comments and recommendations contained, up to the time of its publication, in the reports of the Committee and other associated bodies. The Committee noted that the publication reaffirms the validity of recommendations that are still appropriate and points out the problems associated with those that are no longer valid in the light of technical advances.

2.2.1 Safety evaluation of flavouring agents

Flavouring agents with other functional uses

The Committee noted that a small number of the chemicals submitted for evaluation as flavouring agents have other uses in foods, for example as solvents, acidity regulators or preservatives, and that the Committee has previously evaluated them for these uses under the normal procedures for food additives. The Committee re-affirmed its position that the safety evaluation of these chemicals should include consideration of existing safety evaluations for other uses and that their evaluation as flavouring agents should make reference to the previous evaluations.

Flavouring agents that undergo chemical change in food

The Committee noted that some flavouring agents are added to food in the expectation that they will undergo chemical conversion during food processing or storage to form the compounds that provide the flavouring

effect in the food as consumed. Such agents can be evaluated by the Procedure for the Safety Evaluation of Flavouring Agents only if the breakdown and reaction products can be chemically identified. The Committee therefore stressed that information on breakdown and transformation products should be made available in submissions of data on flavouring agents for evaluation.

2.3 **Project to update principles and methods of risk assessment of chemicals in food**

The Committee was informed of progress being made on the Project, which was initiated by FAO and WHO. General principles and methods for the assessment of food additives, contaminants, residues of veterinary drugs and pesticides and food ingredients that have been developed over the years and published in Environmental Health Criteria, Nos 70 (Annex 1, reference 76) and 104 (3) and in reports of JECFA and the Joint FAO/WHO Meeting on Pesticide Residues will be updated and consolidated, and the utility of new assessment procedures will be considered.

The final product will contain a historical background, and the activity will be placed in the context of risk analysis. A framework for incorporation of new principles and methods will be developed. The Project will focus on:

- chemical characterization (including contaminants and natural constituents) and development of specifications for food additives, pesticides and veterinary drugs;
- maximum residue levels for pesticides and veterinary drugs;
- exposure assessment (including short- and long-term intake, deterministic and probabilistic approaches and cumulative and aggregate exposure);
- toxicological test procedures and evaluation (including general issues and specific toxicological end-points);
- human data (clinical studies, epidemiological studies including biomarkers, potentially susceptible populations, allergenicity, intolerance and interactions with the diet);
- evaluations (including such issues as thresholds of toxicological concern, potency estimates, margins of safety, benchmark doses, acute reference doses, special considerations for contaminants, uncertainty and variability, scientific criteria for re-evaluation);
- principles related to specific substances (e.g. flavouring agents and substances consumed in large amounts); and
- a glossary (nomenclature and terminology).

The Committee emphasized the importance of this activity to its work, and encouraged FAO and WHO to proceed with it in a timely manner. It recommended that:

- the approach not be prescriptive, in order to maintain maximum flexibility in evaluating chemicals and in incorporating new methods of assessment;
- the intended audience, including risk managers and potential submitters of data, be clearly defined and kept in mind during all stages of the Project;
- new testing procedures and methods of assessment be validated before they are used; and
- harmonization and cooperation with risk assessment activities of other international and national organizations be considered, as appropriate.

2.3.1 **Specifications of identity and purity of food additives**

The *Project to Update Principles and Methods for Risk Assessment of Chemical in Foods* will include the definition of substances considered for safety by JECFA and the Joint FAO/WHO Meeting on Pesticide Residues. The Committee has a defined process for drafting specifications for food additives; as part of its input to the Project, the Committee considered a paper on the development of food additive specifications that had been prepared for the Project.

In general, the guidance given in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76) for drafting food additive specifications remains valid. However, this document was written before the role of the Committee in risk analysis had been defined, and it does not discuss how formulation of specifications fits into the Committee's work on risk assessment.

In considering this issue, the Committee recommended that:

- The updated principles should explicitly recognize that development of specifications is an integral part of the risk assessment of food additives.
- Risk assessment requires data on the manufacture and composition of an additive at all steps in its development and safety testing. In addition, information is needed on technological function and current and intended uses.
- Risk assessments should include specifications for the material evaluated and the product to be marketed.

- The updated principles should require continuous review of specifications in the light of possible changes in the manufacture, use and consumption of the additive.

2.4 Procedure for evaluating flavouring agents that are members of groups that have been evaluated previously by the Committee

The Committee has evaluated a large number of flavouring agents with the Procedure for the Safety Evaluation of Flavouring Agents. To facilitate the evaluations, the substances have been grouped according to their chemical structure. A number of flavouring agents in development and additional ones in commerce fit into the groups of flavouring agents that have already been evaluated, and the Committee concluded that these flavouring agents should be evaluated and the evaluations documented in a manner consistent with the previous practices and procedures of the Committee. Past evaluations should serve as the basis for evaluating new or additional flavouring agents and, if a substantial body of new data became available, the previous evaluation might need to be reconsidered. The Committee recommended that the *Guidelines for the preparation of working papers (monographs) on flavouring agents*¹ be updated accordingly.

2.5 Risk analysis principles and exposure assessment

The Codex Committee on Food Additives and Contaminants at its Thirty-fourth Session (1) asked JECFA to review and comment on papers that it had prepared, entitled *Application of risk analysis principles for food additives and contaminants* and *Draft principles for exposure assessment of contaminants and toxins in food*. The Expert Committee reviewed these papers and provided comments to the Codex Committee in the form of letters from the Chairman and Vice-Chairman of the present Committee to the Chairman of the Codex Committee.

2.6 Risk analysis terms related to food safety

In considering the definitions of 'hazard' and 'risk' adopted by the Codex Alimentarius Commission for risk assessment, the Committee noted that different definitions have been used by other bodies involved in chemical risk assessment. This could cause problems in communication and interpretation of risk assessments made by different organizations. For example, in the Codex definition, 'hazard' is considered to be *a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect*, whereas some other bodies (e.g. the

¹ Available at <http://www.who.int/pes>

International Union of Pure and Applied Chemistry) consider hazards to be intrinsic chemical and biological properties (potentially adverse effects) of a chemical. JECFA has implicitly tended to the latter interpretation of 'hazard'. Similarly, 'risk' is defined in purely probabilistic terms by some organizations, whereas the Codex definition of 'risk' includes not only the probability of an adverse effect but also its 'severity'. The word 'severity' has been used variously to describe either the magnitude of response or the qualitative nature of the effect of a substance; this difference could also lead to confusion. The term 'severity' is used by the Committee to refer to the magnitude of a response and not to its qualitative nature.

The Committee noted the ongoing Joint IPCS/OECD project to harmonize the terminology used in chemical hazard or risk assessment¹, in which these issues are being addressed, and recommended that the harmonized terminology agreed upon in that project be adopted by the Committee and by the Codex Alimentarius Commission.

2.7 **Consideration of guidelines**

The Committee confirmed that detailed guidelines for the preparation of working papers were required and should be revised regularly on the basis of comments provided by members of the Committee. At its present meeting, the Committee considered guidelines that had been prepared by the Joint Secretariat and made comments and suggestions for improvement.

Comprehensive assessments of the intake of food additives are a recent task of the Committee. Therefore, at the present meeting, specific attention was paid to the *Guidelines for the preparation of working papers on the intake of food additives*. The Committee suggested that intake from dietary sources other than food additives and exposure to non-dietary sources might also be of relevance. The terms for intake assessment used in working papers should be standardized within the Committee and harmonized with those developed by OECD/IPCS (as summarized in the *Glossary of exposure assessment-related terms*²).

A draft guideline for working procedures to be followed by the Committee in elaborating specifications for food additives was considered, and substantial changes and amendments were suggested to the Secretariat.

2.8 **Specifications for flavouring agents**

The Committee concluded that it was not desirable to develop separate specifications for flavouring agents (e.g. benzoic acid) that it has evaluated for other uses. In such cases, the material used for flavouring purposes should comply with the specifications for its use for other technological purposes,

¹ Available at <http://www.ipcsharmonize.org/index.html>

and the list of specifications for flavouring agents should simply make reference to the full specifications monograph.

The Committee recognized that information on secondary components of flavouring agents should be incorporated into specifications when relevant to the safety evaluation. As necessary, this information would include upper limits on the content of such components. The Committee agreed that this information should appear as a distinct item under the heading 'Other requirements'. At the same time, the Committee decided to adjust the format for other items included under 'Other requirements' in the specifications tables to distinguish more clearly between genuine requirements and items included for information. The preamble to the table of specifications published in Food and Nutrition Paper 52 was expanded to explain how the information listed under 'Other requirements' is to be used.

3. **Specific food additives (other than flavouring agents)**

The Committee evaluated several food additives for the first time and re-evaluated others. The evaluations and specifications are summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 **Safety evaluations**

3.1.1 **Alitame**

Alitame is an intense sweetener, with a sweetness potency 2000 times greater than that of sucrose. It is a dipeptide of L-aspartic acid and D-alanine, with a terminal *N*-substituted tetramethylthietanylamine moiety. Alitame is prepared by a multistep synthesis involving the reaction between two intermediates, (*S*)-[2,5-dioxo-(4-thiazolidine)] acetic acid and (*R*)-2-amino-*N*-(2,2,4,4-tetramethyl-3-thietanyl)propanamide. The final product is isolated and purified by crystallization of an alitame-4-methylbenzenesulfonic acid adduct, followed by additional purification steps, and finally recrystallization from water.

Alitame was considered by the Committee at its forty-fourth and forty-sixth meetings (Annex 1, references 116 and 122). Most of the toxicological studies were examined at the forty-fourth meeting, and specifications and a toxicological monograph were prepared; however, an ADI could not be allocated because of concern about deficiencies in the studies of carcinogenicity. At its forty-sixth meeting, the Committee reconsidered this issue in the light of a general discussion of the survival rates of animals in contemporary long-term studies and a further statistical analysis of the data from the two available

studies. At that meeting, the Committee concluded that there was no evidence that alitame is carcinogenic. An ADI of 0–1 mg/kg bw was allocated on the basis of the NOEL of 100 mg/kg bw per day in an 18-month study in dogs. The Committee noted that, although not specifically requested, a further study of tolerance to repeated doses of alitame in persons with diabetes was under way and that, as is customary, the results should be forwarded to the Committee when available.

While consideration of the 90-day study of tolerance in diabetic subjects was on the agenda of the present meeting, it was postponed pending receipt of a final report. The ADI of 0–1 mg/kg bw allocated by the Committee at its forty-sixth meeting was retained.

No data on the dietary intake of alitame were available at the previous meetings of the Committee. At its present meeting, the Committee compared the maximum levels of alitame listed in the Codex draft General Standard on Food Additives (GSFA) with the theoretical maximum level calculated by the budget method (see Annex 1, reference 137). On the assumption that alitame is used in all foods, the theoretical maximum level of alitame, based on the current ADI, was calculated to be 40 mg/kg. The maximum levels in the GSFA are up to 300 mg/kg in a wide range of foods and beverages, with no limit in 'other sugars and syrups' or in table-top sweeteners. Detailed assessments of the intake of alitame when used in foods were therefore required.

National assessments of intake based on recent individual dietary records and GSFA maximum levels were provided by Australia and by New Zealand. These data showed that the ADI might be exceeded by some consumers, at levels representing 148% of the ADI in Australia and 140% of the ADI in New Zealand for consumption at the 95th percentile. These are likely to be overestimates, as the surveys were of short duration (24 h) and because it was assumed that alitame was systematically present in some major food categories. The intake of alitame from 'other sugars and syrups' was not assessed since no manufacturer's use levels were available. In both Australia and New Zealand, the main contributor to the mean estimated intake was sweet bakery wares, which was responsible for 89% of the overall estimated intake from all food categories in Australia and 88% in New Zealand. These estimates are based on data from only two countries, and further work is required to refine the intake estimates with recent data from other countries.

The Committee identified GSFA food category 7 (bakery wares, including bread) as a food group that might contribute to a high intake of alitame, as this group includes foods that are staples in many countries. The Committee therefore suggested that the Codex Committee on Food Additives and Contaminants consider reviewing the standards for alitame.

An addendum to the monograph summarizing the available data on intake was prepared. The specifications for alitame were revised. The addresses of suppliers of some of the chemicals needed for the purity tests were updated. In addition, in line with the decision made by the Committee at its fifty-seventh meeting (Annex 1, reference 154), a brief description of the method of manufacture of alitame was included. The revised specifications also incorporate the heavy metals limits established by the Committee at its fifty-seventh meeting.

3.1.2 **Cross-linked sodium carboxymethyl cellulose**

Cross-linked sodium carboxymethyl cellulose is manufactured by acidifying an aqueous suspension of sodium carboxymethyl cellulose and heating the suspension to achieve cross-linking. The product is then washed and dried. It is also produced during the manufacture of sodium carboxymethyl cellulose by lowering the pH and heating to cause cross-linking. Cross-linked sodium carboxymethyl cellulose is used in tablets of table-top sweeteners and dietary food supplements, as it facilitates disintegration in aqueous solutions, with a maximum level of use of 30 g/kg. It is also widely used as an excipient in pharmaceutical applications.

Cross-linked sodium carboxymethyl cellulose has not been evaluated previously by the Committee. The parent compound, sodium carboxymethyl cellulose, was considered by the Committee in its evaluations of modified celluloses at its fifth, seventh, tenth, thirteenth, seventeenth, twenty-sixth, twenty-seventh, thirtieth and thirty-fifth meetings (Annex 1, references 5, 7, 13, 19, 32, 59, 62, 73 and 88) and of ethyl cellulose and ethylhydroxyethyl cellulose at the twenty-sixth and twenty-seventh meetings (Annex 1, references 59 and 62). At its thirty-fifth meeting, the Committee reviewed new data on seven modified celluloses (Annex 1, reference 89), including sodium carboxy-methyl cellulose. The data consisted of the results of studies in rats on caecal enlargement, effects on caecal flora and developmental toxicity and studies of mutagenicity in vitro. New data were also available from studies in humans, indicating that some individuals may experience laxative effects at a dose as low as 5 g per person per day. The Committee concluded that modified celluloses are of low toxicity and allocated a group ADI 'not specified'¹ to seven modified celluloses: methyl cellulose, ethyl

¹ ADI 'not specified' is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in the individual evaluation, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive that meets this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, sodium carboxy-methyl cellulose, ethyl cellulose and ethylhydroxyethyl cellulose. The Committee pointed out that the laxative properties of these celluloses should be taken into account when they are used as food additives.

Enzymatically hydrolysed sodium carboxymethyl cellulose was evaluated by the Committee at its fifty-first meeting (Annex 1, reference 137). The Committee compared the toxicity and absorption, distribution and metabolism of enzymatically hydrolysed carboxymethyl cellulose with that of the parent compound and concluded that the similarities in the results were consistent with the absence of any toxicologically significant difference between the two. Therefore, the enzymatically hydrolysed form was included in the group ADI 'not specified' with the other seven modified celluloses.

At its present meeting, the Committee reviewed two studies on cross-linked sodium carboxymethyl cellulose and considered its properties in the light of previous evaluations of modified celluloses. Like other modified celluloses, cross-linked sodium carboxymethyl cellulose is considered to be of low toxicity. Because cross-linking renders it insoluble in water, it is less likely to be absorbed than the parent compound.

In a 90-day dietary study in rats, the only treatment-related observations were increased food consumption, decreased body-weight gain and effects on the kidneys of animals receiving cross-linked sodium carboxymethyl cellulose at 50 000 mg/kg of diet, equivalent to 3900–4700 mg/kg bw per day averaged over the study period. The changes in food consumption or body-weight gain were consistent with a 5% reduction in the nutritional content of the diet, as compared with the basal diet fed to control animals, due to addition of the test substance. In contrast to the effects of some other non-digestible substances evaluated previously by the Committee, caecal enlargement was not observed. Mineralization at the corticomedullary junction was observed in the kidneys of some female rats, with a higher incidence in treated groups than in controls. The report equated the renal mineralization to nephrocalcinosis and noted that, although this is a spontaneous change in control female rats, it was related to treatment in female rats fed cross-linked sodium carboxymethyl cellulose at 50 000 mg/kg of diet, but not at 10 000 mg/kg of diet.

Pelvic nephrocalcinosis and corticomedullary nephrocalcinosis were observed in rats given sodium carboxymethyl cellulose or enzymatically hydrolysed carboxymethyl cellulose in a study evaluated by the Committee at its fifty-first meeting (Annex 1, reference 137). In that study, it was also reported that corticomedullary nephrocalcinosis was observed more frequently in females than in males. Because of the inconsistency of the dose–response relationship, however, the Committee at its fifty-first meeting

had not been convinced that these changes were of toxicological concern. The changes observed in the study evaluated at the current meeting appeared to represent a minimal increase in the incidence and severity of a spontaneous lesion, which may be within normal variation. As discussed in the report of the twenty-sixth meeting (Annex 1, reference 59), nephrocalcinosis is a common finding in laboratory rodents. No implications for human hazard have been established. Therefore, the NOEL for cross-linked sodium carboxymethyl cellulose in the above study was 3900 mg/kg bw per day.

A study of developmental toxicity in rats showed no evidence of maternal toxicity or teratogenicity at the highest possible dose of cross-linked sodium carboxymethyl cellulose of 50 000 mg/kg of diet. The NOEL was 4500 mg/kg bw per day.

The Committee noted that evaluation of the results of the 90-day toxicity study was not straightforward, because of nutritional differences between the basal diet given to control animals and the diets supplemented with cross-linked sodium carboxymethyl cellulose. However, taking into account evaluations of other modified celluloses, the Committee concluded that the effects of cross-linked sodium carboxymethyl cellulose were consistent with dietary overloading with a poorly absorbed substance of low toxicity. The Committee included cross-linked sodium carboxymethyl cellulose in the group ADI 'not specified' with the other modified celluloses.

A toxicological monograph and new specifications were prepared.

3.1.3 **Mineral oils (low- and medium-viscosity)**

Explanation

Mineral oils (medium- and low-viscosity) comprise a subgroup of the family of mineral oils and waxes. They are manufactured from crude mineral oils in various refining steps, such as distillation, extraction and crystallization, and are subsequently purified by acid treatment (oleum method) and/or hydrotreatment (catalytic hydrogenation). Mineral oils (medium- and low-viscosity) are mixtures of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling-points greater than 200 °C.

In order to define the materials more clearly, the Committee at its forty-fourth meeting (Annex 1, reference 116) prepared individual specifications for microcrystalline wax and for two groups of mineral oils: mineral oils (high-viscosity) and mineral oils (medium- and low-viscosity). The latter group was furthermore divided into three classes. For the purpose of characterization of the different types of oils and waxes, the criteria viscosity, average relative molecular mass and carbon number at 5% distillation-point were used in the specifications (see Table 1).

Food-grade mineral oils were last evaluated by the Committee at its forty-fourth meeting (Annex 1, reference //6), when four 90-day studies conducted in Fischer 344 rats with a range of mineral oils and waxes representative of materials in commercial use were reviewed. The materials tested included low-, medium- and high-viscosity mineral oils (N10(A), N15(H), P15(H), N70(A), N70(H), P70(H) and P100(H)); paraffin waxes (low-melting-point wax and intermediate-melting-point wax); and microcrystalline waxes (high-melting-point wax and high-sulfur wax). All the substances, with the exception of the microcrystalline waxes, appeared to accumulate in the tissues of the animals to varying degrees, depending on the material and dose. Except for P70(H) and P100(H) oils, there was evidence of accumulation of the mineral hydrocarbons and effects indicative of a reaction to a foreign body at one or more doses. The types of effects seen were similar and included

Table 1

Classification of mineral hydrocarbons

Name	ADI (mg/kg bw) ^a	Viscosity at 100 °C (mm ² /s)	Average relative relative molecular mass	Carbon number at 5% distillation-point
Microcrystalline wax	0–20 ^{a,b}	≥ 11	≥ 500	≥ 25
High-melting-point wax		Not included in the present studies		
Low-melting-point wax	Withdrawn ^c		No specification	
Low-melting-point wax		3.3	380	22
Mineral oil (high viscosity)	0–20 ^a	> 11	> 500	≥ 28
P100		11	520	29
Mineral oil (medium and low viscosity) class I	0–10 ^d	8.5–11	480–500	≥ 25
P70		9.0	480	27
Medium-viscosity liquid petroleum		8.7	480	25
P70(H)		8.6	480	27
Mineral oil (medium and low viscosity) class II	0–0.01 ^{e,f}	7.0–8.5	400–480	≥ 22
N70(H)		7.7	420	23
Mineral oil (medium and low viscosity) class III	0–0.01 ^{e,f}	3.0–7.0	300–400	≥ 17
P15(H)		3.5	350	17
N15(H)		3.5	330	17

P100 oil, crude: paraffinic, viscosity (40 °C): 100 mm²/s; P70 oil, crude: paraffinic, viscosity (40 °C): 70 mm²/s; P70(H) oil, crude: paraffinic, viscosity (40 °C): 70 mm²/s, hydrotreated (catalytic hydrogenation); N70(H) oil, crude: naphthenic, viscosity (40 °C): 70 mm²/s, hydrotreated (catalytic hydrogenation); P15(H) oil, crude: paraffinic, viscosity (40 °C): 15 mm²/s, hydrotreated (catalytic hydrogenation); N15(H) oil, crude: naphthenic, viscosity (40 °C): 15 mm²/s, hydrotreated (catalytic hydrogenation)

^a Established by the Committee at its forty-fourth meeting

^b Group ADI

^c Previous ADI 'not specified'

^d Established at the present meeting

^e Temporary group ADI

^f Extended at the present meeting

focal histiocytosis, increased weights of liver, lymph nodes, spleen and kidneys, granulomas or microgranulomas of the liver, haematological changes typical of a mild, chronic inflammatory reaction and biochemical changes indicative of mild hepatic damage.

The ADI of 0–20 mg/kg bw established at the forty-fourth meeting (Annex 1, reference 116) for mineral oils with the specifications of high-viscosity oils and of high-melting-point and high-sulfur waxes was based on NOELs at the highest dose tested (2% in the diet) in 90-day studies in Fischer 344 rats. The NOELs for all the other materials except P70(H) oil (i.e. class II and III medium- and low-viscosity oils and low-melting-point wax) were based on an increased incidence of histiocytosis in the lymph nodes at the next highest dose. For P70(H) oil, the NOEL was based on an increased incidence of pigmented macrophages in male rats at a dietary concentration of 2%, a minor effect considered of doubtful biological significance. Accordingly, because effects were observed at all doses, the ADIs for low-melting-point and intermediate-melting-point waxes were withdrawn. A group temporary ADI of 0–0.01mg/kg bw was allocated for class II and III medium- and low-viscosity mineral oils, the temporary nature of the ADI being due to uncertainty about the long-term significance of the inflammatory response to accumulated dietary mineral hydrocarbons. A temporary ADI of 0–1 mg/kg bw was allocated for P70(H) oil. These temporary ADIs were extended at the fifty-first meeting (Annex 1, reference 137).

At its forty-fourth meeting, the Committee considered that, although the types of effects seen were essentially reactions to a foreign body, it was possible that a prolonged inflammatory response of the type observed could result in functional changes in the immune system and that this aspect required further investigation. It also noted that the oils and waxes for which high NOELs were observed contained a greater proportion of hydrocarbon components of high relative molecular mass (high carbon number) and had higher viscosities than those materials with a low NOEL, which contained a greater proportion of hydrocarbon components of lower relative molecular mass (low carbon number). The Committee required information about the compositional factors in mineral oils that influence their absorption and toxicity. It also required a study of at least 1 year's duration on one of these materials in Fischer 344 rats, including an assessment of immune function at appropriate times and an investigation of the kinetics of accumulation of the material, to determine whether a plateau is reached. It recommended that a reversal period of 1 year be included, in order to determine whether the granulomatous hepatic lesions observed in rats in the 90-day studies were fully reversible.

At its present meeting, the Committee reviewed a number of new studies, including the results of a combined 2-year study of toxicity and carcino-

genicity and a 1-year study of toxicity with a 1-year recovery period with P70(H) and P100(H) oils (with the specifications of class-I medium- and low-viscosity mineral oils and of high-viscosity mineral oil, respectively) conducted in parallel. In addition, the Committee received and reviewed a number of studies conducted with low- and medium-viscosity mineral oils, including: a 2-year study of the carcinogenicity of a medium-viscosity liquid petroleum (class I medium- and low-viscosity mineral oil); studies of pharmacokinetics and studies of humoral immune function after administration to P15(H) mineral oil (class III medium- and low-viscosity mineral oil) in Fischer 344 and Sprague-Dawley rats; and a 90-day study of histopathological responses and compositional analysis of absorbed hydrocarbons with N15(H), N70(H) and P70(H) oils (classes III, II and I medium- and low-viscosity mineral oils, respectively). Several studies of low-melting-point paraffin wax were also reviewed by the Committee, as they were considered to provide information relevant for the evaluation of low- and medium-viscosity mineral oils relating to the difference in response to mineral hydrocarbons in Fischer 344 and Sprague-Dawley rats.

Toxicological data

In the long-term studies of toxicity, Fischer 344 rats were given a diet containing P70(H) or P100(H) oil, at concentrations that were varied to provide constant doses ranging from 60 to 1200 mg/kg bw per day, for up to 24 months. At 24 months, treatment with either test material was associated with mild treatment-related effects, which included: dose-related increases in the absolute and relative weights of the mesenteric lymph nodes; an increase in the severity of reticuloendothelial-cell hyperplasia (previously described as focal histiocytosis) of the mesenteric lymph nodes; increases in the incidence and/or severity of vacuolation of periportal hepatocytes; and an increased incidence of sinusoidal dilatation in the liver of male rats only, which was not dose-related. These effects were observed even at the lowest dose tested, 60 mg/kg bw per day. There were no treatment-related effects on survival with either material. Neither P70(H) nor P100(H) was carcinogenic in this study.

The effects observed at 12 months were marginal and included a slight increase in the severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes in male and female rats treated with P70(H) at the highest dose, the only dose for which histopathological data were available, as well as statistically significantly increased absolute and relative weights of mesenteric lymph nodes in males at this dose. Because of the marginal nature of these changes at 12 months, the reversibility of the lesions at the end of the recovery period could not be assessed; however, the results did indicate lack of progression of tissue alterations after dietary exposure to mineral hydrocarbons for 12 months.

No special indicators of immune function were measured in the long-term studies, although standard end-points of haematology, clinical chemistry, organ weight and histopathology, indicative of immune function, were evaluated. The only alteration in these end-points was exacerbation of mild cellular infiltration of the mesenteric lymph nodes, which had not been observed previously after administration for 90 days.

Aside from the earlier onset and increased severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes and the greater increase in the weights of mesenteric lymph nodes in response to dietary administration of P70(H), there was little difference in response to the two oils. None of the effects at 24 months was reported in 90-day studies at concentrations up to 2% of the diet (equivalent or equal to 2000 mg/kg bw per day) of either material. The pigmented macrophages observed with P70(H) oil at 2% in the diet (equivalent to 2000 mg/kg bw per day) in the 90-day study reviewed at the forty-fourth meeting, on which the ADI was based, were not observed after 24 months' intake at doses up to 1200 mg/kg bw per day.

Accumulated hydrocarbon was detected in the liver, mesenteric lymph nodes and spleen; the amounts of hydrocarbon could be quantified only in the liver. In the liver, a steady-state level of hydrocarbon accumulation appeared to have been reached in rats treated with the highest dose (1200 mg/kg bw per day) by 3 months, the only dose for which data on the complete time course were available. The concentration of P70(H) reached in the liver at any given time was about twice that of P100(H). The concentrations of mineral hydrocarbons in the liver returned to control levels within 12 months of cessation of treatment.

Medium-viscosity liquid petroleum (corresponding to class I medium- and low-viscosity mineral oils, including P70(H) oil) was not carcinogenic when fed to Fischer 344 rats at a concentration of 2.5% or 5% in the diet (equivalent to 1250 and 2500 mg/kg bw per day). Increased incidence or severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes was also observed in rats of each sex in this study at both dietary concentrations.

The pharmacokinetics of single doses of P15(H) oil given by gavage at a dose of 34 or 340 mg/kg bw over 96 h was studied in female Fischer 344 and Sprague-Dawley rats, with [¹⁴C]1-eicosanycyclohexane as the tracer. In one of the studies, the animals received a 2-week dietary pretreatment with low-viscosity mineral oil. The faeces were the major route of elimination, and urinary excretion was a minor route. Elimination of radiolabel by either route was more rapid in Sprague-Dawley than in Fischer 344 rats. Estimates of the area under the curve for serum concentrations of mineral hydrocarbons indicated that the bioavailability of mineral hydrocarbon was greater in Fischer 344 rats. Rats of this strain also accumulated and retained a larger

proportion of the administered dose in the liver (2% vs 0.1–0.5%) and, to a much lesser extent (about 100-fold), in the mesenteric lymph nodes. While a longer delay in faecal excretion of radiolabel was seen in Fischer 344 rats than in Sprague-Dawley rats in one study, suggesting a strain-related difference in intestinal absorption of mineral hydrocarbons, this difference was not observed in two similar studies. Rather, the results suggested that Sprague-Dawley rats metabolized and excreted radiolabelled mineral hydrocarbon tracer more efficiently than Fischer 344 rats, which exhibited deposition and retention in the liver. Dietary pretreatment with mineral oil did not affect the extent to which hydrocarbon accumulated in the liver of either strain of rats. In another study, the rate of metabolism of *n*-heptadecane by liver microsomes in vitro differed by rat strain, but not sex, in the order Wistar > Sprague-Dawley > Fischer 344. Metabolism of *n*-octadecane was negligible in all strains, consistent with the findings for hydrocarbons with more than 17 carbons in microsomal systems in vitro.

New short-term studies in which the responses of female Fischer 344 and Sprague-Dawley rats to dietary administration of P15(H) oil (for 120 and 90 days, respectively) at concentrations of up to 2% were compared indicated a more severe response in the Fischer 344 strain. The effects seen in these rats were: increased weights of the liver, mesenteric lymph nodes and spleen; granulomatous inflammation of the liver; and reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes. In Sprague-Dawley rats, only statistically significant increases in liver weight and reticuloendothelial-cell hyperplasia of the lymph nodes, mostly of minimal severity, were observed. The increased liver weights in Fischer 344 rats receded during a 30-day recovery period. Reversibility was not assessed in Sprague-Dawley rats.

The results of the new 90-day study in female Fischer 344 rats with low-melting-point wax and with N15(H), N70(H) and P70(H) oils at a dietary concentration of 2% (equal to approximately 2000 mg/kg bw per day at 90 days) confirmed the previous findings in lymph nodes, liver and spleen, with an additional finding of inflammation of the cardiac mitral valve, which had not been seen previously with this material. This effect occurred at a lower incidence than with low-melting-point wax in this study.

The Committee reviewed the results of a study of T-dependent humoral immune function in response to 90 days' administration of P15(H) oil in female Fischer 344 and Sprague-Dawley rats. No effects were observed in the Sprague-Dawley rats. Interpretation of the results in the Fischer 344 rats was complicated by the increase in spleen weight that resulted from treatment. No data were provided to determine whether the increased spleen weight was associated with a change in the proportion of spleen cell types, which could alter interpretation of the results.

The responses of female Fischer 344 and Sprague-Dawley rats to mineral hydrocarbons were also compared in a 90-day toxicity study with low-melting-point wax at a dietary concentration of 0.2% or 2.0%. This substance had induced the most severe effects in similar studies in Fischer 344 rats with a range of food-grade mineral hydrocarbons, in which, in general, female rats had a stronger response to these materials. The results for Fischer 344 rats in the new study confirmed the findings of the previously evaluated 90-day studies with low-melting-point wax: altered haematological and hepatic clinical chemical parameters, increased weights of the liver, mesenteric lymph nodes and spleen; hepatic vacuolation (affecting both hepatocytes and Kupffer cells) and granulomatous inflammation; reticuloendothelial-cell hyperplasia of mesenteric and cervical lymph nodes; and focal inflammation of the cardiac mitral valve. In addition, increased severity of extramedullary haematopoiesis of the spleen, differential effects on serum immunoglobulin concentrations and lipid-like material in crystalline form in hepatocytes and Kupffer cells were observed in this strain. In contrast, the only effects observed in female Sprague-Dawley rats were increased liver weights and slightly increased reticulo-endothelial-cell hyperplasia of the mesenteric lymph nodes and severity of hepatocellular and Kupffer-cell vacuolation. The crystalloid structures and inflammatory response in the liver were absent in this strain.

Tissue accumulation of mineral hydrocarbon after administration of low-melting-point wax also showed a clear strain difference in terms of degree, onset and the tissues affected. Quantifiable amounts of hydrocarbon were detected in the liver and mesenteric lymph nodes of Fischer 344 rats in a time- and dose-related manner, starting at 30 days in the livers of animals at the higher dose and occurring in both tissues in animals at both doses by 90 days. However, only the mesenteric lymph nodes of Sprague-Dawley rats had quantifiable amounts of hydrocarbon, and only at 90 days in animals at the higher dose. This observation was confirmed in a qualitative investigation of the accumulation of low-melting-point wax in the liver and Kupffer cells of female Fischer 344 and Sprague-Dawley rats after administration of a diet containing 2% wax for 14, 30 or 60 days. Detectable amounts were found in liver cells of Fischer 344 rats by 14 days, but none was found at any time in Sprague-Dawley rats.

In a separate study of low-melting-point wax, feeding a diet containing 2% resulted in alterations in Kupffer-cell function and morphology after 60 days in female Fischer 344, but not Sprague-Dawley, rats. Effects were also seen on selected parameters (i.e. leukocytes, serum liver enzyme activities and hepatic histopathology) that have been associated with administration of low-melting-point wax in this strain, and these were accompanied by accumulation of mineral hydrocarbon in the liver.

No new information was available on the compositional elements of mineral oils that affect their absorption or toxicity.

As the materials tested in the long-term studies reviewed at the present meeting, P70(H) and P100(H) oils, were not associated with induction of liver granulomas in Fischer 344 rats, the studies did not help the Committee to determine the long-term consequences or reversibility of the liver granulomas that had been seen in previous studies in response to consumption of low- and medium-viscosity mineral oils and low-melting-point waxes by Fischer 344 rats. In addition, the Committee was unable to interpret the effects in the study of humoral immune function in response to dietary administration of P15(H) oil.

The results of the studies on the effects of P15(H) oil and low-melting-point wax in Fischer 344 and Sprague-Dawley strains indicated that the more extensive response of Fischer 344 rats, in particular that of females, is associated with greatly enhanced retention of mineral hydrocarbons in the tissues, which is probably due to a reduced ability to metabolize absorbed hydrocarbons. The Committee concluded that additional studies are needed in order to determine whether the Fischer 344 rat is an appropriate model of human response to dietary intake of food-grade mineral hydrocarbons. In particular, elucidation of the metabolic differences between Fischer 344 rats and other strains and species, including humans, would be useful.

Neither P70(H) nor P100(H) oil was carcinogenic in the combined study of toxicity and carcinogenicity reviewed by the Committee at its present meeting. The effects observed even at the lowest dose, i.e. enhanced reticuloendothelial-cell hyperplasia, increased weights of mesenteric lymph nodes and increased incidence and grade of vacuolation of hepatocytes, were shown not to progress to more severe effects, and there was no indication that accumulated test material contributed to suppression or activation of an inflammatory response. Consequently, these effects were considered to be indicators of exposure to mineral hydrocarbon rather than adverse effects. At the present meeting, the NOEL for P70(H) oil was identified as the highest dose tested in the combined study of toxicity and carcinogenicity in rats, 1200 mg/kg bw per day, to which a safety factor of 100 was applied. An ADI of 0-10 mg/kg bw was allocated for class I medium- and low-viscosity mineral oils, which include P70(H) oil. An ADI of 0-20 mg/kg bw for P100(H) oil already exists (see Table 1).

No data were available that would permit allocation of a full ADI for medium- and low-viscosity mineral oils in classes II and III. The Committee noted that the new information reviewed at the present meeting indicated that the observed effects of these mineral oils, on which the temporary ADI is based, may be strain- and sex-specific. The Committee therefore extended the

temporary group ADI of 0–0.01 mg/kg bw for class II and III medium- and low-viscosity mineral oils until 2006, pending information on the relevance to humans of the response of Fischer 344 and Sprague-Dawley rats to these materials. In order for the data to be applicable to as wide a range of mineral oils as possible, the Committee suggested that commercial mineral oils of the lowest viscosity be used in such studies. Additional studies might be required, depending on the outcome of these studies.

Intake

The major food uses of mineral oils are in vegetable and fruit coatings and in packaging, bakery and confectionery manufacture and grain and seed dust control, and these uses are also the major sources of dietary intake of mineral oils in some countries. Dietary intake was assessed from data on the levels of use in foods and on migration from coatings and packaging materials into foods, combined with national data on food consumption in the 1990s. In both the United Kingdom and the USA, the average total intake of mineral hydrocarbons (excluding petroleum jelly) from food use was estimated to be 0.47 mg/kg bw per day; the intake at the 90th percentile of consumption by the population of the United Kingdom was 0.80 mg/kg bw per day. Class III medium- and low-viscosity mineral oils (including P15(H) oil) accounted for 0.21 mg/kg bw per day in the United Kingdom and 0.25 mg/kg bw per day in the USA; these values are 21 and 25 times the temporary ADI of 0–0.01 mg/kg bw, respectively, whereas class I and II medium- and low-viscosity mineral oils and high-viscosity mineral oil (including P70(H) and N70(H) oils, respectively) accounted for 0.18 and 0.19 mg/kg bw per day, respectively. As these different categories of mineral oil have different ADIs, but data on intake are not available for separate categories, the intake of each category cannot be compared with the corresponding ADI. Use of solid hydrocarbons (e.g. microcrystalline wax (high-melting-point wax) and paraffin wax (low-melting-point wax)) accounted for the remainder of the total intake.

The intake of high-viscosity and class I, II and III medium- and low-viscosity mineral oils that have migrated into food from coating and packaging materials was estimated to be 0.001 mg/kg bw per day in both the United Kingdom and the USA, while the combined intake of paraffin wax and microcrystalline wax from this source was estimated to be 0.005 and 0.006 mg/kg bw per day, respectively.

Naturally occurring hydrocarbons are widely distributed in many edible plants and animals, and they contribute significantly to the overall dietary intake of hydrocarbons. For example, the dietary intake of naturally occurring hydrocarbons was estimated to be 0.47, 0.25 and 0.19 mg/kg bw per day in the populations of the European Union, the United Kingdom and the USA,

respectively. It is clear, therefore, that account should be taken of intakes from naturally occurring hydrocarbons when evaluating the safety of mineral oils.

Mineral oil is included in the Codex draft GSFA in numerous food categories, with maximum use levels of 200–3000 mg/kg and, in some categories, in accordance with good manufacturing practice. The Committee suggested that the Codex Committee on Food Additives and Contaminants consider reviewing the standards for mineral oil, particularly in view of the fact that the intake of class III medium- and low-viscosity mineral oil is more than 20 times higher than its temporary ADI of 0–0.01 mg/kg bw.

An addendum to the toxicological monograph, including data on intake, was prepared. The existing specifications for mineral oils (medium- and low-viscosity) were revised, with minor editorial changes.

3.1.4 **Nitrate and nitrite**

Nitrite

Explanation

Nitrite occurs in the environment, in food and water, and is produced endogenously. It is also used as a food additive, mainly as a preservative, antimicrobial agent and colour fixative. It is used in foods such as cheese and cheese products, raw and processed meats, edible casings, processed fish and fish products and roe.

Nitrite was reviewed by the Committee at its sixth, eighth, seventeenth, twentieth and forty-fourth meetings (Annex 1, references 6, 8, 32, 41 and 116). At its sixth meeting, the Committee allocated an ADI of 0–0.4 mg/kg bw to this substance, expressed as sodium nitrite. This ADI was based on a marginal reduction in body-weight gain at a dose of 100 mg/kg bw per day in a long-term study in rats. At its seventeenth meeting, the Committee lowered the ADI for sodium nitrite to 0–0.2 mg/kg bw and made it temporary. At that time, the Committee used a safety factor higher than normal (500) because the ADI was based on a marginal effect and there is a possibility of endogenous formation of *N*-nitroso compounds from nitrite and *N*-nitrosatable compounds present in food and the gastrointestinal tract. At its twentieth meeting, the Committee considered the reports of a WHO task group and of a working group of the International Agency for Research on Cancer on *N*-nitroso compounds but concluded that they did not provide sufficient evidence to revise the temporary status of the ADI.

After that meeting, numerous toxicological and epidemiological data became available, which the Committee considered at its forty-fourth meeting. Nitrite both with and without nitrosatable precursors was found to be genotoxic in vitro in several tests, but, with the exception of a test in *Drosophila*

melanogaster, the results obtained in vivo were negative. The results of carcinogenicity studies with nitrite were negative, except those in which extremely high doses of both nitrite and nitrosatable precursors were administered simultaneously or premixed in the diet. In addition, the epidemiological data provided no evidence for an association between exposure of humans to nitrite and nitrate and the risk for cancer.

At its forty-fourth meeting, the Committee noted that several controlled laboratory studies had shown that *N*-nitroso compounds are formed endogenously when both nitrite and *N*-nitrosatable compounds are present at high concentrations; it observed, however, that quantitative data were available only on those *N*-nitroso compounds that are readily formed endogenously, such as *N*-nitrosoproline, which is not carcinogenic. As there was no quantitative evidence of the endogenous formation of carcinogenic *N*-nitroso compounds at the levels of intake of nitrite and nitrosatable precursors achievable in the diet, a quantitative risk assessment of nitrite on the basis of endogenously formed *N*-nitroso compounds was considered to be inappropriate. The Committee at its forty-fourth meeting therefore based its safety evaluation on studies of toxicity with nitrite. The NOELs in these studies were 5.4 mg/kg bw per day (expressed as nitrite ion) in a 90-day toxicity study in rats, in which hypertrophy of the zona glomerulosa was observed, and 6.7 mg/kg bw per day (expressed as nitrite ion) in a 2-year study in rats in which effects on the heart and lungs were observed. On this basis, the Committee allocated an ADI of 0–0.06 mg/kg bw, expressed as nitrite ion, by applying a safety factor of 100.

At its present meeting, the Committee reviewed the data that had become available on nitrite since its forty-fourth meeting, including data on the relevance and adverse nature of certain effects, with new information on the mode of action of effects on the adrenals and on toxicokinetics.

Toxicological and epidemiological data

The Committee considered a new study of toxicokinetics in human volunteers given sodium nitrite in their drinking-water. The maximum plasma nitrite concentrations were observed 15–30 min after dosing, and nitrite disappeared rapidly from plasma, with a half-life of approximately 30 min. The bioavailability of sodium nitrite was similar and virtually total after low and high oral doses. An intravenous dose of sodium nitrite of 290–380 mg per person induced methaemoglobinaemia, with maximum percentages of 8.4–12%. After oral administration, the maximum concentration of methaemoglobin was reached 0.70 h after dosing.

Several new studies were available on the acute and short-term toxicity of nitrite in humans, in which the severity of methaemoglobinaemia was reported after accidental, high intake of nitrite. Most were case reports of poisoning

incidents. None of these studies added data that could be used for the safety evaluation.

The Committee considered studies recently completed within the National Toxicology Program in the USA. In 14-week studies of the toxicity of sodium nitrite administered in drinking-water to mice at doses equal to 0, 90, 190, 340, 750 or 990 mg/kg bw per day and to rats at doses equal to 0, 30, 55, 120, 200 or 310 mg/kg bw per day, elevated levels of methaemoglobinaemia were observed in rats in all treatment groups, but not in mice. The Committee considered a level of methaemoglobin formation of up to 5% not to be adverse. The lowest NOEL value was observed in a study in male rats and was equal to 55 mg/kg bw per day (37 mg/kg bw per day expressed as nitrite ion) on the basis of reduced sperm motility at higher doses. Reduced sperm motility was also seen in mice at higher doses. The results of 2-year carcinogenicity studies with sodium nitrite added to drinking-water of mice and rats at a concentration of 750, 1500 or 3000 mg/l (equal to 45, 90 and 160 mg/kg bw per day in mice and to 35, 70 and 130 mg/kg bw per day in rats) led to the conclusion that nitrite is not carcinogenic in rats or male mice. The authors noted that there was equivocal evidence of carcinogenic activity of sodium nitrite in female mice, in view of the positive trend in the incidence of squamous-cell papilloma and carcinoma (combined) in the forestomach. As no evidence of genotoxicity in vivo or of cytotoxicity in the forestomach was found, the mode of action of sodium nitrite in inducing forestomach neoplasia is unclear.

A study of reproductive toxicity in mice given drinking-water containing sodium nitrite at concentrations up to 0.24% w/w (equivalent to 240 mg/kg bw per day) did not reveal adverse effects on reproductive performance or on end-points examined at necropsy.

New studies on genotoxicity were available. Sodium nitrite was mutagenic in a test for reverse mutation but did not induce micronucleus formation in the bone marrow or peripheral erythrocytes of mice treated in vivo, consistent with previous results.

A few studies on the endogenous formation of *N*-nitroso compounds after intake of nitrite or nitrate and of nitrosatable compounds (amines and amides) and the possible association with cancer were available. In addition, one long-term study of toxicity and carcinogenicity was reviewed, in which rats were fed fishmeal at various concentrations concomitantly with sodium nitrite. Dose-dependent increases in the incidences and multiplicity of atypical renal tubules, adenomas and renal-cell carcinomas were found. The concentration of *N*-nitrosodimethylamine in the stomach contents after 4 weeks of treatment with 64% fishmeal plus 0.12% sodium nitrite was twice that measured after administration of 8% fishmeal plus 0.12% sodium nitrite.

However, the diets used in this study were considered to be nutritionally inappropriate for the rat: they had a high protein content, resulting in abnormally high renal nitrogen clearance. These studies did not provide additional insight for the safety evaluation of nitrite.

A number of epidemiological studies of the relationship between the intake of nitrite and cancer risk have been published since the forty-fourth meeting. At its present meeting, the Committee ranked the study designs according to their capacity to provide evidence of a relationship. In the descriptions below, relative risk estimates are given for those studies in which levels of intake of nitrite were provided. Four of the studies were cross-sectional, involving measurement of nitrite in, e.g., salivary or gastric juice in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore, these studies cannot contribute to a causal interpretation of the results of studies of nitrite and cancer risk.

Nine (longitudinal) case-control studies on previous nitrite intake and various cancer types were reviewed. For oral and laryngeal cancer, no association was found with nitrite intake. One study conducted in the USA reported a positive association with oesophageal cancer, with odds ratios of 1.0 (reference category), 1.2 and 1.6 for persons with a daily nitrite intake of < 1.1 mg, 1.1–1.6 mg and > 1.6 mg, respectively. The odds ratios and the trend across odds ratios were not statistically significant, however. The association between nitrite intake and oesophageal cancer was stronger, and it was significant for persons with a history of canker sores. Another study in the USA, however, found no association between nitrite intake and oesophageal cancer, nor with the subtypes adenocarcinoma and squamous-cell carcinoma; a positive association was found only with gastric cancer other than of the cardia. A positive association with gastric cancer was also reported in an Italian case-control study (average consumption, 2.4 mg/day), while no association was found in a French study (average consumption, 1.9 mg/day).

An association of borderline significance was found between nitrite intake and urinary bladder cancer in men but not women of Japanese descent, nor in whites of either sex, in Hawaii, USA. Although a positive association was reported from a study in the USA between brain tumours in children and their mothers' consumption of processed meat, no association was found with nitrite intake during gestation or in childhood in a recent case-control study from Israel. One study on nasopharyngeal cancer among Taiwanese reported no association with nitrite intake in adulthood (as reported by cases and controls), but a positive association was found with childhood nitrite

intake as recalled by the mothers of the cases and controls. The validity of recall of remote dietary intake is questionable, however.

Two prospective cohort studies have been conducted on nitrite intake and cancer risk. A cohort study from the Netherlands, with 6 years of follow-up, on dietary nitrite and gastric cancer risk reported relative risks of 1.0 (reference category), 1.2, 1.2, 0.9 and 1.4 for increasing mean quintiles of nitrite intake of 0.01, 0.04, 0.09, 0.16 and 0.35 mg/day, respectively. Neither the relative risks nor the trend was significant. A Finnish cohort study, with 24 years of follow-up, reported no association with the incidence of stomach, colorectal, or head-and-neck tumours. The average nitrite intake by this cohort was reported to be 5.3 mg/day.

Thus, some studies indicated increased risks for oesophageal and gastric cancer; however, other studies — particularly prospective cohort studies — revealed no such association. The results for brain tumours in children and for urinary bladder cancer in adults were equivocal. Wide variation between the studied populations in the recorded intake of nitrite was noted. In none of these studies was a possible interaction between nitrite and nitrosatable amines evaluated in respect of cancer risk. The results of these studies and those of the epidemiological studies considered by the Committee at its forty-fourth meeting do not provide evidence that nitrite is carcinogenic to humans. In addition, studies on nitrate intake and cancer risk (of relevance because of the conversion of nitrate to nitrite) also did not provide evidence of a positive association.

A 6-week study was performed in young adult and old rats to determine whether older animals are more sensitive to the toxic effects of nitrite, particularly with regard to the known effects on the kidney and on the functions of some hormones (insulin, thyroxine). No age-related differences were found. The hypertrophy of the zona glomerulosa in the adrenals of rats of this strain was seen only at an intake equivalent to 50 mg/kg bw per day, expressed as nitrite ion.

Several studies were available on the mechanism of action of nitrite on the vascular system, blood pressure and the adrenals in rats. Doses of 50 mg/kg bw or higher, expressed as nitrite ion, are known to lower blood pressure by causing vasodilatation. The Committee calculated that an oral dose equivalent to 160 mg/kg bw of nitrate ion induced a reduction in blood pressure of 15–20 mm Hg.

Administration of nitrite to rats resulted in minimal hypertrophy of the zona glomerulosa of the adrenal gland and secretion of aldosterone, which was reversible after 60 days. Inhibition of angiotensin-converting enzyme indicated that the effect was produced indirectly via stimulation of the renin–angiotensin axis.

The Committee concluded that the minimal hypertrophy reflected physiological adaptation to small fluctuations in blood pressure and should not be considered a direct toxic action on the adrenals. This conclusion implies that the safety evaluation should not be based on the NOEL for minimal hypertrophy of the adrenal zona glomerulosa, used by the Committee at its forty-fourth meeting, but on NOELs for other end-points. The NOEL of 5.4 mg/kg bw per day (expressed as nitrite ion) was therefore considered to be no longer relevant, as it was based on the indirect effect on the adrenals described above. The Committee established an ADI of 0–0.07 mg/kg bw, expressed as nitrite ion, on the basis of the NOEL of 6.7 mg/kg bw per day for effects on the heart and lung in a 2-year study in rats and a safety factor of 100.

Nitrite causes methaemoglobinaemia. As this might occur after a single dose, it would be appropriate to establish an acute reference dose for nitrite. The data available for review at the present meeting related primarily to its long-term toxicity and were not relevant for establishing an acute reference dose. The Committee recommended that the acute toxicity of nitrite be reviewed at a future meeting.

Intake

Ideally, all sources of nitrite should be included in an intake assessment; however, only food and water were included in the assessments made at this meeting. The intake of nitrite from non-food additive sources, on the basis of the concentrations in food derived from European studies and the GEMS/Food regional diets and with an addition for water was below the ADI (40–50% of the ADI for the various diets). Major contributors to the estimated intake from sources other than food additives are cereals (35–60% of the estimated intake) and water (20–40% of the estimated intake).

The estimated intakes from use of nitrite as a food additive derived from national assessments were above the ADI, assuming maximum levels in the Codex draft GSFA. The intake of nitrite by consumers at the mean represented 460–500% of the ADI, while that of consumers at the 95th percentile represented 1300–1400% of the ADI. The intakes of nitrite from food additive use based on maximum permitted levels in national standards also exceeded the ADI (consumers at the mean, 100–130% of the ADI; consumers at the 95th percentile, 320–360% of the ADI). The estimated intakes based on the GSFA do not reflect reality, as the calculations include the GSFA maximum levels, which often are significantly higher than the typical levels of addition to foods by manufacturers. The calculations also do not take into consideration degradation of nitrite over time and do not reflect the concentrations in foods as consumed. The intakes are based on 24-h food consumption data, which tend to result in overestimates of intake by consumers at high percentiles.

More realistic estimates of the intake of nitrite from all dietary sources, submitted in total diet and duplicate diet studies, showed mean intakes representing 5–15% of the ADI, although the intakes by consumers at higher percentiles approached the ADI and exceeded it in one study, representing 55–330% of the ADI. A major contributor to the total intake estimated from these studies was beverages (specific types not stated), which accounted for 30–35% of the total estimated intake, owing more to the high volumes consumed than to high concentrations of nitrite. Analysed concentrations in foods as consumed were used in these studies. These results confirm that the estimates of intake from use of nitrite as a food additive at the listed maximum levels in the GSFA or national maximum permitted levels are overestimates.

The Committee recommended that assessments of the intake of nitrite should also include sources other than food additives, such as vegetables and drinking-water. Studies with more accurate methods of assessment should be conducted, based on analysed concentrations of nitrite in foods. Furthermore, foods that are ‘ready to consume’ should be included to account for losses of the chemicals over time and during food storage, preparation and cooking, as occurs for nitrite.

The Committee recommended that the Codex Committee on Food Additives and Contaminants reconsider the list of maximum levels of nitrite in the GSFA, as the estimated intakes might exceed the ADI.

An addendum to the toxicological monograph, including an assessment of intake, was prepared.

Nitrate

Explanation

Nitrate occurs in the environment, in air, food (particularly in vegetables and fruits) and water, and is produced endogenously. It is also used as a food additive, mainly as a preservative and anti-microbial agent. It is used in foods such as cheese and cheese products, raw and processed meats, edible casings, processed fish and fish products and spirits and liqueurs.

Nitrate was reviewed by the Committee at its sixth, eighth, seventeenth and forty-fourth meetings (Annex 1, references 6, 8, 32 and 116). At its sixth meeting, the Committee allocated an ADI of 0–5 mg/kg bw, expressed as sodium nitrate. This ADI was based on a NOEL of 500 mg/kg bw per day for body-weight gain at a higher dose in a long-term study in rats and a short-term study of toxicity in dogs, with a safety factor of 100. This ADI was retained by the Committee at its eighth and seventeenth meetings. After the latter meeting, numerous toxicological and epidemiological data became

available, which were considered by the Committee at its forty-fourth meeting.

At that meeting, the Committee concluded that nitrite should also be considered, because nitrate is readily converted in humans to nitrite. The rat was considered an unsuitable animal model for assessing the toxicity of nitrate, as it does not convert nitrate to nitrite in a quantitatively similar way to humans. Nevertheless, as the data on the toxicity of nitrate in other animal species were considered limited, the toxicity of nitrite in rats and the conversion rate of nitrate to nitrite were also evaluated.

The Committee at its forty-fourth meeting concluded that nitrate itself was not genotoxic, and the results of studies of carcinogenicity with nitrate were negative except when extremely high doses of both nitrate and nitrosatable precursors were administered. The available epidemiological data were considered to provide no evidence for an association between exposure of humans to nitrite and the risk for cancer. On the basis of this information, the NOEL of 370 mg/kg bw per day, expressed as nitrate ion, in a long-term study in rats was considered to be the most appropriate for the safety evaluation. When the proportion of nitrate converted to nitrite in humans was taken as 5% for the average individual and 20% for those with a high level of conversion, and when the NOEL for nitrite (6 mg/kg bw per day, expressed as nitrite ion) was used to calculate the 'transposed' NOEL for nitrate, expressed as nitrate ion, these values were estimated to be 160 and 40 mg/kg bw per day for average and high responders, respectively. As these figures were derived in part from data on human pharmacokinetics, use of a safety factor of less than 100 was considered to be justified.

On the basis of the NOEL of 370 mg/kg bw per day, expressed as nitrate ion, and a safety factor of 100, an ADI of 0–5 mg/kg bw expressed as sodium nitrate or 0–3.7 mg/kg bw expressed as nitrate ion was allocated. On the basis of the 'transposed' NOEL for nitrate of 160 mg/kg bw per day for normally responding persons (5% rate of conversion) and a safety factor of 50, an ADI of 0–3.2 mg/kg bw, expressed as nitrate ion, could be allocated. These two methods of deriving the ADI for nitrate thus resulted in similar figures, and the Committee at its forty-fourth meeting therefore retained the previously established ADI of 0–3.7 mg/kg bw, expressed as nitrate ion.

The Committee at its present meeting reviewed data relevant to the evaluation of nitrate which had become available since its forty-fourth meeting. Studies illustrating the relevance and severity of certain effects and other supporting data were also considered. New information on metabolism and toxicokinetics were included. Data on the putative health benefits of nitrate were not assessed, as this is not a safety issue and is therefore not within the purview of the Committee.

The few new studies on the toxicokinetics and metabolism of nitrate in animals that have become available since the forty-fourth meeting of the Committee confirm that the rat is not a good surrogate species for humans in this respect, as it does not show salivary transport of nitrate and therefore has limited conversion of nitrate to nitrite.

In a study of the conversion of nitrate to nitrite in humans, in which sodium nitrate was administered in drinking-water at a single dose of 7.3 mg/kg bw, expressed as nitrate ion, neither blood pressure nor methaemoglobin concentration was affected. The nitrite concentration of the gastric juice was approximately six times higher after administration of nitrate in combination with pretreatment with omeprazole at 40 mg/day (which increased the gastric pH) than after nitrate alone. Nitrate was absorbed rapidly, the concentration in plasma increasing within 10 min, and the half-life of nitrate in plasma was about 6.5 h; about 70% of the dose was excreted in urine within 10 h of dosing. The plasma concentration of nitrite did not change after nitrate administration. About 8% of the total nitrate administered was converted to nitrite in saliva, as found in other studies. The Committee at its forty-fourth meeting concluded that the range of nitrate conversion is 5–7% for normal individuals and 20% for individuals with a high rate of conversion.

The results of studies in humans that considered the potential of high intake of nitrate to cause methaemoglobinaemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinaemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinaemia. No increase in methaemoglobin concentration was seen in volunteers after a single administration of sodium nitrate in drinking-water providing a dose of 7.3 mg/kg bw, expressed as nitrate ion.

A study in humans showed that nitrate in vegetable matrices and from other sources, such as drinking-water, is almost totally bioavailable.

As nitrate shares a common transport mechanism with iodide, studies were conducted to determine whether nitrate affects thyroid function. A 28-day study with volunteers given sodium nitrate in drinking-water at a concentration equivalent to 15 mg/kg bw per day (11 mg/kg bw per day expressed as nitrate ion) showed no effects on thyroid function and no increase in the per cent of methaemoglobinaemia. A 90-day study of toxicity in rats showed that sodium nitrate at a dose of 50 mg/kg bw per day did not affect the thyroid or the zona glomerulosa of the adrenals.

In studies in humans, consumption of drinking-water containing sodium nitrate at a concentration of 2800 mg/l concomitantly with volatile *N*-nitro-

satable amines in the diet (in cod, salmon or shrimp) led to a two- to threefold increase in urinary excretion of *N*-nitrosodimethylamine and *N*-nitroso-piperidine.

Several studies were reviewed on the effect of administration of nitrate on the release of nitric oxide at the junction of the oesophagus and the stomach in humans, which, it was speculated, might be associated with an increased incidence of cancer at this site. However, no such association has been observed in epidemiological studies.

A number of epidemiological studies have been published since the forty-fourth meeting of the Committee on the relationship between nitrate intake and cancer risk. At its present meeting, the Committee ranked the study designs according to their capacity to provide evidence of a relationship. In the descriptions below, relative risk estimates are given for those studies in which levels of intake of nitrate were provided.

Six ecological (correlation) studies were reported on nitrate in drinking-water and mortality from or incidence of cancer. Elevated risks were found for prostate cancer and for brain tumours (each in one study), but the results of six studies on gastric cancer were conflicting. The results of ecological studies (in which populations are the units of measurement) cannot be extrapolated to the individual level. Furthermore, most of the ecological studies were based on limited data on nitrate concentrations and on cancer mortality rates (rather than incidence rates), and none took an induction period for cancer into account.

Three of the studies were cross-sectional, involving measurement of, e.g., salivary nitrate in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore these studies could not contribute to a causal interpretation of the results of studies of nitrate intake and cancer risk.

Seven case-control studies on nitrate in drinking-water and/or food and cancers at various sites were reviewed. In the studies on nitrate in drinking-water, conflicting results were reported with regard to an association with non-Hodgkin lymphoma, and no association was found with brain tumours. In the studies on dietary nitrate, no association was found with oral, oesophageal, gastric or testicular cancer. No other cancer sites have been studied.

Three prospective cohort studies have been conducted on nitrate intake and cancer risk. A cohort study in the Netherlands, with 6 years of follow-up, found no significant association between the incidence of gastric cancer and

intake of nitrate from food or drinking-water, with relative risks for increasing quintiles of total nitrate intake of 1.0 (reference quintile), 1.2, 0.7, 0.9 and 0.9 for mean intakes of 60, 85, 100, 120 and 180 mg/day, respectively. Neither the relative risks nor the trend across relative risks was significant. A further analysis of the effect of nitrate within tertiles of vitamin C intake also did not reveal a positive association between nitrate intake and gastric cancer. A Finnish cohort study on dietary nitrate, with 24 years of follow-up, reported no association with the risks for tumours of the stomach, colorectum or head and neck. The average nitrate intake in this cohort was reported to be 77 mg/day. A cohort study in Iowa, USA, with 11 years of follow-up, revealed no consistent association between intake of nitrate from drinking-water and the risks for cancers at many sites, and an inverse association was reported with cancers of the uterus and rectum. Positive associations with nitrate intake were observed only for cancers of the ovary and urinary bladder, although it was not possible to determine whether other factors in drinking-water were responsible for these associations. In addition, no evidence of a dose-response relationship was found for any of the cancer sites addressed in the study in Iowa. The cohort studies included control for various potential confounders, such as intake of vegetables, age and smoking.

Overall, the epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. These data, combined with the results of the epidemiological studies considered by the Committee at its forty-fourth meeting, did not provide evidence that nitrate is carcinogenic to humans.

A number of studies were performed to determine whether there are associations between nitrate intake in drinking-water and insulin-dependent diabetes mellitus, neural tube defects or sudden infant death syndrome. In none of these studies was a hypothesis proposed for the mechanism of an association. Two studies were conducted on the incidence of insulin-dependent diabetes mellitus and nitrate intake via drinking-water. One study in Yorkshire, United Kingdom, suggested a positive association, but the authors considered that the finding required confirmation. A study in the Netherlands with a larger number of subjects did not show a positive association. The two studies on nitrate intake and neural tube defects also showed no association. In a recent ecological study in Sweden, a correlation was reported between nitrate concentration in drinking-water and the occurrence of sudden infant death syndrome; however, no confounding factors were taken into account. The Committee concluded that it would be premature to include these observations in its safety assessment.

The Committee concluded that the pivotal observed toxic effects of nitrate are consequent on its conversion to nitrite *in vivo*. The Committee at its present meeting established an ADI of 0–0.07 mg/kg bw for nitrite (see earlier

part of this section). As the new data on nitrite would not provide a basis for a significant change in the previous ADI for nitrate, the Committee retained the ADI of 0–5 mg/kg bw expressed as sodium nitrate, or 0–3.7 mg/kg bw, expressed as nitrate ion, established at its forty-fourth meeting.

Intake

Ideally, all sources of nitrate should be included in an intake assessment; however, contributions from air were not included here. Nitrate intake from sources other than food additives, based on concentrations in food derived from European studies and the GEMS/Food regional diets and with an addition for water, was below the ADI (10–70% of the ADI for the various diets). Major contributors to intake were vegetables (30–65% of the estimated intake) and water (5–45% of the estimated intake). The nitrate content of both groundwater and surface water is highly variable, and wide variations are seen between countries.

The estimated intakes from use of nitrate as a food additive derived from national assessments were below the ADI, assuming the maximum levels in the Codex draft GSFA. The intake by consumers at the mean was about 10% of the ADI, while the intake of those at the 95th percentile was 30–35% of the ADI, on the basis of national data on food consumption. The intakes of nitrate from food additive use based on maximum permitted levels in national standards were also below the ADI (consumers at the mean, 10% of the ADI; consumers at the 95th percentile, 35% of the ADI). Major contributors from its use as a food additive were processed comminuted meats in national assessments (50–55% of estimated intake) and raw meats on the basis of GSFA maximum levels (50–55% of the estimated intake) from national food consumption data. These intakes were based on 24-h food consumption data, which tend to result in overestimates of the intake of consumers at high percentiles.

Assessments of intake of nitrate from all dietary sources, including drinking-water, showed that they were below the ADI. In total diet studies in the United Kingdom, the mean intakes were between 40% and 45% of the ADI and those by consumers at the 95th percentile were 60–75% of the ADI. Duplicate diet studies showed mean intakes representing 30–35% of the ADI and intakes by consumers at the 95th percentile representing 95–125% of the ADI. These studies included one of vegetarians, who are likely to have higher intakes of nitrate owing to their greater consumption of vegetables; however, their intakes were not considerably different from those of the general population. Major contributors to total dietary intake of nitrate were vegetables, which accounted for 70–75% of the total estimated intake. The Committee noted that the food additive use of nitrate is not a major source of intake.

The Committee recommended that the Codex Committee on Food Additives and Contaminants reconsider the maximum levels of nitrate in the GSFA, as the estimated intakes of nitrate might exceed the ADI.

The Committee recommended that assessments of the intake of nitrate also include sources other than food additives, such as vegetables and drinking-water. Studies should be conducted in which more accurate methods of assessment are used, which provide estimates of intake based on analysed concentrations of chemicals in foods. Studies should be conducted in different seasons to account for natural variations in concentrations in foods such as vegetables.

An addendum to the toxicological monograph, including an assessment of intake, was prepared.

3.1.5 **Salatrim**

Explanation

Salatrim is the acronym for short- and long-chain acyltriglyceride molecules. Salatrim preparations are tailored mixtures of triglycerides containing at least one long-chain fatty acid moiety (principally stearic acid) and one or two short-chain fatty acid moieties (acetic, propionic and/or butyric acid). Salatrim is synthesized by non-enzymatic inter-esterification of triacetin, tripropionin, tributyrin or their mixtures with hydrogenated canola, soya bean, cottonseed or sunflower oils. The physical properties of salatrim may be fitted to its food applications by selecting specific short-chain fatty acids and hydrogenated oils for synthesis. Salatrim is intended for use as a low-calorie fat in soft sweets, coatings (e.g., wafers and confectionery), dairy products (including spreads), margarines and bakery products.

Salatrim of various compositions was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference 137), when it concluded that the available studies did not provide an adequate basis for evaluating the safety and the nutritional effects of salatrim. At that meeting, the Committee recommended that additional, appropriately designed studies be performed to assess fully both the toxicological and nutritional consequences of ingestion of salatrim.

The Committee noted at its forty-ninth meeting that the specifications for salatrim permit compositions that include a triglyceride mixture with up to 0.87 g of stearate per gram of fat, but that the available biological data did not provide information on materials of such a composition. Since that time, the sponsor has proposed that the specifications for salatrim be limited to preparations with $\leq 70\%$ by weight of total saturated long-chain fatty acids. Studies were available on the biological effects in humans of salatrim containing up to 0.61 g of stearate per gram of fat.

The Committee previously evaluated studies on the caloric value of salatrim, taking into account the fact that short-chain fatty acids supply fewer kilocalories per gram than long-chain fatty acids. Moreover, it concluded that the claim of reduced absorption of stearic acid has not been proven for humans. In addition, as there is no specific composition for salatrim, a single caloric value could not be assigned.

In evaluating the safety of salatrim at its forty-ninth meeting, the Committee considered studies in which salatrim of various compositions was administered to rats or minipigs at concentrations of up to 10% in the diet. These studies showed no toxicologically significant effects, but the study in minipigs was considered to have been of insufficient duration. The Committee also discussed one home-based trial and four clinic-based studies with volunteers who ingested up to 60 g salatrim per person per day. The studies indicated that the consumption of diets containing salatrim, particularly at 60 g per day, was associated with an increased incidence of mild gastrointestinal symptoms and significantly elevated serum enzyme activities.

Thus, the Committee concluded at its forty-ninth meeting that the available studies in experimental animals and humans did not provide an adequate basis for a toxicological evaluation. In addition, as the studies were not optimized to detect potential nutritional effects, the Committee concluded that they did not provide an adequate basis for a nutritional evaluation.

In response to the Committee's earlier request, four additional home-based trials in humans and one study of nutrition in rats were submitted for consideration at the present meeting.

Toxicological data

The Committee concluded that the newly submitted study of nutrition in rats did not resolve the issue of the extent to which salatrim might reduce energy intake since, as stated previously, the rat is not a good model for humans with respect to stearic acid absorption.

The aim of the home-based trials was to investigate plasma lipid profiles in small groups of adults ingesting salatrim at 30 g/day for up to 5 weeks. Diets containing salatrim did not affect the blood concentration of total high-density or low-density lipoprotein cholesterol. The post-prandial increase in serum triglyceride concentration was lower after ingestion of a meal containing 30 g of salatrim than after ingestion of a meal containing 30 g of oleate or cocoa butter. In one study, it was reported that some of the volunteers complained of gastric discomfort, nausea or feeling 'bloated' or 'satiated and averse to eating until the early evening' after a breakfast containing 30 g of salatrim. However, adequate controls were not included in this study, and it was not clear if these subjective symptoms were related to ingestion of

salatrim. The Committee was informed that, in one of these trials, serum liver enzyme activities were found to be within the normal range; however, these results were not included in the published manuscript.

Overall, the Committee concluded that the new studies did not respond to the questions raised at its forty-ninth meeting and the available information was inadequate for a toxicological evaluation. The Committee noted that, in general, toxicological studies in experimental animals should be conducted at doses that allow an adequate margin of safety in relation to anticipated human intake. Such safety margins cannot feasibly be attained with substances, such as salatrim, that are intended for use as major components of food. Additionally the rat is not a good model for humans with respect to stearic acid absorption, and further studies in experimental animals would not contribute to the safety evaluation of salatrim. The Committee therefore paid particular attention to the results of the studies in humans. These studies indicated that adverse effects (gastrointestinal and hepatic effects) might be experienced by adults consuming diets containing salatrim at 60 g/day (equivalent to 1 g/kg bw per day). The available information was not adequate to evaluate whether effects might occur at intakes of less than 60 g/day, nor if the effects differ with the composition of salatrim.

Intake

Data on the projected intake of salatrim were assessed by the Committee at its forty-ninth meeting. These data, with small revisions, and with projections of intake based on data on consumption in the United Kingdom, were submitted to the present meeting. Manufacturers' use levels for salatrim vary from 1–2 g/100 g in some dairy products and crackers to more than 80 g/100 g in margarine.

The mean intake of salatrim after hypothetical substitution of added fats by this substance in all food categories would be 45 g/day, but this value is unrealistic. The mean intake of salatrim based on hypothetical substitution of fats in specified food categories would vary from 11 to 13 g/day. Intake by adolescent consumers at the 90th percentile would reach 35 g/day. On a gram per kilogram body-weight basis, the highest intake would be that of children aged less than 2 years, as those at the 90th percentile of consumption would have an intake of 1.5 g/kg bw per day. Children aged 1–6 years who are consumers of the category 'chocolates and confectionery' at the 90th percentile would have an intake of salatrim from this food category alone of 1.1 g/kg bw per day, which is higher than the intake reported to have effects in adults in clinical studies.

The Committee concluded that the available data did not provide an adequate basis for evaluating the safety of salatrim and recommended that additional studies be performed in humans to assess fully the safety of salatrim covering

the range of compositions intended for use. The studies should be designed appropriately for safety evaluation and should include investigation of the gastrointestinal and hepatic effects observed in previous studies.

A monograph reproducing the previous monograph and incorporating the information that had become available since the previous evaluation was prepared. The updated monograph includes corrections to the information provided to the Committee at its forty-ninth meeting.

Specifications

The existing specifications for salatrim were revised. The assay was amended by introducing a maximum upper limit of 70% by weight of saturated long-chain fatty acids. Calculation of the weight per cent of saturated long-chain fatty acids in salatrim requires that the molar ratio of short-chain to long-chain fatty acids be determined with analysis for fatty acid butyl esters by gas chromatography (method I in the original specifications). Therefore, the alternative method II, with identification of fatty acid residues by proton nuclear magnetic resonance spectrometry, was considered to have no further value and was deleted.

3.2 Revision of specifications

3.2.1 *Amyloglucosidase from *Aspergillus niger*, var.*

Amyloglucosidase from *Aspergillus niger*, var. is a hydrolytic enzyme produced by controlled fermentation of this organism. The enzyme is isolated from the growth medium and is used to hydrolyse α -glucosidic linkages in starch.

The existing specifications for amyloglucosidase from *A. niger*, var. were revised, and a new procedure was incorporated for assaying enzyme activity in products, including those containing glucose. The tentative designation to the specifications was removed.

3.2.2 *Limits for heavy metals in colours and acidity regulators*

At its fifty-fifth meeting (Annex 1, reference 149), the Committee began implementation of a systematic 5-year programme to replace the outdated test for heavy metals (as lead) in all existing food additive specifications with appropriate limits for individual metals of concern. At the present meeting, the heavy metals and arsenic limits of 52 colours and 44 acidity regulators were reviewed

Colours

In response to the call for data, some proposed limits and supporting data were received for a number of colours from biological sources ('natural colours'). No data were received for synthetic or inorganic colours.

The proposed changes to the current limits are:

For natural colours

- The limits for arsenic were maintained because information was received that the plant sources for certain natural colours grow in areas where the level of arsenic in groundwater is high. The Committee agreed to review these limits at a future meeting when arsenic is on the agenda for evaluation.
- The proposed limit for lead was set at 2 mg/kg, except in certain natural colours containing higher levels or levels set at meetings following the forty-ninth meeting of the Committee. The limit of 2 mg/kg is consistent with the previously agreed policy.
- No limits were proposed for cadmium.
- Limits for mercury were proposed for two natural colours.
- Limits for heavy metals (as lead) were deleted.

For synthetic colours in accordance with the Committee's policy:

- Limits for arsenic were deleted.
- The limit for lead was set at 2 mg/kg.
- No limits were proposed for cadmium.
- Limits for mercury were deleted.
- Limits for heavy metals (as lead) were deleted.

For inorganic colours

The Committee concluded that, because of the high levels of heavy metals in the present specifications for aluminium powder, iron oxides and titanium dioxide, the specifications should be reconsidered. Therefore the Committee maintained the existing limits and decided to call for data on the raw materials, manufacturing methods and analytical data on impurities for review at a future meeting.

Acidity regulators

No data were received. The Committee followed its past practice of including:

- a limit of 3 mg/kg of arsenic for phosphates and calcium compounds;
- a limit of 4 mg/kg of lead for phosphates, but 2 mg/kg for other substances.
- Levels of cadmium and mercury were not considered to be of concern and were not specified.
- Limits for heavy metals (as lead) were deleted.

The conclusions of the Committee on limits for heavy metals in colours and acidity regulators are summarized in Table 2.

Table 2
Limits for heavy metals in 52 colours and 44 acidity regulators

Category	Food additive	INS	As	Pb	Cd	Hg
Colour	Allura red AC	0129	-	2	-	-
Colour	Aluminum powder	0173	3	20	-	-
Colour	Amaranth	0123	-	2	-	-
Colour	Annatto extracts(oil & alkali extracted)	0160 b	3	2	-	1
Colour	Annatto extracts(solvent extracted)	0160 b	3	2	-	1
Colour	β -Apo-8'-carotenal	0160 e	-	2	-	-
Colour	β -Apo-8'-carotenic acid ethyl ester	0160 f	-	2	-	-
Colour	Azorubine	0122	-	2	-	-
Colour	Beet red	0162	3	2	-	-
Colour	Blackcurrant extract	0163 ii	-	2	-	-
Colour	Brilliant Black BN	0151	-	2	-	-
Colour	Brilliant Blue FCF	0133	-	2	-	-
Colour	Brown FK	0154	-	2	-	-
Colour	Brown HT	0155	-	2	-	-
Colour	Canthaxanthin	0161 g	-	2	-	-
Colour	Caramel colours	0150 a,b,c,d	1	2	-	-
Colour	Carmines	0120	-	5	-	-
Colour	β -Carotene, synthetic	0160 a(i)	-	2	-	-
Colour	β -Carotene from <i>Blakeslea trispora</i>	0160 a	-	2	-	-
Colour	Carotenes (algae)	0160 a(ii)	-	5	-	-
Colour	Carotenes (vegetable)	0160 a(ii)	-	5	-	-
Colour	Carthamus red	-	-	5	-	-
Colour	Carthamus yellow	-	-	5	-	-
Colour	Chlorophyllins, copper complexes	0141 ii	3	5	-	-
Colour	Chlorophylls	0140	3	5	-	-
Colour	Chlorophylls, copper complexes	0141 i	3	5	-	-
Colour	Cochineal extract	0120	-	5	-	-
Colour	Curcumin	0100	-	2	-	-
Colour	Erythrosine	0127	-	2	-	-
Colour	Fast Green FCF	0143	-	2	-	-
Colour	Fast Red E	-	-	2	-	-
Colour	Grape skin extract	0163 ii	3	2	-	-
Colour	Green S	0142	-	2	-	-
Colour	Indigotine	0132	-	2	-	-
Colour	Iron oxides	0172	3	10	10	1
Colour	Lithol rubine BK	0180	-	2	-	-
Colour	Mixed carotenoids	-	-	5	-	-
Colour	Paprika oleoresin	0160 c	3	2	-	-
Colour	Patent Blue V	0131	-	2	-	-
Colour	Ponceau 4R	0124	-	2	-	-
Colour	Quinoline Yellow	0104	-	2	-	-
Colour	Red 2G	0128	-	2	-	-
Colour	Riboflavin	0101 i	-	2	-	-
Colour	Riboflavin 5'-phosphate sodium	0101 ii	-	2	-	-
Colour	Riboflavin from <i>Bacillus subtilis</i>	0101 i	-	1	-	-
Colour	Saffron	-	3	2	-	-
Colour	Sunset Yellow FCF	0110	-	2	-	-
Colour	Tagetes extract	-	-	2	-	-
Colour	Tartrazine	0102	-	2	-	-
Colour	Titanium dioxide	0171	3	10	-	1
Colour	Turmeric oleoresin	0100 ii	3	2	-	-
Colour	Vegetable carbon	0153	3	2	-	-

Table 2 (continued)

Category	Food additive	INS	As	Pb	Cd	Hg
Acidity regulator	Acetic acid, glacial	0260	-	2	-	-
Acidity regulator	Ammonia solution	0527	-	2	-	-
Acidity regulator	Ammonium carbonate	0503 i	-	2	-	-
Acidity regulator	Ammonium dihydrogen phosphate	0342 i	3	4	-	-
Acidity regulator	Calcium citrates	0333	-	2	-	-
Acidity regulator	Calcium dihydrogen phosphate	0341 i	3	4	-	-
Acidity regulator	Calcium DL-malate	0352 ii	-	2	-	-
Acidity regulator	Calcium hydroxide	0526	-	2	-	-
Acidity regulator	Calcium lactate	0327	-	2	-	-
Acidity regulator	Calcium oxide	0529	-	2	-	-
Acidity regulator	Diammonium hydrogen phosphate	0342 ii	3	4	-	-
Acidity regulator	Dicalcium pyrophosphate	0450 vi	3	4	-	-
Acidity regulator	Dipotassium hydrogen phosphate	0340 ii	3	4	-	-
Acidity regulator	Disodium hydrogen phosphate	0339 ii	3	4	-	-
Acidity regulator	Disodium pyrophosphate	0450 i	3	4	-	-
Acidity regulator	Hydrochloric acid	0507	-	1	-	-
Acidity regulator	Magnesium hydroxide carbonate	0504 ii	-	2	-	-
Acidity regulator	Magnesium hydroxide	0528	-	2	-	-
Acidity regulator	Magnesium DL-lactate	0329	-	2	-	-
Acidity regulator	Phosphoric acid	0338	3	4	-	-
Acidity regulator	Potassium carbonate	0501 i	-	2	-	-
Acidity regulator	Potassium dihydrogen citrate	0332	-	2	-	-
Acidity regulator	Potassium hydrogen carbonate	0501 ii	-	2	-	-
Acidity regulator	Potassium hydroxide	0525	-	2	-	-
Acidity regulator	Sodium acetate	0262 I	-	2	-	-
Acidity regulator	Sodium carbonate	0500 I	-	2	-	-
Acidity regulator	Sodium dihydrogen citrate	0331 i	-	2	-	-
Acidity regulator	Sodium dihydrogen phosphate	0339 i	3	4	-	-
Acidity regulator	Sodium DL-malate	0350 ii	-	2	-	-
Acidity regulator	Sodium fumarate	0365	-	2	-	-
Acidity regulator	Sodium hydrogen carbonate	0500 ii	-	2	-	-
Acidity regulator	Sodium hydrogen DL-malate	0350 i	-	2	-	-
Acidity regulator	Sodium hydroxide	0524	-	2	-	-
Acidity regulator	Sodium sesquicarbonate	0500 iii	-	2	-	-
Acidity regulator	Sulfuric acid	0513	-	2	-	-
Acidity regulator	Triammonium citrate	0380	-	2	-	-
Acidity regulator	Tripotassium citrate	0332 ii	-	2	-	-
Acidity regulator	Tripotassium phosphate	0340 iii	3	4	-	-
Acidity regulator	Trisodium citrate	0331 iii	-	2	-	-
Acidity regulator	Trisodium phosphate	0339 iii	3	4	-	-

INS, International Numbering System

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

Six groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references 116, 122, 131, 137, 143, 149 and 154). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference 122). The structural classes are as follows:

- Class I. Flavouring agents that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity by the oral route.
- Class II. Flavouring agents that have structural features that are less innocuous than those of substances in Class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Flavouring agents that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting:

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

Endogenous substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Intake data

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in the European Union during the previous year.

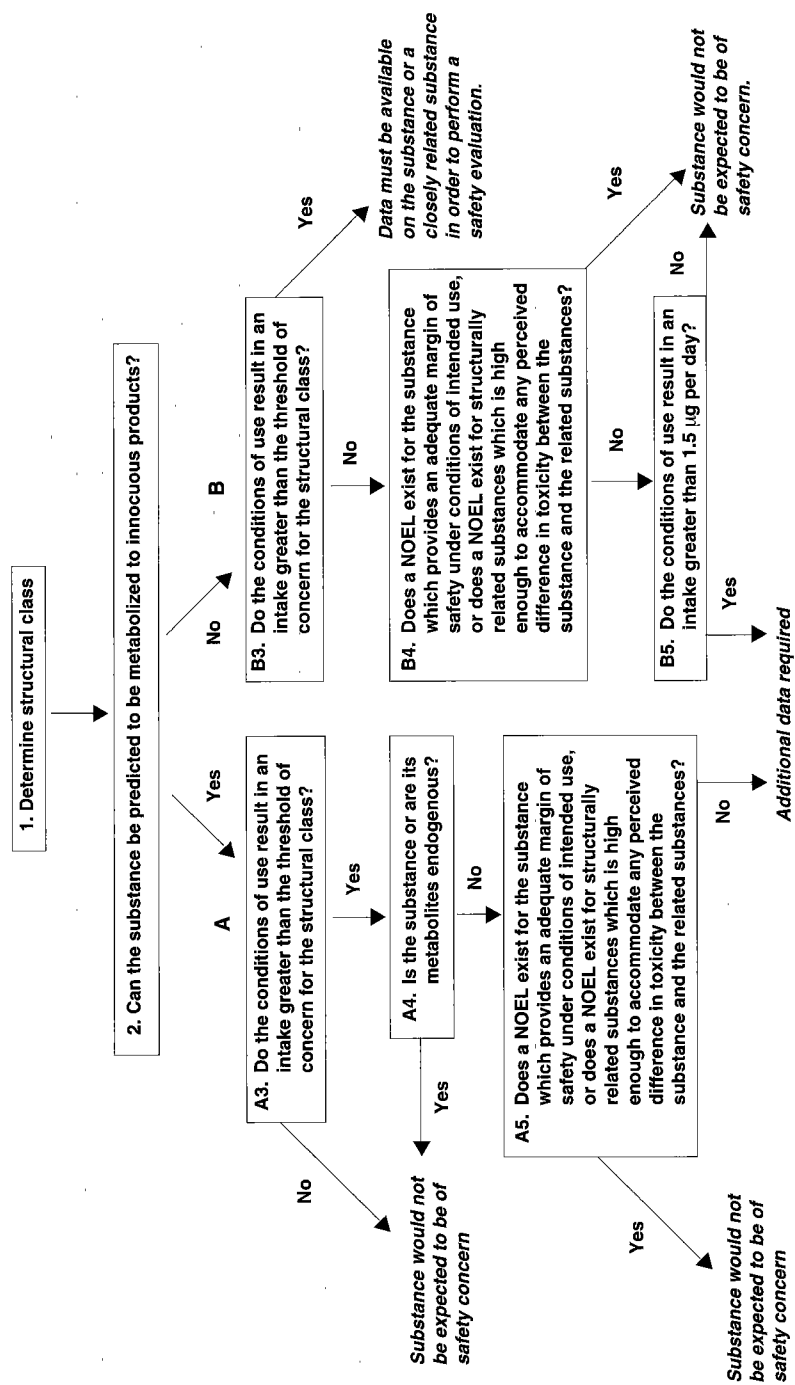


Figure 1
Procedure for the safety evaluation of flavouring agents

Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the National Academy of Sciences National Research Council (under contract to the Food and Drug Administration) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added in a number of broad food categories.

In using the data from these surveys to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported in Europe and 80% of the amount used is reported in the USA and that the total amount used in food is consumed by only 10% of the population.

$$\text{Intake} = \frac{\text{annual volume of production (kg)} \times 10^9 \text{ (}\mu\text{g/kg)}}{\text{population of consumers} \times 0.6 \text{ or } 0.8 \times 365 \text{ days}}$$

(μg per person per day)

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

Several of the flavouring agents that were evaluated at the present meeting were not included in the above surveys or were placed on the market after the surveys were conducted. Intakes of these flavouring agents were estimated on the basis of anticipated use by the manufacturer in the USA, and the standard formula was applied.

4.1.1 ***Alicyclic primary alcohols, aldehydes, acids and related esters***

The Committee evaluated a group of 26 flavouring agents that included alicyclic primary alcohols, aldehydes, acids and related esters (see Table 3), using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). The Committee had not evaluated any of these agents previously.

Twenty of the 26 flavouring agents in this group are terpenoid alcohols, aldehydes or carboxylic acids or their related esters. Thirteen are common components of food and have been detected in vanilla, bourbon, rum, mango, rosemary, grapefruit juice, mandarin peel oil and blackberries.

Estimated daily per capita intake

The total annual volume of the 26 flavouring agents in this group that is produced is approximately 170 kg in Europe and 130 kg in the USA. None of the agents is produced in an annual volume greater than 55 kg. Most of the total annual volume in Europe is accounted for by (2,2,3-trimethylcyclopent-3-en-1-yl)acetaldehyde (No. 967; campholenic aldehyde), *p*-mentha-

Table 3

Summary of results of safety evaluation of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents

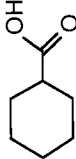
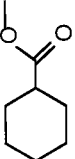
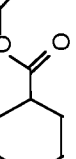
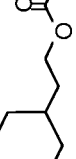
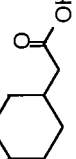
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^a Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Structural class I						
Cyclohexanecarboxylic acid	961	98-89-5 	A3 – No Europe: 0.07 USA: 4	N/R	See note 1	No safety concern
Methyl cyclohexanecarboxylate	962	4630-82-4 	A3 – No Europe: 0.09 USA: 0.01	N/R	See notes 1,2	No safety concern
Ethyl cyclohexanecarboxylate	963	3289-28-9 	A3 – No Europe: ND USA: 0.1	N/R	See notes 1,2	No safety concern
Cyclohexaneethyl acetate	964	21722-83-8 	A3 – No Europe: 1 USA: ND	N/R	See notes 2,3	No safety concern
Cyclohexaneacetic acid	965	5292-21-7 	A3 – No Europe: 0.1 USA: 0.4	N/R	See note 3	No safety concern

Table 3 (continued)

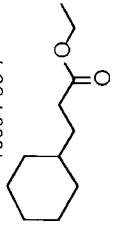
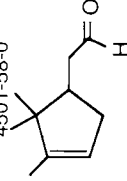
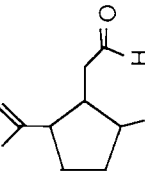
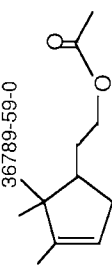
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{a,b} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Ethyl cyclohexanepropionate	966	10094-36-7 	A3 – No Europe: ND USA: 0.1	N/R	See notes 1,2	No safety concern
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	4501-58-0 	A3 – No Europe: 6 USA: ND	N/R	See note 4	No safety concern
<i>cis</i> -5-Isopropenyl- <i>cis</i> -2-methylcyclopentan-1-carboxaldehyde	968	55253-28-6 	A3 – No Europe: 0.01 USA: ND	N/R	See note 4	No safety concern
Campholene acetate	969	36789-59-0 	A3 – No Europe: 0.07 USA: ND	N/R	See notes 2,4	No safety concern

Table 3 (continued)

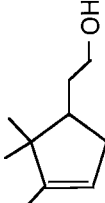
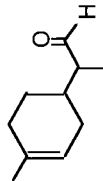
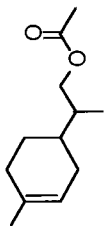
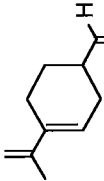
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{ab} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
α -Campholenic alcohol	970	1901-38-8 	A3 – No Europe: 0.01 USA: ND	N/R	See note 4	No safety concern
<i>p</i> -Menth-1-ene-9-al	971	29548-14-9 	A3 – No Europe: 0.1 USA: ND	N/R	See note 4	No safety concern
1- <i>p</i> -Menthen-9-yl acetate	972	17916-91-5 	A3 – No Europe: 1 USA: ND	N/R	See notes 2,4	No safety concern
<i>p</i> -Mentha-1,8-dien-7-al	973	2111-75-3 	A3 – No Europe: 2 USA: 2	N/R	See note 4	No safety concern

Table 3 (continued)

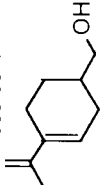
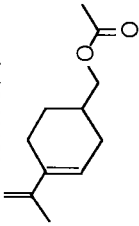
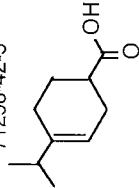
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{a,b} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
<i>p</i> -Mentha-1,8-dien-7-ol	974	536-59-4 	A3 – No Europe: 2 USA: 1	N/R	See note 4	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	15111-96-3 	A3 – No Europe: 0.4 USA: 0.07	N/R	See notes 2,4	No safety concern
1,2,5,6-Tetrahydrocuminic acid	976	71298-42-5 	A3 – No Europe: 0.01 USA: ND	N/R	See note 1	No safety concern

Table 3 (continued)

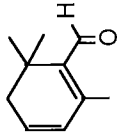
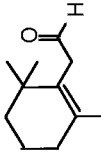
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{ab} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	977	116-26-7 	B3 – No Europe: 4 USA: 0.07	Yes. The NOELs of 120 mg/kg bw per day for perillyl alcohol in 90-day studies in rats and dogs and NOELs of 10 mg/kg bw per day for α -ionone and β -ionone in a 90-day study in rats are > 2 million and > 200 000 times, respectively, the estimated intake of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal when used as a flavouring agent.		No safety concern
2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	978	472-66-2 	A3 – No Europe: 0.3 USA: 2	N/R	See note 3.	No safety concern

Table 3 (continued)

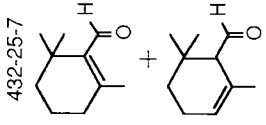
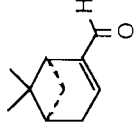
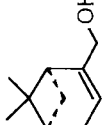
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{ab} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethyl-1-cyclohexen-1-carboxaldehyde	979	432-25-7 	A3 – No Europe: 0.4 USA: ND	N/R	See note 1	No safety concern
2-Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	564-94-3 	A3 – No Europe: 4 USA: 7	N/R	See note 4	No safety concern
Myrtenol	981	515-00-4 	A3 – No Europe: 0.4 USA: 0.03	N/R	See note 4	No safety concern

Table 3 (continued)

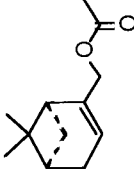
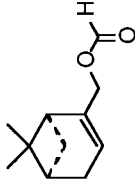
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{ab} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Myrtenyl acetate	982	1079-01-2 	A3 – No Europe: 0.3 USA: 0.04	N/R	See note 4	No safety concern
Myrtenyl formate	983	72928-52-0 	A3 – No Europe: 0.4 USA: ND	N/R	See note 4	No safety concern

Table 3 (continued)

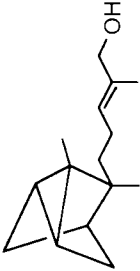
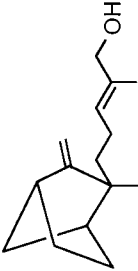
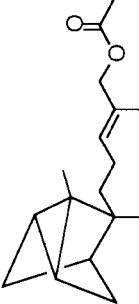
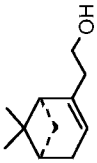
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{ab} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Santalol (α and β)	984	115-71-9  α	A3 – No Europe: 0.04 USA: ND	N/R	See note 4	No safety concern
		77-42-9  β				
Santalyol acetate	985	1323-00-8 	A3 – No Europe: ND USA: 0.01	N/R	See note 4	No safety concern

Table 3 (continued)

Flavouring agent	No.	CAS No. and structure	Steps A3 and B3 ^{a,b} Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
10-Hydroxymethylene-2-pinene	986	128-50-7 	A3 – No Europe: ND USA: 0.01	N/R	See note 4	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation

^a Step 2: Except for No. 977, all the flavouring agents in this group were predicted to be metabolized to innocuous products.

^b The threshold for human intake for structural class I is 1800 µg/day. All intake values are expressed in µg/day. The combined intake of all flavouring agents in this group is 22 and 18 µg/person per day in Europe and the USA, respectively.

Notes:

1. Metabolized by β-oxidation, aromatization and conjugation with glycine and glucuronic acid
2. Metabolized by hydrolysis followed by oxidation and/or conjugation
3. Metabolized by β-oxidation and eliminated largely by metabolism via the citric acid cycle
4. Metabolized largely by oxidation of the side-chain to the corresponding carboxylic acid, which is excreted unchanged and as conjugates

1,8-dien-7-al (No. 973; perillaldehyde), *para*-mentha-1,8-dien-7-ol (No. 974; perillyl alcohol), 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980; myrtenal). In the USA, approximately 91% of the total annual volume is accounted for by cyclohexanecarboxylic acid (No. 961), *para*-mentha-1,8-dien-7-al (No. 973; perillaldehyde), 2,6,6-trimethyl-1-cyclohexen-1-acetaldehyde (No. 978; β -cyclocitral) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980; myrtenal). The estimated daily *per capita* intake of 11 of the substances in this group is $\leq 10 \mu\text{g}$.

Absorption, distribution, metabolism and elimination

The number of potential pathways of metabolism of alicyclic substances increases as the number and types of functional groups and ring substituents in the molecule increase. The cyclohexane derivatives (carboxylic acids and their related esters) in this group (Nos 961–966) can undergo β -oxidation and subsequent ring cleavage and/or aromatization of the cyclohexane ring and/or can be excreted in urine as cyclohexane carboxylic acid and its glycine and glucuronide conjugates. If there is an even number of carbons in the side-chain, β -oxidative cleavage leads to ring-opened oxygenated metabolites, primarily dicarboxylic acids, which are metabolized via the citric acid cycle. If there is an odd number of carbons in the side-chain, β -oxidative cleavage leads to a cyclohexane carboxylic acid derivative that may subsequently undergo dehydrogenation to yield unsaturated derivatives. The two non-terpenoid substances containing a cyclohexene ring (Nos 978 and 979) would be metabolized and eliminated by similar pathways. The substance with two endocyclic double bonds, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), is a structural analogue of dehydrodihydroionol (No. 397), which was evaluated by the Committee at its fifty-first meeting (Annex 1, reference 137), and, like that agent, cannot be predicted to be metabolized to innocuous products.

The flavouring agents with a cyclopentane or cyclopentene ring (Nos 967–970) also have an oxygenated side-chain, and this is predicted to be the main site of oxidative metabolism prior to excretion of more polar acid metabolites. The processes of side-chain and alicyclic ring oxidation were considered by the Committee at its fifty-first meeting (Annex 1, reference 137).

Monocyclic terpenoid primary alcohols (e.g., No. 974, perillyl alcohol) and aldehydes (e.g., Nos 971 and 973) would be oxidized to yield the corresponding carboxylic acid, while the esters (Nos 972 and 975) would undergo hydrolysis prior to oxidation. The acid metabolites of the above agents and 1,2,5,6-tetrahydrocuminic acid (No. 976) would be conjugated with glucuronic acid and excreted mainly in the urine. In a minor pathway,

the aldehyde can be reduced to the alcohol and excreted as the glucuronic acid conjugate. If an endocyclic double-bond is present, the flavouring agent or its metabolite could be reduced by the action of gut microflora. The acid metabolite may also undergo aromatization of the ring to yield a hippuric acid derivative. Bicyclic and tricyclic compounds (e.g., Nos 980–986) would undergo similar metabolism to the monocyclic terpenoids and, in addition, may undergo ring oxidation.

Four agents in this group contain an α,β -unsaturated carbonyl group (Nos 973, 977, 979 and 980), which is a structural alert for toxicity. The Committee, at previous meetings, devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference 154) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with the use of compounds such as flavouring agents. These protective processes include conjugation with glutathione and reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid).

Application of the Procedure for the Safety Evaluation of Flavouring Agents
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned all 26 agents to structural class I.

Step 2. The simple cyclohexane and cyclohexene derivatives (Nos 961–966, 978 and 979), the cyclopentane and cyclopentene compounds (Nos 967–970) and the simple mono-, bi- and tricyclic terpenoid derivatives (Nos 971–976 and 980–986) were predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded *via* the A (left-hand) side of the decision tree (see Figure 1). The remaining substance, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), cannot be predicted to be metabolized to innocuous products and was therefore evaluated *via* the B (right-hand) side of the decision tree.

Step A3. The daily *per capita* intakes of each of the 25 flavouring agents evaluated at this step were below the daily human intake thresholds for structural class I (1800 μg per person), indicating that they present no safety concern when consumed as flavouring agents at current estimated levels.

Step B3. The daily *per capita* intake of the monocyclic substance with two endocyclic double-bonds evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), was below the threshold for daily human intake of compounds of structural class I, and its evaluation therefore proceeded to step B4.

Step B4. As the agent evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), is structurally related to perillyl alcohol

(No. 974), data on the toxicity of perillyl alcohol were used to evaluate its safety. Perillyl alcohol given by intragastric gavage changed the weights of several organs in female rats when given at 400 mg/kg bw per day, but not at 120 mg/kg bw per day, in a 90-day study; changes in organ weights were not reported in male rats. Doses of 40, 120 and 400 mg/kg bw per day produced hyperexcitability and salivation, which the authors considered may have been due to its irritating properties. A daily dose of 120 mg/kg bw was well tolerated by dogs in a 90-day study. The daily intake of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) is 0.052 µg/kg bw in Europe and 0.001 µg/kg bw in the USA. The margin of safety between these intakes and 120 mg/kg bw per day is > 2 000 000. The compound also shares structural similarities with α-ionone and β-ionone (Nos 388 and 389), which were evaluated by the Committee at its fifty-first meeting (Annex 1, reference 137). The NOELs for these compounds were 10 mg/kg bw per day in a 90-day study in rats, providing a margin of safety of about 200 000. Therefore, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) would not be a safety concern.

Consideration of combined intakes

In the unlikely event that all 26 flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for structural class I. Therefore, the combined intake of the agents in this group would not represent a safety concern.

Conclusions

The Committee concluded that none of the 26 flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters would present a safety concern at the current estimated levels of intake.

A monograph summarizing the data on this group of flavouring agents was prepared.

4.1.2 Phenethyl alcohol, aldehyde, acid and related acetals and esters

The Committee evaluated 43 flavouring agents that are derivatives of phenethyl alcohol (No. 987) and phenoxyethyl alcohol (see Table 4). The group includes 39 phenethyl derivatives, comprising phenylacetaldehyde (No. 1002), phenylacetic acid (No. 1007) and structurally related esters and acetals. The group also includes four phenoxyethyl alcohol derivatives: phenoxyacetic acid (No. 1026), the sodium salt of a structurally related phenoxyacetic acid (No. 1029), a phenoxyethyl ester (No. 1027) and a phenoxyacetic acid ester (No. 1028). The evaluations were conducted with the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). None of these agents has previously been evaluated by the Committee.

Table 4

Summary of results of safety evaluations of phenethyl alcohol, aldehyde, acid, and related acetals and esters^a

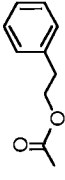
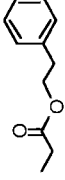
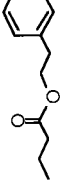
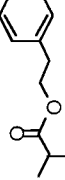
Flavouring agent	No.	CAS No. and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Structural class I							
Phenethyl alcohol	987	60-12-8 	No Europe: 1400 USA: 330	N/R	N/R	See note 1.	No safety concern
Phenethyl formate	988	104-62-1 	No Europe: 2 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl acetate	989	103-45-7 	No Europe: 100 USA: 60	N/R	N/R	See note 2.	No safety concern
Phenethyl propionate	990	122-70-3 	No Europe: 1 USA: 3	N/R	N/R	See note 2.	No safety concern
Phenethyl butyrate	991	103-52-6 	No Europe: 30 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl isobutyrate	992	103-48-0 	No Europe: 20 USA: 60	N/R	N/R	See note 2.	No safety concern

Table 4 (contd)

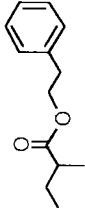
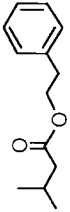
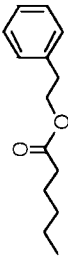
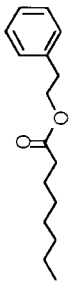
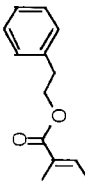
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenethyl 2- methylbutyrate	993	24817-51-4 	No Europe: 0.4 USA: ND	N/R	N/R	See note 2.	No safety concern
Phenethyl isovalerate	994	140-26-1 	No Europe: 100 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl hexanoate	995	6290-37-5 	No Europe: 10 USA: 2	N/R	N/R	See note 2.	No safety concern
Phenethyl octanoate	996	5457-70-5 	No Europe: 30 USA: 0.1	N/R	N/R	See note 2.	No safety concern
Phenethyl tiglate	997	55719-85-2 	No Europe: 0.3 USA: 1	N/R	N/R	See note 2.	No safety concern
Phenethyl senecioate	998	42078-65-9 	No Europe: 2 USA: ND	N/R	N/R	See note 2.	No safety concern

Table 4 (contd)

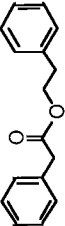
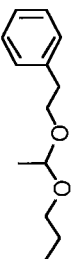
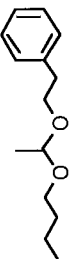
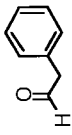
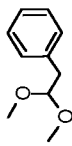
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenethyl phenylacetate	999	102-20-5 	No Europe: 40 USA: 80	N/R	N/R	See note 3.	No safety concern
Acetaldehyde phenethyl propyl acetal	1000	7493-57-4 	No Europe: 0.1 USA: 6	N/R	N/R	See note 4.	No safety concern
Acetaldehyde butyl phenethyl acetal	1001	64577-91-9 	No Europe: 0.01 USA: ND	N/R	N/R	See note 4.	No safety concern
Phenylacetaldehyde	1002	122-78-1 	No Europe: 40 USA: 60	N/R	N/R	See note 5.	No safety concern
Phenylacetaldehyde dimethyl acetal	1003	101-48-4 	No Europe: 20 USA: 40	N/R	N/R	See note 6.	No safety concern

Table 4 (contd)

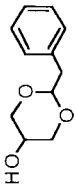
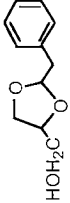
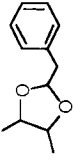
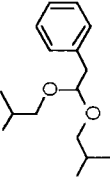
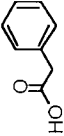
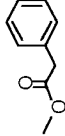
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenylacetaldehyde glyceryl acetal	1004	29895-73-6  or 	No Europe: 0.1 USA: 1	N/R	N/R	See note 6.	No safety concern
Phenylacetaldehyde 2,3-butylene glycol acetal	1005	5468-06-4 	No Europe: ND USA: 1	N/R	N/R	See note 6.	No safety concern
Phenylacetaldehyde diisobutylacetal	1006	68345-22-2 	No Europe: 30 USA: 0.4	N/R	N/R	See note 6.	No safety concern
Phenylacetic acid	1007	103-82-2 	No Europe: 290 USA: 60	N/R	N/R	See note 7.	No safety concern
Methyl phenylacetate	1008	101-41-7 	No Europe: ND USA: 20	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

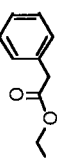
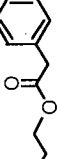
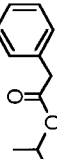
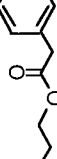
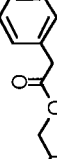
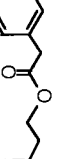
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Ethyl phenylacetate	1009	101-97-3 	No Europe: 130 USA: 20	N/R	N/R	See note 8.	No safety concern
Propyl phenylacetate	1010	4606-15-9 	No Europe: ND USA: 0.3	N/R	N/R	See note 8.	No safety concern
Isopropyl phenylacetate	1011	4861-85-2 	No Europe: 0.07 USA: ND	N/R	N/R	See note 8.	No safety concern
Butyl phenylacetate	1012	122-43-0 	No Europe: 3 USA: 3	N/R	N/R	See note 8.	No safety concern
Isobutyl phenylacetate	1013	102-13-6 	No Europe: 20 USA: 20	N/R	N/R	See note 8.	No safety concern
Isoamyl phenylacetate	1014	102-19-2 	No Europe: 30 USA: 30	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

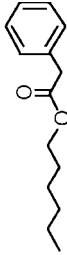
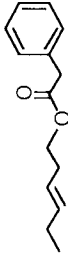
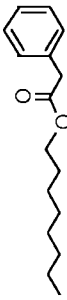
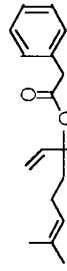
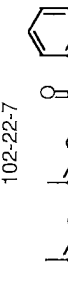
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Hexyl phenylacetate	1015	5421-17-0 	No Europe: 8 USA: ND	N/R	N/R	See note 8.	No safety concern
3-Hexenyl phenylacetate	1016	42436-07-7 	No Europe: 1 USA: 0.05	N/R	N/R	See note 8.	No safety concern
Octyl phenylacetate	1017	122-45-2 	No Europe: 0.004 USA: 0.006	N/R	N/R	See note 8.	No safety concern
Rhodinyl phenylacetate	1018	10486-14-3 	No Europe: 0.001 USA: ND	N/R	N/R	See note 9.	No safety concern
Linalyl phenylacetate	1019	7143-69-3 	No Europe: 0.09 USA: ND	N/R	N/R	See note 9.	No safety concern
Geranyl phenylacetate	1020	102-22-7 	No Europe: 2 USA: 2	N/R	N/R	See note 9.	No safety concern

Table 4 (contd)

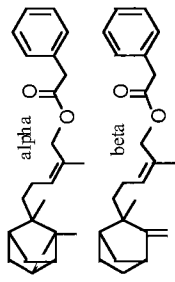
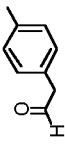
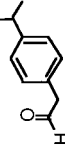
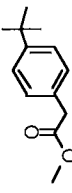
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Citronellyl phenylacetate	1021	139-70-8 	No Europe: 1 USA: 2	N/R	N/R	See note 9.	No safety concern
Santalyl phenylacetate	1022	1323-75-7 	No Europe: ND USA: 1	N/R	N/R	See note 9.	No safety concern
<i>p</i> -Tolylacetaldehyde	1023	104-09-6 	No Europe: 6 USA: 3	N/R	N/R	See note 5.	No safety concern
<i>p</i> -Isopropylphenylacetaldehyde	1024	4395-92-0 	No Europe: 0.1 USA: 0.01	N/R	N/R	See note 5.	No safety concern
Methyl <i>para-tert</i> -butylphenylacetate	1025	3549-23-3 	No Europe: 20 USA: 20	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

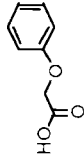
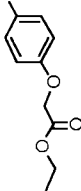
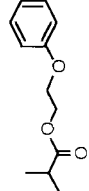
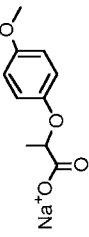
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Structural class III							
Phenoxyacetic acid	1026	122-59-8 	No Europe: 40 USA: 0.1	N/R	N/R	See note 10.	No safety concern
Ethyl (<i>p</i> -tolyl-oxy)acetate	1027	67028-40-4 	No Europe: 0.1 USA: ND	N/R	N/R	See note 11.	No safety concern
2-Phenoxyethyl isobutyrate	1028	103-60-6 	Yes Europe: 2 USA: 110	No	Yes. The NOEL of 15 mg/kg bw per day for the related chemical No. 1027 and the NOEL of 250 mg/kg bw per day for the related chemical No. 1029 are >1000 times the estimated intake of 2-phenoxyethyl isobutyrate when used as a flavouring agent.	See note 12.	No safety concern

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for metabolites human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Sodium 2-(4-methoxyphenoxy)propanoate	1029	13794-15-5 	No Europe: ND USA: 6	N/R	N/R	See note 13.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation.

^a Step 2: All the flavouring agents in this group were predicted to be metabolized to innocuous products.

^b The thresholds for human intake for classes I and III are 1800 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/person per day. The combined intakes of flavouring agents in class I are 2300 and 920 µg/person per day in Europe and the USA, respectively. The combined intakes of flavouring agents in class III are 42 and 120 µg/person per day in Europe and the USA, respectively.

Notes:

1. Oxidized to phenylacetic acid and either completely oxidized or conjugated and excreted primarily in the urine
2. Hydrolysed to phenethyl alcohol (see note 1) and the corresponding acid, which is further oxidized to carbon dioxide and water
3. Hydrolysed to phenethyl alcohol (see note 1) and phenylacetic acid (see note 7)
4. Hydrolysed to phenethyl alcohol (see note 1) and the corresponding aldehyde, which is further oxidized to carbon dioxide and water
5. Phenylacetaldehyde derivative is oxidized to phenylacetic acid derivative (see note 7).
6. Hydrolysed to phenylacetaldehyde (see note 5) and the corresponding alcohol, which is further oxidized to carbon dioxide and water
7. Phenylacetic acid is excreted as the glutamine acid conjugate.
8. Hydrolysed to phenylacetic acid derivative (see note 7) and the corresponding alcohol, which is completely oxidized.
9. Hydrolysed to phenylacetic acid (see note 7) and terpene alcohol, which is further oxidized to a polar, excretable metabolite.
10. Excreted unchanged in the urine
11. Hydrolysed to *para*-tolylloxy acetic acid and ethanol; the acid is excreted unchanged.
12. Hydrolysed to isobutyric acid and 2-phenoxyethanol, which is oxidized to phenoxyacetic acid and excreted unchanged in the urine.
13. Excreted mainly unchanged and as the *O*-demethylated metabolite, (±)2-(4-hydroxyphenoxy) propionic acid.

Twenty of the 39 flavouring agents in this group are natural components of foods. Phenethyl alcohol has a mild rose aroma. The agents have been detected in a wide range of products; for example, the parent alcohol and its derivatives have been detected in beans, fruits, vegetables, cheeses, milk, oils and alcoholic and non-alcoholic beverages. Only one phenoxyacetic acid derivative is a natural component of food: sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) has been detected in coffee.

Estimated daily per capita intake

The total annual volume of production of the 43 phenethyl alcohol and phenoxyethyl alcohol derivatives in this group is approximately 17 000 kg in Europe and 7800 kg in the USA. About 75% and 45% of the total annual volume in Europe and the USA, respectively, is accounted for by use of phenethyl alcohol (No. 987), its corresponding acetate ester (No. 989) and phenylacetic acid (No. 1007). The total annual volume of production of the four derivatives of phenoxyethyl alcohol (Nos 1026–1029) is approximately 260 kg in Europe and 870 kg in the USA, accounting for about 2% and 11% of the total annual volumes in Europe and the USA, respectively.

Five flavouring agents in the group account for the highest estimated intakes. These are 1400 mg/day for phenethyl alcohol (No. 987), 290 µg/day for phenylacetic acid (No. 1007), 130 µg/day for ethyl phenylacetate (No. 1009) and 100 µg/day for phenethyl acetate (No. 989) and for phenethyl isovalerate (No. 994) in Europe and 330 µg/day for phenethyl alcohol (No. 987) and 110 µg/day for phenoxyethyl isobutyrate (No. 1028) in the USA. The intakes of all the other flavouring agents in the group ranged from 0.001 to 80 µg/day, the intake of about 70% of them being less than 25 µg/day.

Absorption, distribution, metabolism and elimination

Phenethyl and phenylacetate esters and phenylacetaldehyde acetals are rapidly hydrolysed in vivo to yield phenethyl alcohol (No. 987), phenylacetic acid (No. 1007) and phenylacetaldehyde (No. 1002), respectively. Phenethyl alcohol and phenylacetaldehyde are both oxidized to phenylacetic acid, which is conjugated and excreted primarily in the urine. Similarly, the phenoxyethyl and phenoxyacetate esters, 2-phenoxyethyl isobutyrate (No. 1028) and ethyl (*para*-tolylloxy)acetate (No. 1027), respectively, are anticipated to be hydrolysed to their component acids and alcohols. Phenoxyethyl alcohol is rapidly oxidized to phenoxyacetic acid, which in turn is rapidly absorbed and excreted primarily unchanged in the urine. Sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) is also rapidly absorbed and excreted primarily unchanged, with a small amount of the *O*-demethylated metabolite, (±)2-(4-hydroxyphenoxy) propionic acid. Therefore, all the flavouring agents in this group were predicted to hydrolyse and/or oxidize

to yield phenylacetic acid or a phenoxyacetic acid derivative that is excreted either free or in conjugated form.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned phenylacetic acid (No. 1007) to structural class I because it is a normal component of human urine. The other 38 phenethyl alcohol derivatives were also assigned to structural class I because they are simple aromatic compounds with a primary oxygenated functional group.

The Committee assigned all four phenoxyacetic acid derivatives in this group to structural class III. These are aromatic substances with an ether linkage between the benzene ring and an unsaturated side-chain with a primary oxygenated functional group.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. Their evaluation therefore proceeded via the A (left-hand) side of the decision tree.

Step A3. The estimated daily intakes of each of the 39 flavouring agents in structural class I are below the threshold for daily human intake for that class (1800 µg per person). According to the Procedure, the safety of these 39 flavouring agents raises no concern when they are consumed at currently estimated levels.

The estimated daily intakes of three of the four flavouring agents in structural class III are below the threshold for daily human intake for that class (90 µg per person). According to the Procedure, the safety of these three flavouring agents (Nos 1026, 1027 and 1029) raises no concern when they are consumed at their currently estimated levels. The daily intake of 2-phenoxyethyl isobutyrate (No. 1028) in the USA (110 mg /person) exceeds the threshold for daily human intake for compounds in structural class III (90 mg per person). Accordingly, the evaluation of this substance proceeded to step A4.

Step A4. 2-Phenoxyethyl isobutyrate (No. 1028) is not endogenous. Therefore, its evaluation proceeded to step A5.

Step A5. Although no NOEL was available for 2-phenoxyethyl isobutyrate (No. 1028), data were available on the toxicity of two structurally related flavouring agents in this group (Nos 1027 and 1029). The NOEL of 15 mg/kg bw per day for ethyl (*para*-tolylloxy)acetate (No. 1027) in a 90-day dietary study in rats and the NOEL of 250 mg/kg bw per day for sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) in another 90-day dietary study in rats provide adequate margins of safety (> 1000 times) in relation to the estimated daily per capita intake of 2-phenoxyethyl isobutyrate (No. 1028) in Europe (0.03 µg/kg bw) and the USA (2 µg/kg bw). The Committee therefore concluded that the safety of this agent would not be a concern.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 39 of the phenethyl alcohol derivatives (Nos 987–1025) were consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for daily human intake of compounds in structural class I (1800 mg per person). At the levels of intake associated with their use as flavouring agents, all 39 agents are expected to be metabolized efficiently, without saturating metabolic pathways. The same holds true for two of the four flavouring agents in structural class III: the estimated combined intakes of phenoxyacetic acid (No. 1026) and 2-phenoxyethyl isobutyrate (No. 1028) would exceed the threshold for daily human intake of compounds in structural class III (90 mg per person). At the levels of intake associated with their use as flavouring agents, both agents are expected to be metabolized efficiently, without saturating metabolic pathways. The other two agents, ethyl (*para*-tolylloxy)acetate (No. 1027) and sodium 2-(4-methoxyphenoxy)propanoate (No. 1029), are not converted to a common metabolite, and there is no need to consider their combined intake from use as flavouring agents. On the basis of the evaluation of all the data, combined intake would raise no safety concern.

Conclusions

The Committee concluded that none of the 43 flavouring agents in this group would present a safety concern at current estimated levels of intake. No data on toxicity were required for application of the Procedure to 42 of the 43 flavouring agents, as they were predicted to be metabolized to innocuous agents and the estimated intakes were below the human intake threshold associated with the relevant structural class. Data on the toxicity of related agents were used to evaluate 2-phenoxyethyl isobutyrate (No. 1028). Data on the toxicity and metabolism of phenethyl alcohol, phenylacetaldehyde, phenylacetic acid, phenoxyethanol and phenoxyacetic acid and related agents were consistent with the results of the safety evaluation.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.3 Sulfur-containing heterocyclic compounds

The Committee evaluated a group of 30 flavouring agents comprising sulfur-containing heterocyclic compounds (see Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). The group is composed of both five- and six-member S-containing aromatic and non-aromatic heterocyclic compounds, including thiazole itself and derivatives of thiazole, dithiazine, thiazoline and thiophene. Thiazole is a five-membered aromatic heterocyclic compound containing sulfur and nitrogen atoms in the 1- and 3-ring positions, respectively (Nos 1030–1044 and 1054–1057).

Table 5

Summary of the results of safety evaluations of sulfur-containing heterocyclic compounds^a

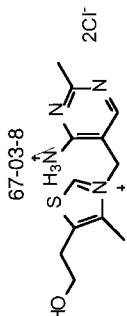
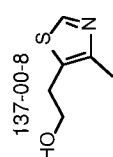
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metab- olized to innocuous products?	Step A3 / B3 ^b Does intake exceed threshold for structural class?	Step A4 Is the flav- ouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Structural class II							
Thiamine hydrochloride	1030	 67-03-8	Yes ^c A side	Yes Europe: 2900 USA: 1200	No	A5 Yes. The NOEL of 36 mg/kg bw per day is > 500 times the estimated intake of thiamine hydrochloride when used as a flavouring agent.	No safety concern
4-Methyl-5-thiazoleethanol	1031	 137-00-8	Yes ^d A side	No Europe: 170 USA: 380	N/R	N/R	No safety concern
Thiazole	1032	 288-47-1	No B side	No Europe: 0.01 USA: 0.07	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 900 000 times the estimated intake of thiazole when used as a flavouring agent.	No safety concern

Table 5 (continued)

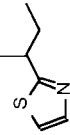
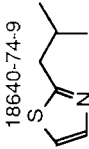
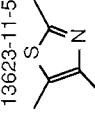
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-(1-Methylpropyl)- thiazole	1033	18277-27-5 	No B side	No Europe: 0.03 USA: 0.01	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 1 million times the estimated intake of 2-(1-methylpropyl)-thiazole when used as a flavouring agent.	No safety concern
2-Isobutylthiazole	1034	18640-74-9 	No B side	No Europe: 3 USA: 0.4	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is 10 000 times the estimated intake of 2-isobutylthiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl- thiazole	1035	3581-91-7 	No B side	No Europe: 0.2 USA: 0.4	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 200 000 times the estimated intake of 4,5-dimethylthiazole when used as a flavouring agent.	No safety concern
2,4,5-Trimethyl- thiazole	1036	13623-11-5 	No B side	No Europe: 1 USA: 0.3	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 90 000 times the estimated intake of 2,4,5-trimethylthiazole when used as a flavouring agent.	No safety concern

Table 5 (continued)

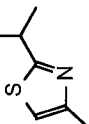
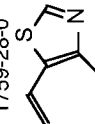
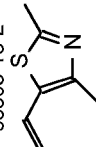
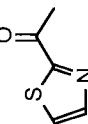
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Isopropyl-4-methylthiazole	1037	15679-13-7 	No B side	No Europe: 22 USA: 10	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 2000 times the estimated intake of 2-isopropyl-4-methylthiazole when used as a flavouring agent.	No safety concern
4-Methyl-5-vinylthiazole	1038	17759-28-0 	No B side	No Europe: 2 USA: 0.2	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 20 000 times the estimated intake of 4-methyl-5-vinylthiazole when used as a flavouring agent.	No safety concern
2,4-Dimethyl-5-vinylthiazole	1039	65505-18-2 	No B side	No Europe: ND USA: 0.007	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day is > 9 million times the estimated intake of 2,4-dimethyl-5-vinylthiazole when used as a flavouring agent.	No safety concern
2-Acetylthiazole	1041	24295-03-2 	No B side	No Europe: 11 USA: 10	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day is > 200 000 times the estimated intake of 2-acetylthiazole when used as a flavouring agent.	No safety concern

Table 5 (continued)

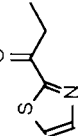
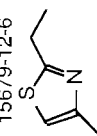
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Propionylthiazole	1042	43039-98-1 	No B side	No Europe: ND USA: 0.2	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day for the related flavouring agent No. 1041 is > 10 million times the estimated intake of 2-propionylthiazole when used as a flavouring agent.	No safety concern
4-Methylthiazole	1043	693-95-8 	No B side	No Europe: 0.1 USA: 0.05	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 400 000 times the estimated intake of 4-methylthiazole when used as a flavouring agent.	No safety concern
2-Ethyl-4-methylthiazole	1044	15679-12-6 	No B side	No Europe: 4 USA: 1	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 10 000 times the estimated intake of 2-ethyl-4-methylthiazole when used as a flavouring agent.	No safety concern

Table 5 (continued)

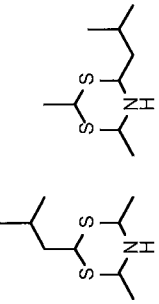
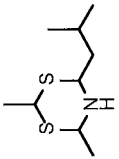
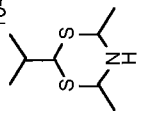
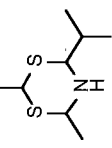
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flav- ouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1046	101517-87-7  18%  62%	No B side	No Europe: 0.1 USA: 0.05	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day is > 5 million times the estimated intake of a mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine when used as a flavouring agent.	No safety concern
2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1047	104691-41-0  27%  44%	No B side	No Europe: ND USA: 0.07	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day is > 10 million times the estimated intake of a mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine when used as a flavouring agent.	No safety concern

Table 5 (continued)

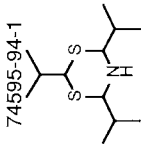
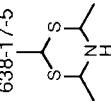
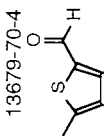
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2,4,6-Triisobutyl- 5,6-dihydro-4 <i>H</i> - 1,3,5-dithiazine	1048	74595-94-1 	No B side	No Europe: ND USA: 2.6	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day for the related flavouring agent No. 1046 is > 200 000 times the estimated intake of 2,4,6- triisobutyl-5,6-dihydro-4 <i>H</i> - 1,3,5-dithiazine when used as a flavouring agent.	No safety concern
2,4,6-Trimethyl- dihydro-4 <i>H</i> -1,3,5- dithiazine	1049	638-17-5 	No B side	No Europe: ND USA: 3.3	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day for the related flavouring agent No. 1046 is > 5 million times the estimated intake of 2,4,6- trimethyldihydro-4 <i>H</i> -1,3,5- dithiazine when used as a flavouring agent.	No safety concern
5-Methyl-2-thio- phenecarboxy- aldehyde	1050	13679-70-4 	No B side	No Europe: 1 USA: 0.01	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 20 million times the estimated intake of 5-methyl- 2-thiophene-carboxyaldehyde when used as a flavouring agent.	No safety concern

Table 5 (continued)

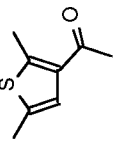
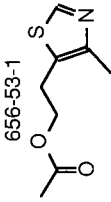
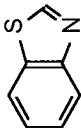
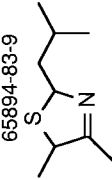
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metab- olized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
3-Acetyl-2,5- dimethylthiophene	1051	2530-10-1 	No B side	No Europe: 22 USA: 0.2	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 700 000 times the estimated intake of 3-acetyl-2,5-dimethylthiophene when used as a flavouring agent.	No safety concern
4-Methyl-5- thiazoleethanol acetate	1054	656-53-1 	Yes ^c A side	No Europe: 10 USA: 3	N/R	N/R	No safety concern
Structural class III							
Benzothiazole	1040	95-16-9 	No B side	No Europe: 1 USA: 0.2	N/R	B5 Yes. The NOEL of 5.1 mg/kg bw per day is > 20 000 times the estimated intake of benzothiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl-2- isobutyl-3- thiazoline	1045	65894-83-9 	No B side	No Europe: 0.01 USA: 4	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day for the related flavouring agent No. 1059 is > 10 000 times the estimated intake of 4,5-dimethyl-2-isobutyl-3-thiazoline when used as a flavouring agent.	No safety concern

Table 5 (continued)

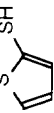
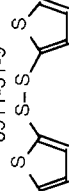
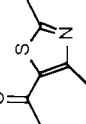
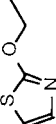
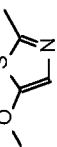
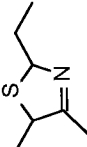
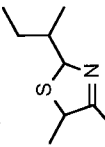
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Thienyl mercaptan	1052	7774-74-5 	No B side	No Europe: 0.01 USA: 0.03	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 700 million times the estimated intake of 2-thienyl mercaptan when used as a flavouring agent.	No safety concern
2-Thienyl disulfide	1053	6911-51-9 	No B side	No Europe: ND USA: 0.07	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day is > 200 million times the estimated intake of 2-thienyl disulfide when used as a flavouring agent.	No safety concern
2,4-Dimethyl-5- acetylthiazole	1055	38205-60-6 	No B side	No Europe: 0.01 USA: 2	N/R	B5 Yes. The NOEL of 24 mg/kg bw per day is > 10 million times the estimated intake of 2,4-dimethyl-5-acetylthiazole when used as a flavouring agent.	No safety concern
2-Ethoxythiazole	1056	15679-19-3 	No B side	No Europe: 0.01 USA: 0.12	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day for the related flavouring agent No. 1041 is > 20 million times the estimated intake of 2-ethoxythiazole when used as a flavouring agent.	No safety concern

Table 5 (continued)

Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous products?	Step A3 /B3 ^{a,b} Does intake exceed threshold for structural class?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 / B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Methyl-5-methoxythiazole	1057	38205-64-0 	No B side	No Europe: ND USA: 0.01	N/R	B5 Yes. The NOEL of 8.6 mg/kg bw per day is > 40 million times the estimated intake of 2-methyl-5-methoxythiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	76788-46-0 	No B side	No Europe: ND USA: 0.01	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day for the related flavouring agent No. 1059 is > 6 million times the estimated intake of 4,5-dimethyl-2-ethyl-3-thiazoline when used as a flavouring agent.	No safety concern
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	65894-82-8 	No B side	No Europe: ND USA: 5	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day is > 10 000 times the estimated intake of 2-(2-butyl)-4,5-dimethyl-3-thiazoline when used as a flavouring agent.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation

^a Step 1. Twenty-one flavouring agents are in structural class II and nine flavouring agents are in structural class III.

^b The thresholds for human intake for structural classes II and III are 540 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intake of flavouring agents in structural class II is 3100 µg/day in Europe and 1600 µg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 1 µg/day in Europe and 11 µg/day in the USA.

^c Enzymatically cleaved to yield 4-methyl-5-thiazoleethanol (No. 1031) and 2-methyl-4-amino-5-hydroxymethylpyrimidine. The thiazole and pyrimidine fragments are further oxidized to yield 4-methylthiazole-4-acetic acid and the 5-pyrimidine carboxylic acid derivative, respectively, which, together with thiamine, are excreted in the urine. Can also be converted to 2-methyl-4-amino-5-formylaminopyrimidine and thiamine acetic acid.

^d Oxidized to yield 4-methylthiazole-4-acetic acid and excreted in the urine (See note c)

^e Hydrolysed to yield 4-methylthiazole-4-acetic acid and excreted in urine (See note c)

Dithiazine is a six-membered non-aromatic heterocyclic compound containing two sulfur atoms and one nitrogen atom in the 1-, 3- and 5-positions, respectively (Nos 1046–1049). Thiazoline (partially reduced thiazole) is a five-membered non-aromatic heterocyclic compound containing sulfur and nitrogen atoms in the 1- and 3-positions, respectively (Nos 1045, 1058 and 1059). Thiophene is a five-membered aromatic heterocyclic compound containing a sulfur atom in the 1-position (Nos 1050–1053). Except for thiazole (No. 1032), all the flavouring agents in this group are ring-substituted with one or more of the following functional groups: alkyl, alkenyl, aryl, alcohol, keto, thiol and disulfide.

The Committee has not previously evaluated any of the members of this group.

Eighteen flavouring agents in the group have been detected as natural components of food, and quantitative data have been reported on the natural occurrence of eight agents (Nos 1032, 1034, 1036, 1040, 1041, 1043 and 1050). The foods in which one or more of these flavouring agents can be found include lean meat, beans, nuts, whole-grain cereals, fish, coffee, milk, beer, peanuts, popcorn, pork liver, shrimp, tomato, potato, grapes and apples.

Estimated daily per capita intake

The total annual volume of production of the 30 sulfur-containing heterocyclic compounds in this group was reported to be 22 000 kg in Europe and 12 000 kg in the USA. These values are equivalent to total daily *per capita* intakes of 3100 µg in Europe and 1600 µg in the USA.

In both Europe and the USA, thiamine hydrochloride (vitamin B1; No. 1030) and its principal metabolite, 4-methyl-5-thiazoleethanol (No. 1031), accounted for approximately 98% of the total *per capita* dietary intake of the flavouring agents in this group. In Europe, thiamine hydrochloride and 4-methyl-5-thiazoleethanol accounted for approximately 92% (2900 µg/day) and 6% (170 µg/day), respectively, of the total *per capita* intake. In the USA, thiamine hydrochloride and 4-methyl-5-thiazoleethanol accounted for approximately 75% (1200 µg/day) and 23% (380 µg/day), respectively, of the total *per capita* intake of the flavouring agents in this group.

The estimated intakes of the remaining flavouring agents are between 0.01 and 22 µg/day in both Europe and the USA, with most intakes below 4 µg/day.

Absorption, distribution, metabolism and elimination

Thiazole and its derivatives are metabolized primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms; however, other routes of metabolism, involving ring cleavage, are possible. Derivatives of dithiazine, which are cyclic sulfides, are expected to be metabolized primarily by

S-oxidation to yield the corresponding sulfoxides and sulfones. Thiazoline is predicted to be similarly metabolized. Thiamine hydrochloride (No. 1030) at an intake of less than 5 mg/day is readily absorbed in the small intestine by an active transport system, followed by conversion to the coenzyme, thiamine pyrophosphate. Thiamine is metabolized primarily to a pyrimidine and a thiazole (4-methyl-5-thiazoleethanol, No. 1031) fragment. These fragments are further oxidized and excreted in the urine.

Thiophene derivatives are metabolized primarily by S-oxidation, followed by conjugation with glutathione; however, other routes of metabolism, involving ring cleavage, are also possible. The resulting mercapturic acid derivative is eliminated in the urine. The aromatic-fused thiazole, 2-acetylthiazole (benzothiazole; No. 1041), which lacks ring substituents, is metabolized by thiazole ring cleavage, yielding a series of free and conjugated *ortho*-aminophenyl sulfone and sulfoxide metabolites.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

The stepwise evaluation of the 30 sulfur-containing heterocyclic compounds is described in detail below and summarized in Table 5.

Step 1. All the flavouring agents in this group are heterocyclic compounds, and five of these (Nos 1040, 1050, 1051, 1052 and 1053) are also aromatic¹. One of the aromatic heterocyclic flavouring agents, benzothiazole (No. 1040) is unsubstituted and was therefore placed in structural class III. Of the other four substituted compounds, two (Nos 1050 and 1051) are common constituents of food and were therefore classified in structural class II, while the other two (Nos 1052 and 1053) are not common constituents of food and were therefore placed in structural class III.

Of the 25 non-aromatic heterocyclic flavouring agents, 19 (Nos 1030–1039, 1041–1044, 1046–1049 and 1054) are or are structurally closely related to common constituents of food and were therefore placed in structural class II. The remaining six flavouring agents (Nos 1045 and 1055–1059) were classified in structural class III.

Step 2. The data on most individual members of the group were, in most cases, insufficient to allow conclusions about their probable metabolic fate. The metabolism of thiamine hydrochloride (No. 1030) and its metabolite, 4-methyl-5-thiazoleethanol (No. 1031), is well characterized, and both are metabolized to innocuous products. 4-Methyl-5-thiazoleethanol acetate (No. 1054) is expected to be readily hydrolysed to 4-methyl-5-thiazole-

¹ According to the decision-tree process, an 'aromatic' compound has at least one benzene, furan, thiophene, pyridine or pyrrole ring, however substituted and regardless of whether it is fused to another ring.

ethanol. The evaluation of all three flavouring agents therefore proceeded via the A (left-hand) side of the scheme. For all other flavouring agents in the group, the evaluation proceeded via the B (right-hand) side of the scheme.

Step A3. With respect to those flavouring agents evaluated via the A side of the scheme, the estimated daily *per capita* intakes of 4-methyl-5-thiazoleethanol (No. 1031) and 4-methyl-5-thiazoleethanol acetate (No. 1054) are below the threshold for human intake for structural class II (540 µg), and therefore these flavouring agents are not expected to raise any safety concern. The daily *per capita* intake of thiamine hydrochloride (No. 1030) is 2900 µg in Europe and 1200 µg in the USA. The intake of this flavouring agent therefore exceeds the threshold for human intake of compounds in structural class II, and its evaluation proceeded to step A4.

Step A4. Thiamine hydrochloride (No. 1030) is not endogenous; therefore, its evaluation proceeded to step A5.

Step A5. The NOEL of 36 mg/kg bw per day for thiamine hydrochloride (No. 1030) in a 90-day dietary study in rats is more than 500 times the estimated intake of this substance from its use as a flavouring agent in Europe (48 µg/kg bw per day) and more than 1000 times the estimated intake in the USA (22 µg/kg bw per day). Thiamine hydrochloride would therefore not be expected to be a safety concern.

Step B3 With regard to those flavouring agents evaluated via the B side of the scheme, the estimated daily *per capita* intakes of all 18 flavouring agents in structural class II (Nos 1032–1039, 1041–1044 and 1046–1051) are below the threshold for human intake for structural class II (540 µg per person per day). Similarly, the estimated daily *per capita* intakes of all nine flavouring agents in structural class III (Nos 1040, 1045, 1052, 1053 and 1055–1059) are below the threshold for human intake for compounds in this class (90 µg per person per day). The evaluation of all of these flavouring agents therefore proceeded to step B4.

Step B4 The NOEL for 2,4-dimethyl-5-vinylthiazole (No. 1039) in a 90-day dietary study in rats was 0.92 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agents, thiazole (No. 1032), 2-(1-methylpropyl)thiazole (No. 1033), 2-isobutylthiazole (No. 1034), 4,5-dimethylthiazole (No. 1035), 2,4,5-trimethylthiazole (No. 1036), 2-isopropyl-4-methylthiazole (No. 1037), 4-methyl-5-vinylthiazole (No. 1038), 4-methylthiazole (No. 1043) and 2-ethyl-4-methylthiazole (No. 1044).

The NOEL for 2-acetylthiazole (No. 1041) in a 90-day dietary study in rats was 50 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agent, 2-propionylthiazole (No. 1042).

The NOEL for 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) in a 90-day dietary study in rats was 1.2 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agents, 4,5-dimethyl-2-isobutyl-3-thiazoline (No. 1045) and 4,5-dimethyl-2-ethyl-3-thiazoline (No. 1058).

The NOEL for a mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1046) and of a mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1047) in a 14-day dietary study in rats was 11 mg/kg bw per day, and this NOEL is appropriate for the structurally related agents, 2,4,6-triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine (No. 1048) and 2,4,6-trimethyldihydro-4*H*-1,3,5-dithiazine (No. 1049).

The NOEL for 2-thienyl disulfide (No. 1053) in a 90-day dietary study in rats was 290 mg/kg bw per day, and this NOEL is appropriate for the structurally related agents 5-methyl-2-thiophene carboxyaldehyde (No. 1050), 3-acetyl-2,5-dimethylthiophene (No. 1051) and 2-thienyl mercaptan (No. 1052).

The NOEL for benzothiazole (No. 1040) in a 90-day dietary study in rats was 5.1 mg/kg bw per day.

The NOEL for 2,4-dimethyl-5-acetylthiazole (No. 1055) in a dietary study in rats was 24 mg/kg bw per day.

The NOEL for 2-methyl-5-methoxythiazole (No. 1057) in a 90-day dietary study in rats was 8.6 mg/kg bw per day.

For all the above-mentioned flavouring agents (Nos 1032–1053, 1055–1059), therefore, a NOEL exists either for the substance itself, which provides an adequate margin of safety under the intended conditions of use, or for a structurally related substance, which is high enough to accommodate any perceived difference in toxicity between the substance itself and the related substance.

The NOEL used to evaluate each flavouring agent and the margins of safety they provide on the basis of current levels of intake are summarized in Table 6. On the basis of these data, these flavouring agents would not be expected to be a safety concern.

Consideration of combined intakes from use as flavouring agents

Although the 30 flavouring agents in this group are unlikely to produce common metabolites, consideration of their combined intake is appropriate since all are likely to be conjugated with glutathione before excretion.

Table 6

Margins of safety for the sulfur-containing heterocyclic compounds used as flavouring agents proceeding via the B side of the Procedure

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
Structural class II						
Thiazole	1032	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.001	> 900 000
2-(1-Methylpropyl)thiazole	1033	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.0005	> 1 million
2-Isobutylthiazole	1034	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.05	> 10 000
4,5-Dimethylthiazole	1035	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.004	> 200 000
2,4,5-Trimethylthiazole	1036	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.01	> 90 000
2-Isopropyl-4-methylthiazole	1037	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.4	> 2000
4-Methyl-5-vinylthiazole	1038	2,4-dimethyl-5-vinylthiazole	1039	0.92 ^a	0.04	> 20 000
2,4-Dimethyl-5-vinylthiazole	1039	NA	NA	0.92 ^a	0.0001	> 9 million
2-Acetylthiazole	1041	NA	NA	50 ^a	0.2	> 200 000
2-Propionylthiazole	1042	2-Acetylthiazole	1041	50 ^a	0.003	> 10 million
4-Methylthiazole	1043	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.002	> 400 000
2-Ethyl-4-methylthiazole	1044	2,4-Dimethyl-5-vinylthiazole	1039	0.92 ^a	0.06	> 10 000
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1046	NA	NA	11 ^b	0.002	> 5 million
2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1047	NA	NA	11 ^b	0.001	> 10 million
2,4,6-Triisobutyl-5,6-dihydro-4 <i>H</i> -1,3,5-dithiazine	1048	A mixture of 2-Isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	1046	11 ^b	0.04	> 200 000
2,4,6-Trimethyldihydro-4 <i>H</i> -1,3,5-dithiazine	1049	A mixture of 2-Isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	1046	11 ^b	0.002	> 5 million
5-Methyl-2-thiophene-carboxaldehyde	1050	2-Thienyl disulfide	1053	290 ^a	0.01	> 20 million
3-Acetyl-2,5-dimethylthiophene	1051	2-Thienyl disulfide	1053	290 ^a	0.4	> 700 000

Table 6 (continued)

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
5-Methyl-2-thiophene-carboxaldehyde	1050	2-Thienyl disulfide	1053	290 ^a	0.01	> 20 million
3-Acetyl-2,5-dimethylthiophene	1051	2-Thienyl disulfide	1053	290 ^a	0.4	> 700 000
Structural class III						
Benzothiazole	1040	NA	NA	5.1 ^a	0.02	> 20 000
4,5-Dimethyl-2-isobutyl-3-thiazoline	1045	2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	1.2 ^a	0.07	> 10 000
2-Thienyl mercaptan	1052	2-Thienyl disulfide	1053	290 ^a	0.0004	> 700 million
2-Thienyl disulfide	1053	NA	NA	290 ^a	0.001	> 200 million
2,4-Dimethyl-5-acetylthiazole	1055	NA	NA	24 ^a	0.03	> 10 million
2-Ethoxythiazole	1056	2-Acetylthiazole	1041	50 ^a	0.002	> 20 million
2-Methyl-5-methoxythiazole	1057	NA	NA	8.6 ^a	0.0002	> 40 million
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	1.2 ^a	0.0002	> 6 million
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	NA	NA	1.2 ^a	0.09	> 10 000

NA, not applicable

^a90-day study in rats^b14-day study in rats

In the unlikely event that all 21 flavouring agents in structural class II were to be consumed simultaneously on a daily basis, the estimated combined *per capita* intakes (3100 µg/day in Europe and 1600 µg/day in the USA) would exceed the threshold of human intake for their structural class (540 µg per person). Since, at these levels of intake, the conjugation capacity of the glutathione pool is unlikely to be depleted, this level of intake is not anticipated to be a safety concern.

In the unlikely event that the nine flavouring agents in structural class III were to be consumed simultaneously on a daily basis, the estimated combined *per capita* intakes (1 µg/day in Europe and 11 µg/day in the USA) would not exceed the threshold for human intake for their structural class (90 µg per person).

Conclusions

On the basis of the predicted metabolism and the NOELs for some members of the group, the Committee concluded that the 30 sulfur-containing heterocyclic compounds in this group would not raise safety concerns at current levels of intake.

The Committee noted that, for the three flavouring agents that were predicted to be metabolized to innocuous products, the available data on toxicity were consistent with the results of the safety evaluation.

A toxicological monograph was prepared.

4.1.4 Sulfur-substituted furan derivatives

The Committee evaluated a group of 33 flavouring agents that includes thiofuran and thiofurfuryl derivatives (see Table 7), using the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). The Committee has not previously evaluated any member of the group.

Eighteen of the 33 flavouring agents in this group have been reported to occur naturally in foods. Thiofurfuryl and thiofuran derivatives have been detected mainly in coffee and cooked meats.

Estimated daily per capita intake

The total annual volume of production of the 33 sulfur-substituted furan derivatives is approximately 370 kg in Europe and 210 kg in the USA. Approximately 65% of the total annual volume in Europe and 40% of that in the USA arises from use of one substance, furfuryl mercaptan (No. 1072), with annual volumes of 240 kg in Europe and 82 kg in the USA. The estimated *per capita* intake of furfuryl mercaptan is 40 µg/day in Europe and 11 µg/day in the USA. The estimated daily intakes of each of the other 32 flavouring agents in this group are less than 5 µg per day in Europe and in the USA.

Table 7

Summary of results of safety evaluations of sulfur-substituted furan derivatives used as flavouring agents^a

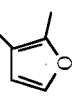
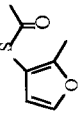
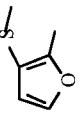
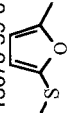
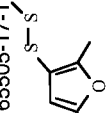
Flavouring agent	No.	CAS No. and structure	Step B3 ^b Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Structural class II					
2-Methyl-3-furanthiol	1060	28588-74-1 	No Europe: 0.6 USA: 0.9	Yes. The NOEL of 5 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-furanthiol when used as a flavouring agent.	No safety concern
Ethanoic acid, S-(2-methyl-3-furanyl) ester	1069	55764-25-5 	No Europe: 0.01 USA: 0.07	Yes. The NOEL of 5 mg/kg bw per day for the related substance No. 1060 is > 1 million times the estimated intake of ethanoic acid, S-(2-methyl-3-furanyl) ester when used as a flavouring agent.	No safety concern
2-Methyl-3-(methylthio)furan	1061	63012-97-5 	No Europe: 1 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of 2-methyl-3-(methylthio)furan when used as a flavouring agent.	No safety concern
2-Methyl-5-(methylthio)furan	1062	13678-59-6 	No Europe: 1 USA: 0.02	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of 2-methyl-5-(methylthio)furan when used as a flavouring agent.	No safety concern
Methyl 2-methyl-3-furyl disulfide	1064	65505-17-1 	No Europe: 0.9 USA: 0.05	Yes. The NOEL of 1.2 mg/kg bw per day is > 10 000 times the estimated intake of methyl 2-methyl-3-furyl disulfide when used as a flavouring agent.	No safety concern

Table 7 (continued)

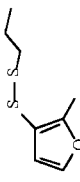
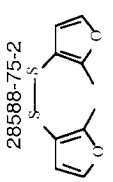
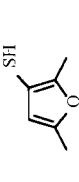
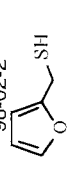
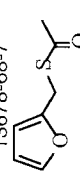
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Propyl 2-methyl-3-furyl disulfide	1065	61197-09-9 	No Europe: ND USA: 0.7	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 100 000 times the estimated intake of propyl 2-methyl-3-furyl disulfide when used as a flavouring agent.	No safety concern
Bis(2-methyl-3-furyl) disulfide	1066	28588-75-2 	No Europe: 0.3 USA: 0.7	Yes. The NOEL of 0.45 mg/kg bw per day is > 10 000 times the estimated intake of bis(2-methyl-3-furyl) disulfide when used as a flavouring agent.	No safety concern
2,5-Dimethyl-3-furanthiol	1063	55764-23-3 	No Europe: 0.03 USA: 0.7	Yes. The NOEL of 5 mg/kg bw per day for the related substance No. 1060 is > 100 000 times the estimated intake of 2,5-dimethyl-3-furanthiol when used as a flavouring agent.	No safety concern
Furfuryl mercaptan	1072	98-02-2 	No Europe: 40 USA: 11	Yes. The NOEL of 3 mg/kg bw per day is > 1000 times the estimated intake of furfuryl mercaptan when used as a flavouring agent.	No safety concern
Furfuryl thioacetate	1074	13678-68-7 	No Europe: 0.5 USA: 0.05	Yes. The NOEL of 0.83 mg/kg bw per day is > 100 000 times the estimated intake of furfuryl thioacetate when used as a flavouring agent.	No safety concern

Table 7 (continued)

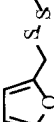
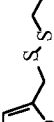
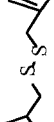
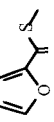
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Furfuryl methyl sulfide	1076	1438-91-1 	No Europe: 1 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of furfuryl methyl sulfide when used as a flavouring agent.	No safety concern
Methyl furfuryl disulfide	1078	57500-00-2 	No Europe: 1 USA: 0.04	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 10 000 times the estimated intake of methyl furfuryl disulfide when used as a flavouring agent.	No safety concern
Furfuryl propyl disulfide	1079	2527366-36-0 	No Europe: ND USA: 3	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 10 000 times the estimated intake of furfuryl propyl disulfide when used as a flavouring agent.	No safety concern
2,2'-(Dithiodimethylene) difuran	1081	4437-20-1 	No Europe: 4 USA: 0.7	Yes. The NOEL of 3 mg/kg bw per day for the related substance No. 1072 is > 10 000 times the estimated intake of 2,2'-(dithiodimethylene) difuran when used as a flavouring agent.	No safety concern
Methyl thiofuroate	1083	13679-61-3 	No Europe: 0.4 USA: 0.1	Yes. The flavouring agent is expected to hydrolyse to methanethiol and furoic acid. The NOEL of 0.74 mg/kg bw per day for the related substance No. 1071, which also hydrolyses to furoic acid, is > 100 000 times the estimated intake of methyl thiofuroate when used as a flavouring agent.	No safety concern

Table 7 (continued)

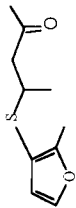
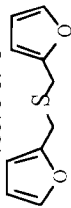
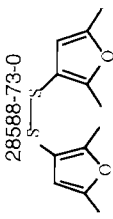
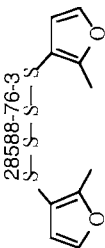
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084	180031-78-1 	No Europe: ND USA: 0.6	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 is > 100 000 times the estimated intake of 4-[(2-methyl-3-furyl)thio]-2-pentanone when used as a flavouring agent.	No safety concern
2-Methyl-3-tetrahydrofuranthiol	1090	57124-87-5 	No Europe: 4 USA: 0.7	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of 2-methyl-3-tetrahydrofuranthiol when used as a flavouring agent.	No safety concern
2,2'-(Thiodimethylene) difuran	1080	13678-67-6 	No Europe: 0.9 USA: 0.005	Yes. The NOEL of 10 mg/kg bw per day is > 100 000 times the estimated intake of 2,2'-(thiodimethylene) when used as a flavouring agent.	No safety concern
Structural class III					
Bis(2,5-dimethyl-3-furyl)disulfide	1067	28588-73-0 	No Europe: 0.01 USA: 0.7	Yes. The NOEL of 0.45 mg/kg bw per day for the related substance No. 1066 is > 10 000 times the estimated intake of bis(2,5-dimethyl-3-furyl)disulfide when used as a flavouring agent.	No safety concern
Bis(2-methyl-3-furyl) tetrasulfide	1068	28588-76-3 	No Europe: ND USA: 0.7	Yes. The NOEL of 0.56 mg/kg bw per day is > 10 000 times the estimated intake of bis(2-methyl-3-furyl) tetrasulfide when used as a flavouring agent.	No safety concern

Table 7 (continued)

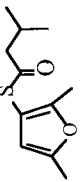
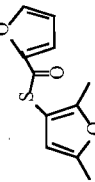
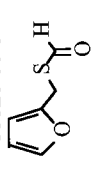
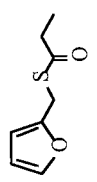
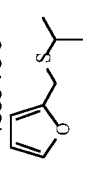
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2,5-Dimethyl-3-furan thioisovalerate	1070	55764-28-8 	No Europe: ND USA: 0.7	Yes. The NOEL of 0.73 mg/kg bw per day is > 10 000 times the estimated intake of 2,5-dimethyl-3-furan thioisovalerate when used as a flavouring agent.	No safety concern
S-2,5-Dimethyl-3-thiofuryl furan	1071	65505-16-0 	No Europe: ND USA: 0.01	Yes. The NOEL of 0.74 mg/kg bw per day is > 1 million times the estimated intake of S-2,5-dimethyl-3-thiofuryl furan when used as a flavouring agent.	No safety concern
Furfuryl thioformate	1073	59020-90-5 	No Europe: 2 USA: 0.02	Yes. The NOELs of 3 and 0.83 mg/kg bw per day for the related substances Nos 1072 and 1074 are > 1 million and > 10 000 times, respectively, the estimated intake of furfuryl thioformate when used as a flavouring agent.	No safety concern
Furfuryl thiopropionate	1075	59020-85-8 	No Europe: 0.01 USA: 0.005	Yes. The NOELs of 3 and 0.83 mg/kg bw per day for the related substances Nos 1072 and 1074 are > 1 million times the estimated intake of furfuryl thiopropionate when used as a flavouring agent.	No safety concern
Furfuryl isopropyl sulfide	1077	1883-78-9 	No Europe: 0.001 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day is > 100 000 times the estimated intake of furfuryl isopropyl sulfide when used as a flavouring agent.	No safety concern

Table 7 (continued)

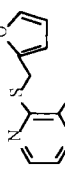
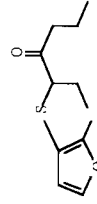
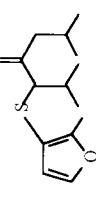
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082	65530-53-2 	No Europe: 0.4 USA: 0.7	Yes. The NOEL of 1.7 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-, 5- or 6-(furfurylthio)pyrazine when used as a flavouring agent.	No safety concern
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085	61295-41-8 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day is > 100 000 times the estimated intake of 3-[(2-methyl-3-furyl)thio]-4-heptanone when used as a flavouring agent.	No safety concern
2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086	61295-51-0 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 is > 100 000 times the estimated intake of 2,6-dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone when used as a flavouring agent.	No safety concern

Table 7 (continued)

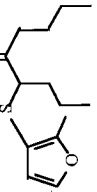
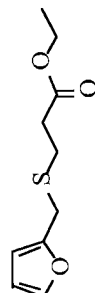
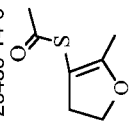
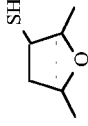
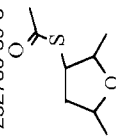
Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087	61295-50-9 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 was > 100 000 times the estimated intake of 4-[(2-methyl-3-furyl)thio]-5-nonanone when used as a flavouring agent.	No safety concern
Ethyl 3-(furfurylthio)propionate	1088	94278-27-0 	No Europe: 0.01 USA: 0.2	Yes. The NOEL of 17 mg/kg bw per day is > 1 million times the estimated intake of ethyl 3-(furfurylthio)propionate when used as a flavouring agent.	No safety concern
2-Methyl-3-thioacetoxy-4,5-dihydrofuran	1089	26486-14-6 	No Europe: ND USA: 0.7	Yes. The NOEL of 8.3 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-thioacetoxy-4,5-dihydrofuran when used as a flavouring agent.	No safety concern
cis- and trans-2,5-Dimethyl-3-tetrahydrofuranthiol	1091	26486-21-5 	No Europe: ND USA: 0.9	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of cis- and trans-2,5-dimethyl-3-tetrahydrofuranthiol when used as a flavouring agent.	No safety concern

Table 7 (continued)

Flavouring agent	No.	CAS No. and structure	Step B3 ^{a,b} Does intake exceed threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
<i>cis</i> - and <i>trans</i> -2,5-Dimethyltetrahydro-3-furyl thioacetate	1092	252736-39-3 	No Europe: ND USA: 2	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of <i>cis</i> - and <i>trans</i> -2,5-dimethyl-tetrahydro-3-furyl thioacetate when used as a flavouring agent.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported

^a Step 2: None of the flavouring agents in this group was predicted to be metabolized to innocuous products.

^b The human intake threshold is 540 µg/person per day for class II and 90 µg/person per day for class III. All intake levels are expressed in µg/person per day. The combined intake of flavouring agents in class II is 56 and 20 µg/person per day in Europe and the USA, respectively. The combined intake of flavouring agents in class III is 2.4 and 8.8 µg/person per day in Europe and the USA, respectively.

Absorption, distribution, metabolism and elimination

No data on the metabolism of these flavouring agents were available; however, all the members of the group contain a reactive divalent sulfur atom attached to the heteroaromatic or heterocyclic ring. Therefore, these flavouring agents are probably metabolized via reactions of the divalent sulfur, like other heteroaromatic ring systems containing sulfur as a side-chain substituent. However, in the absence of data on the disposition of these substances, the metabolic fate of this group of flavouring agents is unknown.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Twenty-nine of the 33 flavouring agents in this group are aromatic heterocyclic compounds bearing ring substituents. Twenty-two of the 29 heteroaromatic *S*-substituted furan derivatives containing only one aromatic ring were assigned to structural class II if they were naturally occurring or to structural class III if they did not occur naturally in foods. The seven remaining heteroaromatic substances contain two aromatic rings. Of these, five agents (Nos 1066, 1067, 1068, 1071 and 1081) would be hydrolysed (thioester) or readily reduced (di- or tetra-sulfides) to mononuclear residues. They were assigned to structural class II or III depending on their natural occurrence. The two remaining substances (Nos 1080 and 1082), which are not readily converted to mononuclear residues, were placed in structural class III.

The remaining four flavouring agents are heterocyclic but not heteroaromatic (Nos 1089–1092). One agent, 2-methyl-3-tetrahydrofuranthiol (No. 1090), occurs naturally in foods and was placed in structural class II. The other three agents were placed in structural class III.

In summary, 18 of the 33 flavouring agents were assigned to structural class II because they are common components of food, while 15 that are not common components of food were assigned to structural class III.

Step 2. None of the flavouring agents in this group was predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded down the B (right-hand) side of the decision tree.

Step B3. The current estimated daily *per capita* intakes of each of the 33 flavouring agents in this group are below the threshold for human intake for the respective structural classes (540 mg/day for structural class II and 90 mg/day for structural class III). Accordingly, the evaluation of all 33 substances in the group proceeded to step B4.

Step B4. The NOEL for 2-methyl-3-furanthiol (No. 1060) in a 90-day dietary study in rats was 5 mg/kg bw per day. This NOEL is appropriate for ethanoic acid, *S*-(2-methyl-3-furanyl) ester (No. 1069) because the acetate ester would

be hydrolysed to 2-methyl-3-furanthiol (No. 1060). This NOEL is also appropriate for the closely structurally related agent, 2,5-dimethyl-3-furanthiol (No. 1063).

The NOEL for furfuryl isopropyl sulfide (No. 1077) in a 90-day dietary study in rats was 1.3 mg/kg bw per day. This NOEL is also appropriate for three structurally related sulfides, 2-methyl-3-(methylthio)furan (No. 1061), 2-methyl-5-(methylthio)furan (No. 1062) and furfuryl methyl sulfide (No. 1076), which would be expected to participate in the same metabolic pathways as furfuryl isopropyl sulfide.

The NOEL for methyl 2-methyl-3-furyl disulfide (No. 1064) in a 90-day dietary study in rats was 1.2 mg/kg bw per day. This NOEL is also appropriate for three structurally related disulfides, propyl 2-methyl-3-furyl disulfide (No. 1065), methyl furfuryl disulfide (No. 1078) and furfuryl propyl disulfide (No. 1079).

The NOEL for bis(2-methyl-3-furyl) disulfide (No. 1066) in a 90-day dietary study in rats was 0.45 mg/kg bw per day. This NOEL is also appropriate for a structurally related bis-disulfide, bis(2,5-dimethyl-3-furyl) disulfide (No. 1067).

The NOEL for furfuryl mercaptan (No. 1072) in a multiple-dose, 13-week study in rats treated by gavage was 3 mg/kg bw per day; and the NOEL for furfuryl thioacetate (No. 1074) in a 90-day dietary study in rats was 0.83 mg/kg bw per day. These NOELs are also appropriate for the esters furfuryl thioformate (No. 1073) and furfuryl thiopropionate (No. 1075), because they are either close structural relatives of furfuryl thioacetate (No. 1074) or are expected to be hydrolysed to furfuryl mercaptan (No. 1072). The NOEL of 3 mg/kg bw per day for furfuryl mercaptan (No. 1072) is also appropriate for 2,2'-(dithiodimethylene) difuran (No. 1081), because this chemical is anticipated to be readily reduced to furfuryl mercaptan.

The NOEL for 2,5-dimethyl-3-thiofuroyl furan (No. 1071) in a 90-day dietary study in rats was 0.74 mg/kg bw per day. This NOEL is appropriate for methyl thiofuroate (No. 1083), because both 2,5-dimethyl-3-thiofuroyl furan and methyl thiofuroate would be hydrolysed to furoic acid.

The NOEL for 3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1085) in a 90-day dietary study in rats was 3.8 mg/kg bw per day. This NOEL is also appropriate for three structurally related thioketones, 4-[(2-methyl-3-furyl)thio]-2-pentanone (No. 1084), 2,6-dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1086) and 4-[(2-methyl-3-furyl)thio]-5-nonanone (No. 1087).

The NOELs for 2,2'-(thiodimethylene) difuran (No. 1080), bis(2-methyl-3-furyl) tetrasulfide (No. 1068), 2,5-dimethyl-3-furan thioisovalerate (No.

1070), 2-methyl-3-, 5- or 6-(furfurylthio)pyrazine (No. 1082) and ethyl 3-(furfurylthio)propionate (No. 1088) in 14-day or 90-day dietary studies in rats were 10, 0.56, 0.73, 1.7 and 17 mg/kg bw per day, respectively.

The NOEL for 2-methylthioacetoxy-4,5-dihydrofuran (No. 1089) in a 1-year dietary study in rats was 8.3 mg/kg bw per day. This NOEL is also appropriate for three structurally related analogues, 2-methyl-3-tetrahydrofuranthiol (No. 1090), *cis*- and *trans*-2,5-dimethyl-3-tetrahydrofuranthiol (No. 1091) and *cis*- and *trans*-2,5-dimethyltetrahydro-3-furyl thioacetate (No. 1092), because these chemicals are expected to be hydrolysed to the corresponding dihydro- or tetrahydrofuranthiol.

The NOEL(s) used to evaluate each flavouring agent and the margins of safety they provide, on the basis of current intake levels, are summarized in Table 8.

Consideration of combined intakes from use as flavouring agents

The hydrolysis of furfuryl thioesters (Nos 1073, 1074 and 1075) or reduction of the disulfides (Nos 1078, 1079 and 1081) in this group would yield the common metabolite furfuryl mercaptan (No. 1072). In the unlikely event that these seven flavouring agents were to be consumed simultaneously on a daily basis, the estimated combined intakes (48 and 15 µg per person per day in Europe and the USA, respectively) would not exceed the threshold for daily human intake of compounds in structural class II (540 µg per person) or III (90 µg per person).

Likewise, hydrolysis of 2-methyl-3-furan thioester (No. 1069) or reduction of the corresponding disulfides (Nos 1064, 1065 and 1066) or tetrasulfide (No. 1068) would yield the common metabolite 2-methyl-3-furanthiol (No. 1060). In the unlikely event that these six flavouring agents were to be consumed simultaneously on a daily basis, the estimated combined daily intake would be less than 5 µg per person in Europe and the USA, which would not exceed the threshold for human daily intake of compounds in structural class II (540 µg per person) or III (90 µg per person).

On the basis of the evaluation of the collective data, the Committee identified no safety concern from combined intake.

Conclusions

The Committee concluded that use of flavouring agents in this group of 33 thiofurfuryl and thiofuran derivatives at current levels of intake would not present a safety concern. In the Procedure, data on toxicity were required for all the evaluations. The Committee noted the absence of data on the metabolic fate of these substances but considered that their potential

Table 8

NOELs and margins of safety for sulfur-substituted furan derivatives used as flavouring agents

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
Structural class II						
2-Methyl-3-furanthiol	1060	Ethanoic acid, S-(2-methyl-3-furyl) ester 2,5-Dimethyl-3-furanthiol	1069 1063	5 ^a	0.015 0.001 0.01 0.014	> 100 000 > 1 000 000 > 100 000 > 10 000
Methyl 2-methyl-3-furyl disulfide	1064	Propyl 2-methyl-3-furyl disulfide Methyl furfuryl disulfide Furfuryl propyl disulfide	1065 1078 1079	1.2 ^a	0.01 0.017 0.04 0.01	> 100 000 > 10 000 > 10 000 > 10 000
Bis(2-methyl-3-furyl) disulfide	1066	Bis(2,5-dimethyl-3-furyl) disulfide	1067	0.45 ^a	0.01	> 10 000
Furfuryl mercaptan	1072	Furfuryl thioformate Furfuryl thiopropionate 2,2'-(Dithiodimethylene) difuran	1073 1075 1081	3 ^a	0.58 0.026 0.0002 0.064	> 1 000 > 100 000 > 1 000 000 > 10 000
Furfuryl thioacetate	1074	Furfuryl thioformate Furfuryl thiopropionate	1073 1075	0.83 ^a	0.008 0.026 0.0002 0.01	> 100 000 > 10 000 > 1 000 000 > 1 000 000
4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084	2-Methyl-3-tetrahydrofuranthiol	1090	54 ^{bc} 12 ^{bc} 10 ^b	0.07 0.014	> 100 000 > 100 000 > 100 000
2,2'-(Thiodimethylene) difuran	1080					

Table 8 (continued)

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
Structural class III						
Bis(2-methyl-3-furyl) tetrasulfide	1068			0.56 ^a	0.01	> 10 000
2,5-Dimethyl-3-furan thioisovalerate	1070			0.73 ^a	0.01	> 10 000
S-2,5-Dimethyl-3-thiofuroyl furan	1071			0.74 ^a	0.0002	> 1 000 000
Furfuryl isopropyl sulfide	1077	Methyl thiofuroate	1083	1.3 ^a	0.002	> 100 000
		2-Methyl-3-(methylthio)furan	1061		0.002	> 100 000
		2-Methyl-5-(methylthio)furan	1062		0.024	> 10 000
		Furfuryl methyl sulfide	1076		0.021	> 10 000
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082			1.7 ^a	0.01	> 100 000
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085			3.8 ^a	0.01	> 100 000
		4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084		0.01	> 100 000
		2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086		0.01	> 100 000
		4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087		0.01	> 100 000
Ethyl 3-(furfurylthio)propionate	1088			17 ^a	0.003	> 1 000 000
2-Methyl-3-thioacetoxo-4,5-dihydrofuran	1089			8.3 ^d	0.01	> 100 000
		2-Methyl-3-tetrahydrofuranthiol	1090		0.07	> 100 000
		cis- and trans-2,5-Dimethyl-3-tetrahydrofuranthiol	1091		0.02	> 100 000
		cis- and trans-2,5-Dimethyltetrahydro-3-furyl thioacetate	1092		0.03	> 100 000

^a90-day study^b14-day study^cThis study was not used in the evaluation, as a 90-day study on a structurally related flavouring agent was available.^d1-year study

metabolism via the reactive divalent sulfur atom and their very low levels of use as flavouring agents were consistent with the outcome of the evaluations.

A monograph summarizing the available safety data on this group of flavouring agents was prepared.

4.1.5 ***Alicyclic ketones, secondary alcohols and related esters***

The Committee evaluated a group of 25 flavouring agents consisting of alicyclic ketones, secondary alcohols and related esters (see Table 9). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). None of these agents has been evaluated previously by the Committee.

Twelve of the 25 substances (Nos 1093, 1098, 1100–1104, 1107, 1108, 1110, 1112 and 1114) have been reported to occur naturally in foods. They have been detected in fruits, vegetables, cheese, meats, seafood, grains, alcoholic beverages, coffee and tea.

Estimated daily per capita intake

The total annual volume of production of the 25 alicyclic ketones, secondary alcohols and related esters is approximately 520 kg in Europe and 310 kg in the USA. The reported annual production of only one agent, 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (No. 1114), is greater than 100 kg (110 kg in Europe). The daily *per capita* intake of each of the agents in this group is $\leq 15 \mu\text{g}$ in Europe and in the USA.

Absorption, distribution, metabolism and elimination

Esters (Nos 1093–1098) in this group are hydrolysed to their corresponding alcohols and carboxylic acids by carboxylesterases, which are found abundantly in hepatocytes. The resulting alicyclic secondary alcohols are conjugated with glucuronic acid and excreted mainly in the urine. Side-chain oxidation of methyl groups may also occur.

Alicyclic ketones (Nos 1100–1117) are reduced to the corresponding secondary alcohol and excreted primarily as glucuronic acid conjugates. If a double bond is present, the chemical may be reduced to the corresponding dihydro derivative. Reduction of the double bond in metabolites excreted in the bile is probably associated with the action of gut microflora. Endocyclic double bonds (Nos 1105, 1107 and 1110–1115) are more prone to reduction than exocyclic double bonds (Nos 1098 and 1106). Alicyclic ketones containing an alkyl side-chain (Nos 1098, 1099 and 1102–1117) can not only follow reductive pathways but can undergo oxidation of the side-chain to form poly-oxygenated metabolites, which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

Table 9

Summary of results of the safety evaluation of alicyclic ketones, secondary alcohols and related esters used as flavouring agents^a

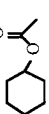
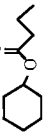
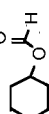
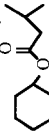
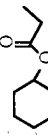
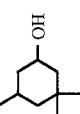
Flavouring agent	No.	CAS No. and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Cyclohexyl acetate	1093	622-45-7 	No Europe: 14 USA: 10	See note 1.	No safety concern
Cyclohexyl butyrate	1094	1551-44-6 	No Europe: ND USA: 0.1	See note 1.	No safety concern
Cyclohexyl formate	1095	4351-54-6 	No Europe: 0.01 USA: 0.2	See note 1.	No safety concern
Cyclohexyl isovalerate	1096	7774-44-9 	No Europe: 0.3 USA: 0.05	See note 1.	No safety concern
Cyclohexyl propionate	1097	6222-35-1 	No Europe: 0.01 USA: 0.05	See note 1.	No safety concern
3,3,5-Trimethylcyclohexanol	1099	116-02-9 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern

Table 9 (continued)

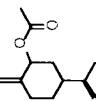
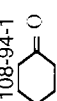
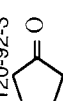
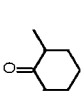
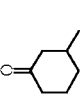
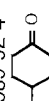
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class II					
<i>cis</i> - and <i>trans</i> - <i>para</i> -1(7),8-Menthadien-2-yl acetate	1098	71660-03-2 	No Europe: ND USA: 0.6	See note 1.	No safety concern
Cyclohexanone	1100	108-94-1 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
Cyclopentanone	1101	120-92-3 	No Europe: 0.02 USA: 0.02	See note 2.	No safety concern
2-Methylcyclohexanone	1102	583-60-8 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
3-Methylcyclohexanone	1103	591-24-2 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
4-Methyl cyclohexanone	1104	589-92-4 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern

Table 9 (continued)

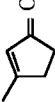
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
1-Methyl-1-cyclopenten-3-one	1105	2758-18-1 	No Europe: 0.07 USA: ND	See note 3.	No safety concern
Structural class II 2-Hexylidene cyclopentanone	1106	17373-89-6 	No Europe: 0.3 USA: 0.01	See notes 3 and 4.	No safety concern
3-Methyl-2-cyclohexen-1-one	1107	1193-18-6 	No Europe: 0.01 USA: 0.1	See note 3.	No safety concern
2,2,6-Trimethylcyclohexanone	1108	2408-37-9 	No Europe: 2.4 USA: 0.04	See note 2.	No safety concern
2-sec-Butylcyclohexanone	1109	14765-30-1 	No Europe: 6 USA: ND	See note 4.	No safety concern
4-Isopropyl-2-cyclohexenone	1110	500-02-7 	No Europe: 0.001 USA: 0.001	See note 3.	No safety concern

Table 9 (continued)

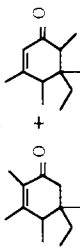
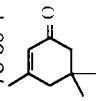
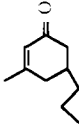
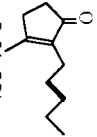
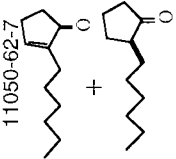
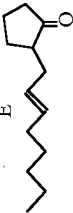
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Tetramethyl ethyl cyclohexenone (mixture of isomers)	1111	17369-60-7 	No Europe: 9 USA: 0.2	See note 3.	No safety concern
Isophorone	1112	78-59-1 	No Europe: 5.4 USA: 0.1	See note 3.	No safety concern
3-Methyl-5-propyl-2- cyclohexen-1-one	1113	3720-16-9 	No Europe: 0.1 USA: 4.1	See note 3.	No safety concern
3-Methyl-2-(2-pentenyl)-2- cyclopenten-1-one	1114	488-10-8 	No Europe: 16 USA: 7.2	See notes 3 and 4.	No safety concern
Isojasmonone	1115	11050-62-7 	No Europe: 0.4 USA: 0.01	See notes 3 and 4.	No safety concern

Table 9 (continued)

Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
(E)-2-(2-Octenyl) cyclopentanone	1116	65737-52-2 	No Europe: 7 USA: 6.6	See note 4.	No safety concern
2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone	1117	68133-79-9 	No Europe: 7.1 USA: 6.6	See note 4.	No safety concern

CAS: Chemical Abstracts Service; ND: No intake data reported

^a Step 2: All of the flavouring agents in this group were predicted to be metabolized to innocuous products.

^b The threshold for human intake is 1800 µg/person per day for class I and 540 µg/person per day for class II. All intake levels are expressed in µg/person per day. The combined intake of flavouring agents in class I is 14 and 10 µg per person per day in Europe and the USA, respectively. The combined intake of flavouring agents in class II is 54 and 26 µg per person per day in Europe and the USA, respectively.

Notes:

1. Detoxicated by hydrolysis of ester and glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid
2. Detoxicated by reduction of the ketone followed by glucuronic acid conjugation of the corresponding alcohol
3. Detoxicated by reduction of the ketone functional group followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone
4. Detoxicated by reduction of the ketone and alkyl side-chain oxidation and excretion

The nine chemicals that are α,β -unsaturated ketones (Nos 1105–1107 and 1110–1116) are subject to glutathione conjugation, with subsequent elimination in the urine as mercapturic acids.

As the α,β -unsaturated carbonyl group is a structural alert for toxicity, the Committee, at previous meetings, has devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference 154) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with use of such compounds as flavouring agents. With respect to α,β -unsaturated ketones, these protective processes include reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid) and conjugation with glutathione. These processes operate for the aliphatic ketones used as flavouring agents.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned six (Nos 1093–1097 and 1099) of the 25 substances to structural class I. These agents are either alicyclic secondary alcohols or are readily hydrolysed to secondary alcohols and simple short-chain carboxylic acids. They are simple aliphatic substances containing secondary hydroxyl groups, which have low toxicity. The remaining 19 compounds, which are monocyclic alkanones (Nos 1100–1117) or have a secondary alcohol attached to a vinyl group (No. 1098), were assigned to structural class II.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded *via* the A (left-hand) side of the decision tree.

Step A3. The estimated daily *per capita* intake of each of the six flavouring agents in structural class I and each of the 19 agents in structural class II is below the threshold for daily human intake of compounds in the respective structural class (i.e., 1800 μg per person for class I and 540 μg per person for class II). According to the Procedure, the safety of these 25 flavouring agents raises no concern when consumed at currently estimated levels.

Table 9 summarizes the evaluations of acyclic ketones, secondary alcohols and related esters used as flavouring agents.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the flavouring agents in structural class I or II were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake of either class.

Conclusions

The Committee concluded that none of the flavouring agents in this group of alicyclic ketones, secondary alcohols and related esters would raise a safety concern at current estimated levels of intake. Other data on the toxicity of alicyclic ketones, secondary alcohols and related esters were consistent with the results of the safety evaluation.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.6 Aliphatic secondary alcohols, ketones and related esters

The Committee evaluated a group of 39 flavouring agents that includes aliphatic acyclic secondary alcohols and ketones and esters derived from aliphatic secondary alcohols (see Table 10), using the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). The Committee had not previously evaluated any of these agents.

Twenty-six of the 39 substances in this group of flavouring agents (Nos 1118, 1120, 1122–1132, 1134–1136, 1139, 1142, 1144, 1147, 1148, 1150–1152, 1154 and 1156) have been reported to occur naturally in foods. They have been found in fruits, juices, spices, vegetables, cocoa, coffee and tea.

Estimated daily per capita intake

The total annual volume of production of the 39 aliphatic secondary alcohols and ketones in this group is approximately 3900 kg in Europe and 1100 kg in the USA. Approximately 73% and 47% of the total annual production volume in Europe and the USA, respectively, is accounted for by 6-methyl-5-hepten-2-one (No. 1120) and 1-octen-3-ol (No. 1152). The daily *per capita* intake of 6-methyl-5-hepten-2-one (No. 1120) and 1-octen-3-ol (No. 1152) was 120 µg and 290 µg in Europe and 44 µg and 23 µg, respectively, in the USA, respectively.

Absorption, distribution, metabolism and elimination

In general, the aliphatic esters in this group hydrolyse to the corresponding secondary alcohols. The secondary alcohols and their corresponding ketones are interconvertible under physiological conditions. In the principal excretion pathway, the ketones are reduced to the corresponding secondary alcohols, which are subsequently conjugated with glucuronic acid and excreted.

When the ketone carbonyl function is located at the 2-position (i.e. a methyl ketone), the methyl group may undergo α -hydroxylation and subsequent oxidation, to yield a corresponding ketocarboxylic acid. The ketoacids are intermediary metabolites (e.g. α -ketoacids), which can undergo oxidative decarboxylation to yield carbon dioxide and simple aliphatic carboxylic acids.

Table 10

Summary of the results of safety evaluations of aliphatic secondary alcohols, ketones and related esters used as flavouring agents^a

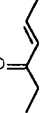
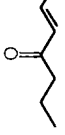
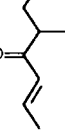
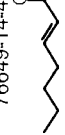
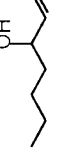
Flavouring agent	No.	CAS No. and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
3-Penten-2-one	1124	625-33-2 	No Europe: 0.3 USA: ND	See notes 1, 3 and 4.	No safety concern
4-Hexen-3-one	1125	2497-21-4 	No Europe: 15 USA: 1	See notes 1 and 3.	No safety concern
2-Hepten-4-one	1126	4643-25-8 	No Europe: 0.01 USA: ND	See notes 1 and 3.	No safety concern
5-Methyl-2-hepten-4-one	1133	81925-81-7 	No Europe: 7 USA: 1	See notes 1 and 3.	No safety concern
3-Octen-2-ol	1140	76649-14-4 	No Europe: 1 USA: ND	See note 1.	No safety concern
(E)-2-Octen-4-ol	1141	20125-81-9 	No Europe: ND USA: 1	See note 1.	No safety concern

Table 10 (continued)

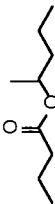
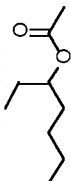
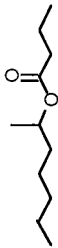
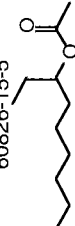
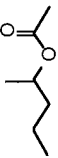
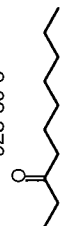
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Pentyl butyrate	1142	60415-61-4 	No Europe: ND USA: 1	See note 2.	No safety concern
(±) Heptan-3-yl acetate	1143	5921-83-5 	No Europe: 4 USA: 3	See note 2.	No safety concern
(±) Heptan-2-yl butyrate	1144	39026-94-3 	No Europe: 4 USA: 3	See note 2.	No safety concern
(±) Nonan-3-yl acetate	1145	60826-15-5 	No Europe: 4 USA: 3	See note 2.	No safety concern
2-Pentyl acetate	1146	626-38-0 	No Europe: 3 USA: 3	See note 2.	No safety concern
Structural class II 3-Decanone	1118	928-80-3 	No Europe: 4 USA: 3	See notes 1 and 3.	No safety concern

Table 10 (*continued*)

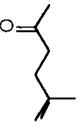
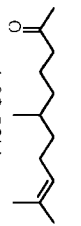
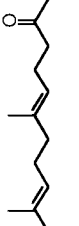
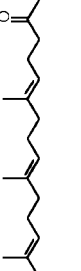
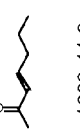
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
5-Methyl-5-hexen-2-one	1119	3240-09-3 	No Europe: ND USA: 0.3	See notes 1, 3 and 4.	No safety concern
6-Methyl-5-hepten-2-one	1120	1110-93-0 	No Europe: 120 USA: 44	See notes 1 and 4.	No safety concern
3,4,5,6-Tetrahydro- pseudionone	1121	4433-36-7 	No Europe: ND USA: 0.01	See notes 1 and 4.	No safety concern
6,10-Dimethyl-5,9- undecadien-2-one	1122	3796-70-1 	No Europe: 49 USA: 2	See notes 1 and 4.	No safety concern
2,6,10-Trimethyl-2,6,10- pentadecatrien-14-one	1123	762-29-8 	No Europe: 0.1 USA: ND	See notes 1 and 4.	No safety concern
3-Hepten-2-one	1127	1119-44-4 	No Europe: 0.2 USA: 0.07	See notes 1, 3 and 4.	No safety concern
3-Octen-2-one	1128	1669-44-9 	No Europe: 1 USA: 1	See notes 1, 3 and 4.	No safety concern

Table 10 (continued)

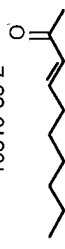
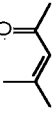
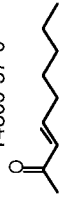
Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Octen-4-one	1129	4643-27-0 	No Europe: 1 USA: 3	See notes 1 and 3.	No safety concern
3-Decen-2-one	1130	10519-33-2 	No Europe: 0.01 USA: ND	See notes 1, 3 and 4.	No safety concern
4-Methyl-3-penten-2-one	1131	141-79-7 	No Europe: 0.4 USA: ND	See notes 1 and 4.	No safety concern
5-Methyl-3-hexen-2-one	1132	5166-53-0 	No Europe: ND USA: 0.1	See notes 1, 3 and 4.	No safety concern
6-Methyl-3,5-heptadien-2-one	1134	1604-28-0 	No Europe: 15 USA: 5	See notes 1, 3 and 4.	No safety concern
(E)-7-Methyl-3-octen-2-one	1135	33046-81-0 	No Europe: ND USA: 2	See notes 1, 3 and 4.	No safety concern
3-Nonen-2-one	1136	14309-57-0 	No Europe: 14 USA: 13	See notes 1, 3 and 4.	No safety concern

Table 10 (continued)

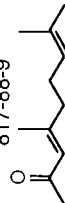
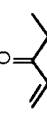
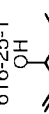
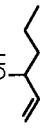
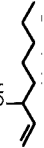
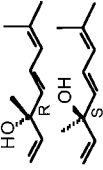
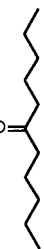
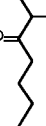
Flavouring agent	No.	CAS No. and structure	Step A3 ^{ab} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
(E) & (Z)-4,8-Dimethyl- 3,7-nonadien-2-one	1137	817-88-9 	No Europe: 7 USA: 7	See notes 1, 3 and 4.	No safety concern
(E)-6-Methyl-3-hepten-2-one	1138	20859-10-3 	No Europe: 4 USA: 3	See notes 1, 3 and 4.	No safety concern
(E,E)-3,5-Octadien-2-one	1139	30086-02-3 	No Europe: 4 USA: 3	See notes 1, 3 and 4.	No safety concern
1-Penten-3-one	1147	1629-58-9 	No Europe: 0.3 USA: 0.1	See notes 1 and 3.	No safety concern
1-Octen-3-one	1148	4312-99-6 	No Europe: 1 USA: 0.1	See notes 1 and 3.	No safety concern
2-Pentyl-1-buten-3-one	1149	63759-55-7 	No Europe: 0.2 USA: ND	See notes 1, 3 and 4.	No safety concern
1-Penten-3-ol	1150	616-25-1 	No Europe: 2 USA: 1	See note 1.	No safety concern

Table 10 (continued)

Flavouring agent	No.	CAS No. and structure	Step A3 ^{a,b} Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
1-Hexen-3-ol	1151	4798-44-1 	No Europe: 1 USA: 2	See note 1.	No safety concern
1-Octen-3-ol	1152	3391-86-4 	No Europe: 290 USA: 23	See note 1.	No safety concern
1-Decen-3-ol	1153	51100-54-0 	No Europe: ND USA: 0.1	See note 1.	No safety concern
(E,R)-3,7-Dimethyl-1,5,7-octatrien-3-ol	1154	20053-88-7 	No Europe: ND USA: 6	See note 1.	No safety concern
6-Undecanone	1155	927-49-1 	No Europe: 4 USA: 3	See note 1.	No safety concern
2-Methylheptan-3-one	1156	13019-20-0 	No Europe: 4 USA: 3	See note 1.	No safety concern

CAS: Chemical Abstracts Service; ND: No intake data reported

^a Step 2: All the flavouring agents in this group were predicted to be metabolized to innocuous products.

^b The human intake threshold is 1800 µg per person per day for class I and 540 µg per person per day for class II. All intake levels are expressed in µg per person per day. The combined intake of flavouring agents in class I is 58 and 16 µg per person per day in Europe and the USA, respectively. The combined intake of flavouring agents in class II is 520 and 120 µg per person per day in Europe and the USA, respectively.

Notes:

1. Detoxicated by reduction of the ketone followed by glucuronic acid conjugation of the corresponding alcohol or direct glucuronic acid conjugation of the secondary alcohol
2. Detoxicated by hydrolysis of the ester and glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid
3. Detoxicated by reduction of the ketone functional group followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone
4. Detoxicated by reduction of the ketone and alkyl side-chain oxidation and excretion

The acid may be completely metabolized in the fatty acid pathway and citric acid cycle. When the substance is an α,β -unsaturated ketone (Nos 1124–1139 and 1147–1149) or secondary alcohol (Nos 1140–1141 and 1150–1154) which is oxidized to an α,β -unsaturated ketone, it can conjugate with glutathione. The glutathione conjugates are converted to the corresponding mercapturic acid derivatives (*N*-acetylcysteine derivative) and excreted.

As the α,β -unsaturated carbonyl group is a structural alert for toxicity, the Committee, at previous meetings, has devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference 154) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with use of such compounds as flavouring agents. With respect to α,β -unsaturated ketones, these protective processes include reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid) and conjugation with glutathione. These processes operate for the aliphatic ketones used as flavouring agents.

Application of Procedure for the Safety Evaluation of Flavouring Substances

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these chemicals, the Committee assigned 11 of the 39 agents to structural class I on the basis that they are acyclic saturated and unsaturated aliphatic ketones with no other functional groups and three or fewer carbons on either side of the keto group (Nos 1124–1126 and 1133) and secondary alcohols (Nos 1140–1141) or esters of secondary alcohols (Nos 1142–1146). The remaining 28 flavouring agents, which are acyclic aliphatic ketones with four or more carbons on either side of the keto group (Nos 1118–1123, 1127–1132, 1134–1139, 1148, 1155 and 1156) and either a ketone (Nos 1147 and 1149) or a secondary alcohol (Nos 1150–1154) attached to a terminal vinyl group, were assigned to structural class II.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded *via* the A (left-hand) side of the decision tree.

Step A3. The estimated daily *per capita* intakes of the 11 flavouring agents in structural class I and the 28 flavouring agents in structural class II are below the threshold for human intake for each class (i.e., 1800 μg per person for structural class I and 540 μg per person for structural class II). According to the Procedure, the safety of these 39 flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 10 summarizes the evaluation of aliphatic secondary alcohols, ketones and related esters used as flavouring agents.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the flavouring agents in this group were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for structural class I or II.

Conclusions

The Committee concluded that none of the flavouring agents in this group of 39 aliphatic secondary alcohols, ketones and related esters would raise a safety concern at the current estimated levels of intake. Other data on the toxicity of aliphatic secondary alcohols, ketones and related esters are consistent with the results of the safety evaluation.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.2 Evaluation of secondary components of flavouring agents

The Committee had previously considered the procedure for evaluating flavouring agents for which the minimum assay figures were below 95%. It has followed the general principle that specifications should be designated as tentative if less than 95% of the material in the commercial product consists of the named compound, and known secondary components should be taken into account in the safety evaluation.

At its present meeting, the Committee considered information on the presence of known secondary components in all 51 flavouring agents evaluated at its forty-sixth through fifty-fifth meetings, for which the minimum assay was less than 95%, in order to assess whether these were covered by the existing safety evaluations of the named compounds.

In most cases, the secondary components of known chemical identity were found to be normal metabolites of the named compound, or to have chemical structures that are sufficiently similar that they fit within the same group evaluations. In two cases, the secondary components were not closely related to the named compound: the safety of 6-hydroxy-3,7-dimethyloctanoic acid (No. 237) was evaluated by reference to the NOEL for a similar compound, while that of the secondary component in 2,3-pentadione (No. 410) was assessed by reference to its likely metabolism.

In the light of all the available data on secondary components present in materials of commerce containing less than 95% of the named compound, the Committee concluded that the secondary components do not raise safety concerns and confirmed its conclusion that the flavouring agents listed in Annex 4 do not present any safety concern at the current estimated levels of intake.

The specifications for the 51 flavouring agents listed in Annex 4 that were originally evaluated at the forty-sixth to the fifty-fifth meetings will be reviewed at a future meeting.

4.3 Chemicals requiring confirmation of flavour use

The Committee received information from the flavour industry (4) concerning 18 flavouring agents for which the evaluations could not be finalized at the fifty-seventh meeting (Annex 1, reference 154), pending information on whether they were in current use as flavouring agents.

Glycerol (No. 909) and *propylene glycol (No. 925)* have a long history of use as solvents and humectants (inter alia). With respect to their use as flavouring agents, the Committee was informed only that these substances provide 'a sweet taste', although propylene glycol was also reported to produce 'a warm burning sensation in the mouth' in certain aqueous applications. With the exception of the category 'meats', the reported flavouring uses of these compounds require high levels of addition, in many cases greater than 10 000 mg/kg (1%), and any 'flavouring' effect might either be a corollary of a more significant technical effect as a carrier or humectant or be related to the acknowledged sweetening properties. On the basis of the information provided, the Committee was not convinced that the reported uses of glycerol and propylene glycol are those of a flavouring agent. The Committee did not finalize the evaluations pending development of a definition of 'flavouring agent'.

Benzoic acid (No 860) and benzoate salts have known uses as preservatives, particularly in certain beverages. The flavouring properties of benzoic acid appear to be well established, and the Committee accepted that this chemical can be added to some foods primarily for its flavouring effect. The Committee noted that the levels used as a flavouring agent are lower than those generally associated with the preservative uses of benzoates, although the possibility of a secondary preservative effect in some flavouring applications cannot be excluded. The group ADI of 0–5 mg/kg bw for benzoic acid and related compounds was maintained at the forty-sixth meeting (Annex 1, reference 122). The contribution of the intake of benzoic acid arising from its use as a flavouring agent to the total intake of benzoic acid and benzoates does not represent a safety concern.

The remaining 15 chemicals were reported to be added to foods as flavouring agents with the expectation that they will be hydrolysed to release flavour molecules, either under storage or during processing of the food. In some cases, hydrolysis is followed by further chemical changes (decarboxylation or oxidation) to generate the ultimate flavouring species. Because the end-products which are ingested by the consumer have been defined (see section

2.2.1), the Committee concluded that it is appropriate to evaluate these 15 chemicals by the Procedure for the Safety Evaluation of Flavouring Agents.

At its fifty-seventh meeting (Annex 1, reference 154), the Committee predicted that all 15 chemicals would be metabolized to innocuous products, and they were therefore evaluated via the left-hand side of the decision tree.

Glyceryl tribenzoate (No. 861) and *propylene glycol dibenzoate* (No. 862) were considered by the Committee at its fifty-seventh meeting in the group of benzyl derivatives (Annex 1, reference 154). The estimated intakes were below the threshold for human intake of their structural class (Step A3). They are partially hydrolysed in food under acidic conditions, giving rise to benzoic acid and glycerol and benzoic acid and propylene glycol. The group ADI of 0–5 mg/kg bw for benzoic acid and related compounds was maintained at the forty-sixth meeting (Annex 1, reference 122). An ADI ‘not specified’¹ was established for glycerol by the Committee at its twentieth meeting (Annex 1, reference 41), and an ADI of 0–25 mg/kg bw was established for propylene glycol at the seventeenth meeting (Annex 1, reference 32). The intakes of the hydrolysis products resulting from the flavouring uses of these agents are included in the relevant ADI. There are no safety concerns in relation to use of these chemicals as flavouring agents.

Butyl para-hydroxybenzoate (No. 870) was considered at the fifty-seventh meeting in the group of hydroxy- and alkoxy-substituted benzyl derivatives (Annex 1, reference 154). The estimated intake was below the threshold for human intake of the structural class (step A3). When baked goods containing this chemical are heated, it is partially hydrolysed to butyl alcohol and *para*-hydroxybenzoic acid. Butyl alcohol (No. 85) was evaluated as a flavouring agent at the forty-ninth meeting and was considered not to be a safety concern (Annex 1, reference 131). The intake of butyl alcohol from the flavouring use of butyl *para*-hydroxybenzoate would not significantly increase the total intake of this alcohol. *para*-Hydroxybenzoic acid was evaluated as a flavouring agent at the fifty-seventh meeting and was considered not to be a safety concern. The formation of *para*-hydroxybenzoic acid from the hydrolysis of butyl *para*-hydroxybenzoate would not significantly increase the total intake of this acid. There is no safety concern in relation to use of this chemical as a flavouring agent.

¹ ADI ‘not specified’ is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in the individual evaluation, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive that meets this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

3-Oxodecanoic acid glyceride (No. 914), 3-oxododecanoic acid glyceride (No. 915), 3-oxotetradecanoic acid glyceride (No. 916) and 3-oxohexadecanoic acid glyceride (No. 917) were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference 154). The estimated intake of No. 914 was above the threshold for human intake of its structural class, class III, while the intakes of the others were below the thresholds for their corresponding structural classes (I and III) (step A3). After absorption, the glycerides would be hydrolysed to glycerol and the corresponding β -keto acid, which are endogenous. All these agents are hydrolysed in food, and flavour arises from the corresponding keto acid and glycerol. The keto acids are unstable and would undergo decarboxylation to the corresponding ketones (2-nonanone, 2-undecanone, 2-tridecanone and 2-pentadecanone, respectively). After absorption, the ketones would be metabolized by reduction to the corresponding aliphatic secondary alcohol, followed by conjugation with glucuronic acid. The ketones (Nos 292, 296, 298 and 299) were assigned to structural class II when they were evaluated at the fifty-first meeting (Annex 1, reference 137) and considered not to be of safety concern at their estimated intakes as flavouring agents. The additional intake arising from intake of agents Nos 914–917 would not result in intakes of the ketones above the threshold for structural class II. There are no safety concerns in relation to use of these chemicals as flavouring agents.

Glyceryl monostearate (No. 918), glyceryl monooleate (No. 919) and propylene glycol monostearate (No. 926) were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference 154). The estimated intake of propylene glycol monostearate (No. 926) was above the threshold for human intake of structural class I, while the intakes of the others were below the intake threshold (Step A3). After absorption, the glyceryl esters would be hydrolysed to glycerol and the corresponding saturated or unsaturated fatty acid, which are endogenous. Mono- and diglycerides (including glycerol monostearate and glyceryl monooleate) were allocated an ADI 'not limited' at the seventeenth meeting of the Committee (Annex 1, reference 32). Propylene glycol monostearate (No. 926) would be hydrolysed to stearic acid and propylene glycol, for which an ADI of 0–25 mg/kg bw was established at the seventeenth meeting. All these agents are hydrolysed in food to the corresponding fatty acid and glycerol and, under certain ageing conditions, may be partially oxidized, imparting a rancid note to certain food flavours. The intake of the hydrolysis products resulting from the flavouring uses of these agents are included in the relevant ADI. There is no safety concern in relation to use of these chemicals as flavouring agents.

Triacetin (No. 920) was considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference 154). The estimated intake exceeded the threshold for human intake of its structural class (Step A3), and triacetin was evaluated under Step A4. When used in foods that are heated or otherwise processed, triacetin would be hydrolysed to glycerol and acetic acid, both of which are endogenous. An ADI 'not specified' was established for glyceryl acetates by the Committee at its twentieth meeting (Annex 1, reference 41), and the flavouring use of triacetin is included in this ADI. There is no safety concern in relation to the intake of triacetin or its hydrolysis products arising from its use as a flavouring agent.

Glyceryl tripropionate (No. 921) and *tributylin* (No. 922) were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference 154). The estimated intakes were below the threshold for human intake of their structural class (Step A3). With heating, the substances are partially or completely hydrolysed to glycerol and the corresponding carboxylic acid. Propionic and butyric acids were evaluated at the forty-ninth meeting (Annex 1, reference 131), and their use as flavouring agents (Nos 84 and 87, respectively) was considered not to represent a safety concern. Glycerol and both acids are endogenous, and the intakes arising from flavouring use of substances Nos 921 and 922 would not significantly alter the total intake of either acid. There is no safety concern in relation to use of these chemicals as flavouring agents.

Glycerol 5-hydroxydecanoate (No. 923) and *glycerol 5-hydroxydodecanoate* (No. 924) were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference 154). The estimated intakes were below the threshold for human intake of their structural class (step A3). With heating, the substances are partially or completely hydrolysed to glycerol and the corresponding carboxylic acid. The acids are unstable and would rapidly cyclize to δ -decalactone and δ -dodecalactone, respectively. These lactones were evaluated at the forty-ninth meeting (Annex 1, reference 131), and their uses as flavouring agents (Nos 232 and 236, respectively) were considered not to represent a safety concern, on the basis of the availability of a NOEL for a structural analogue. The intakes of δ -decalactone and δ -dodecalactone arising from flavouring use of Nos 923 and 924 would not significantly alter the total intakes of either lactone. There is no safety concern in relation to use of these chemicals as flavouring agents.

The conclusions of the present meeting relating to the flavouring properties of these chemicals are summarized in Table 11.

Table 11

Information on flavouring properties of chemicals evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

No.	Chemical	Level of use as a flavouring agent (mg/kg)	Reported flavouring properties	Evaluation based on current intake as a flavouring agent ^b
850	Benzoic acid ^a	≤ 100	Aromatic acid with a sweet-sour, acid note or a balsamic note at concentrations up to 100 mg/kg	No safety concern ^b
861	Glyceryl tribenzoate	≤ 100 (beverages only)	Flavour depends on hydrolysis to benzoic acid and glycerol/propylene glycol	No safety concern ^b
862	Propylene glycol dibenzoate	≤ 100 (beverages only)	Flavour depends on hydrolysis to benzoic acid and glycerol/propylene glycol	No safety concern ^b
870	Butyl- <i>para</i> -hydroxybenzoate	20 (baked goods) 1000 (beverages)	Flavour depends on hydrolysis to butyl alcohol and <i>para</i> -hydroxybenzoic acid	No safety concern ^b
909	Glycerol ^c	40 (meats) 500–23 000 (other foods)	Provides a sweet taste (0.6 times as sweet as cane sugar)	Evaluation not finalized, pending definition of 'flavouring agent' No safety concern ^b
914	3-Oxodecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form 2-nonanone (up to 50 mg/kg).	No safety concern ^b
915	3-Oxododecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form 2-undecanone (up to 50 mg/kg).	No safety concern ^b
916	3-Oxotetradecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form 2-tridecanone (up to 50 mg/kg).	No safety concern ^b
917	3-Oxohexadecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form 2-pentadecanone (up to 50 mg/kg).	No safety concern ^b
918	Glyceryl monostearate ^d	500–3900	Flavour depends on hydrolysis on heating or during storage. The resulting stearic acid has flavouring properties. It may be further oxidized to produce 'rancid' flavour notes.	No safety concern ^b

Table 11 (continued)

No.	Chemical	Level of use as a flavouring agent (mg/kg)	Reported flavouring properties	Evaluation based on current intake as a flavouring agent
919	Glyceryl monooleate ^d	≤ 100	Flavour depends on hydrolysis on heating or during storage. The resulting <i>oleic acid</i> has flavouring properties. It may be further oxidized to produce 'rancid' flavour notes.	No safety concern ^b
920	Triacetin ^e	350 (jams and jellies) ≤ 100 (other foods)	Flavour depends on hydrolysis in heated or otherwise processed foods to <i>glycerol</i> and <i>acetic acid</i>	No safety concern ^b
921	Glyceryl tripropionate (Tripropionin)	≤ 50	Flavour depends on hydrolysis in heated or otherwise processed foods to <i>glycerol</i> and <i>propionic acid</i>	No safety concern ^b
922	Tributyrin	≤ 100	Flavour depends on hydrolysis in heated or otherwise processed foods to <i>glycerol</i> and <i>butyric acid</i>	No safety concern ^b
923	Glycerol 5-hydroxy-decanoate	25	Flavour depends on hydrolysis in heated foods and spontaneous cyclization of the 5-hydroxy acid to δ- <i>decalactone</i> (up to 20 mg/kg)	No safety concern ^b
924	Glycerol 5-hydroxy-dodecanoate	25	Flavour depends on hydrolysis in heated foods and spontaneous cyclization of the 5-hydroxy acid to δ- <i>dodecalactone</i> (up to 20 mg/kg)	No safety concern ^b
925	Propylene glycol ^f	10 (meats) 690–100 000 (other foods)	Provides a mildly sweet taste. In an aqueous environment, may leave a warm burning sensation in the mouth	Evaluation not finalized, pending definition of 'flavouring agent'
926	Propylene glycol stearate ^g	3000–7000	Flavour depends on hydrolysis on heating or during maturation. The resulting <i>stearic acid</i> has flavouring properties. It may be further oxidized to produce a 'rancid' flavour note.	No safety concern ^b

^a Previously evaluated for use as a preservative^b The flavouring uses of this agent are included in the relevant ADI of the agent and/or its hydrolysis products.^c Previously evaluated for use as a carrier and humectant and for other uses^d Previously evaluated (as mono- and di-glycerides) for use as an emulsifier^e Previously evaluated for use as a carrier^f Previously evaluated for use as a carrier and humectant and for other uses^g Previously evaluated for use as an emulsifier

At its fifty-seventh meeting, the Committee mistakenly included *isoamyl pyruvate* (No. 939) in its evaluation of the aliphatic acyclic diols, triols and related substances, even though there was no information on its current use as a flavouring agent (Annex 1, reference 154). Since the fifty-seventh meeting, the flavour industry has provided information that the reported annual volume is approximately 1 kg per year in the USA (5). On the basis of the equation in section 4.1, this corresponds to a daily *per capita* intake of 0.1 µg, which is below the threshold of concern for its structural class (class I, 1800 µg). Therefore, the present Committee confirmed that there is no safety concern in relation to the use of isoamyl pyruvate as a flavouring agent.

4.4 Specifications of purity for flavouring agents

4.4.1 Specifications for flavouring agents evaluated for the first time

Specifications were prepared for 196 flavouring agents being evaluated for the first time and for six flavouring agents that were evaluated for safety at the fifty-seventh meeting but for which specifications were not prepared (Annex 1, reference 154). At the present meeting, none of the 202 specifications was designated 'tentative'. This is the first time since the review of flavouring agents began that no tentative designations were made for new submissions for specifications for flavouring agents and reflects the high quality of the information received.

4.4.2 Reconsideration of existing specifications for flavouring agents

Since 1996, a number of specifications for flavouring agents have been classified as 'tentative' either because of missing physical data or of uncertainty about their use as flavouring agents. In addition the Codex Committee on Food Additives and Contaminants referred 29 specifications of flavouring agents evaluated at the fifty-fifth meeting (Annex 1, reference 151) to the Committee for reconsideration of certain parameters.

The call for data for the present meeting did not include all these substances, but they had been included in previous calls for data and in the reports of previous meetings. As data had been received before the present meeting, the Committee added them to the agenda.

Chemicals used as flavouring agents

At its fifty-seventh meeting, the Committee questioned whether a number of chemicals were used as flavouring agents (Annex 1, reference 154). At its present meeting, it agreed that some of these substances have a flavouring function (see section 4.3), and therefore the tentative designation was deleted. Additionally, data missing on some of the substances had subsequently been supplied. Nevertheless, the specifications for glycerol monostearate (No.

918) and glycerol monooleate (No. 919) were maintained as tentative because of missing or inconsistent data. The specifications for glycerol-5-hydroxydecanoate (No. 923) and glycerol 5-hydroxydodecanoate (No. 924) were also maintained as tentative because the assay method was called into question.

The Committee did not finalize its evaluations of glycerol (No. 909) and propylene glycol (No. 925), pending development of a definition of 'flavouring agent'. The Committee agreed that benzoic acid (No. 850) was used as a flavouring agent but that in this use it should comply with the specifications established for benzoic acid used as a preservative. The Committee recommended that the existing specifications for glycerol, propylene glycol, triacetin (No. 920) and benzoic acid be reviewed at a future meeting, especially in relation to the functional uses of these food additives.

The status of the specifications of these chemicals is listed in Table 12.

Missing physical data

In the request for data for the present meeting, 12 flavouring agents with 'tentative' specifications were listed, with a request for the missing data. These data were provided, and the specifications of these flavouring agents were revised and the tentative status deleted. The Committee also considered the specifications of 10 flavouring agents that were designated 'tentative' at the fifty-seventh meeting and for which data were supplied. The specifications were revised and the tentative designation removed, except in the case of

Table 12
Specifications of chemicals for which functional use as flavouring agents was considered at the present meeting

No.	Chemical	Specifications ^a
861	Glyceryl tribenzoate	S
862	Propylene glycol dibenzoate	S
870	Butyl <i>para</i> -hydroxybenzoate	S
914	3-Oxodecanoic acid glyceride	R
915	3-Oxododecanoic acid glyceride	R
916	3-Oxotetradecanoic acid glyceride	R
917	3-Oxohexadecanoic acid glyceride	R
918	Glyceryl monostearate	R,T
919	Glyceryl monooleate	R,T
921	Glyceryl tripropanoate	S
922	Tributyrin	S
923	Glycerol 5-hydroxydecanoate	S,T
924	Glycerol 5-hydroxydodecanoate	S,T
926	Propylene glycol stearate	R

^a R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing or revised specifications are tentative and comments are invited.

pyruvaldehyde (No. 937), which was maintained as tentative. The status of the specifications of these flavouring agents is provided in Table 13.

Specifications referred to the Committee

Table 14 summarizes the specifications of flavouring agents that were referred to the Committee by the Codex Committee on Food Additives and Contaminants for reconsideration.

5. Future work

1. The Committee concluded that it is not desirable to develop dual specifications, as has been done, for example, for chemicals that were evaluated both by the Procedure for the Safety Evaluation of Flavouring Agents and also as food additives. The Committee recommended that the existing specifications for such chemicals be reviewed at a future meeting, with a view to consolidating and revising them to incorporate all functional uses and new information.

Table 13
Specifications of flavouring agents previously designated as 'tentative'

No.	Chemical	Specifications ^a
111	Lauric acid	R
113	Myristic acid	R
115	Palmitic acid	R
116	Stearic acid	R
390	γ -Ionone	R
490	Allyl thiopropionate	R
814	α -Methylphenethylbutyrate	R
817	4-(<i>para</i> -Tolyl)-2-butanone	R
847	Benzyl 2,3-dimethylcrotonate	R
866	Tolualdehydes (mixed <i>ortho</i> , <i>meta</i> and <i>para</i>)	R
872	Anisyl formate	R
609	1,4-Nonanediol diacetate	R
627	Aconitic acid	R
642	3-Phenylpropyl hexanoate	R
678	α -Amylcinnamyl isovalerate	R
729	Dihydroxyacetophenone	R
752	2-Phenyl3-carboethoxyfuran	R
910	3-Oxohexanoic acid glyceride	R
911	3-Oxooctanoic acid glyceride	R
937	Pyruvaldehyde	S,T
943	Acetaldehyde ethyl <i>cis</i> -3-hexenyl acetal	R
954	Ethyl vanillin propylene glycol acetal	R

^aR, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing specifications are tentative and comments are invited.

Table 14
Specifications of flavouring agents referred to the Committee

No.	Chemical	Specifications ^a
182	Isoamyl laurate	R
230	Hydroxynonanoic acid δ -lactone	S
238	δ -Tetradecalactone	S
250	γ -Methyldecalactone	S
310	Isopropyl isovalerate	R
455	Butyl sulfide	R
476	Ethyl 3-methylthiopropionate	R
483	Ethyl thioacetate	R
492	Methylthio 2-(acetyloxy)propionate	R
493	Methylthio 2-(propionyloxy)propionate	R
540	1,6-Hexanedithiol	R
542	1,9-Nonanedithiol	R
551	2-Mercaptopropionic acid	R
553	Ethyl 3-mercaptopropionate	R
566	Propyl disulfide	R
601	Ethyl 3-hydroxyhexanoate	R
602	Ethyl 3-oxohexanoate	R
606	Laevulinic acid	R
608	Butyl laevulinate	R
614	Diethyl malonate	R
616	Dimethyl succinate	R
617	Diethyl succinate	R
622	Diethyl tartrate	R
624	Diethyl sebacate	R
625	Dibutyl sebacate	R
626	Ethylene brassylate	R
743	Furfuryl 3-methylbutanoate	S
745	5-Methylfurfural	R

^aR, existing specifications revised; S, specifications exist, revision not considered or required.

2. The Committee recommended that the acute toxicity of nitrite be evaluated at a future meeting.
3. The Committee agreed to continue its activity to update the heavy metals specifications in food additives and concluded that specifications for antioxidants and other functional uses should be reviewed at the next meeting on food additives.
4. The Committee recognized the need for a working definition of the term 'flavouring agent' and recommended that such a definition be agreed at a future meeting of the Committee.
5. As noted in section 2.7, the present Committee considered several guidelines that had been prepared by the Joint Secretariat and made comments and suggestions for improvement, including the following:
 - The Technical Data Sheets prepared by Committee experts, which summarize the available technological information, should

provide authors of working papers on intake with information on the levels of use of the additive in current practice.

- Data for intake assessments of food additives should preferably be submitted in a standard format. The Joint Secretariat should publish a *Format for submission of intake data* as a separate document and encourage its use when calling for data.
- The Secretariat should publish amended versions of guidelines for working papers on both the FAO and the WHO Internet sites for further comments and suggestions.
- The Committee recommended that the *Guidelines for the preparation of working papers (monographs) on flavouring agents* be updated to include consideration of the evaluation of flavouring agents that are members of groups that have been evaluated previously.

6. Recommendations

In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role of the recommendations of the Committee in the development of international food standards and of regulations in many countries and the need to maintain consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.

Acknowledgements

This report of the Committee is dedicated to Dr John L. Herrman, who joined WHO as Joint Secretary of the Joint FAO/WHO Joint Committee on Food Additives in 1988. From his first meeting, the thirty-third, he demonstrated the unique management skills required of his position. He has served as Joint Secretary at 27 meetings, culminating in the present one, his last before retirement.

Over the course of his career, he has steadfastly supported the work of the Committee and is largely responsible for the emergence of JECFA as the leading international body on food safety.

As WHO Joint Secretary he has displayed immense dedication and leadership, resulting in his international recognition as a leading scientist in food safety. His timely introduction to the Committee of new concepts in safety evaluation has kept the Committee at the forefront of science, has contributed to its continued success and ensures that its advice is based on the best possible science.

The Committee wishes John Herrman a happy and successful retirement.

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Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
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12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
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24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
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37. *Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances.* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
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40. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. *Summary of toxicological data of certain food additives*. WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others*. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants*. WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives*. FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives)*. FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 16, 1981.
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60. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 17, 1982.
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87. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/2, 1990.
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112. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/6, 1994.
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121. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/8, 1996.
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143. *Evaluation of certain food additives and contaminants* (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
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151. *Compendium of food additive specifications: addendum 8*. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
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153. *Safety evaluation of certain mycotoxins in food*. WHO Food Additives Series, No. 47/ FAO Food and Nutrition Paper 74, 2001.
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Annex 2

Acceptable daily intakes, other recommendations and information on specifications

Food additives evaluated toxicologically

Food additive	Specifications ^a	Acceptable daily intake (ADI) and other toxicological recommendations
Alitame	R	0–1 mg/kg bw ^b
Cross-linked sodium carboxymethyl cellulose	N	Included in the group ADI 'not specified' ^c with other modified celluloses
Mineral oils (low- and medium-viscosity)		
Class I ^d	R	0–10 mg/kg bw
Class II ^e and Class III ^f	R	0–0.01 mg/kg bw (group ADI) (temporary) ^g
Nitrite	S	0–0.07 mg/kg bw (expressed as nitrite ion)
Nitrate	S	0–3.7 mg/kg bw (expressed as nitrate ion)
Salatrim (short- and long-chain acyltriglyceride molecules)	R	Adequate information was not available to evaluate safety and nutritional effects.

^a N, new specifications prepared; R, existing specifications revised; S, existing specifications were not considered.

^b ADI was established at the forty-sixth meeting (WHO Technical Report Series No, 868, 1997). Evaluation of a 90-day study of tolerance in patients with diabetes was postponed.

^c ADI 'not specified' is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive that this criterion must be used with in the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve its effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

^d Including P70(H) oil.

^e Including N70(H) and N70(A) oils.

^f Including P15(H), N15(H), and N10(A) oils

^g See Annex 3.

Food additive considered for specifications only

Food additive	Specifications ^a
Amyloglucosidase from <i>Aspergillus niger</i> , var.	R

^aR, existing specifications revised

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
Cyclohexanecarboxylic acid	961	N	No safety concern
Methyl cyclohexanecarboxylate	962	N	No safety concern
Ethyl cyclohexanecarboxylate	963	N	No safety concern
Cyclohexaneethyl acetate	964	N	No safety concern
Cyclohexaneacetic acid	965	N	No safety concern
Ethyl cyclohexanepropionate	966	N	No safety concern
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	N	No safety concern
cis-5-Isopropenyl-cis-2-methylcyclopentan-1-carboxaldehyde	968	N	No safety concern
Campholene acetate	969	N	No safety concern
α -Campholenic alcohol	970	N	No safety concern
<i>para</i> -Menth-1-en-9-al	971	N	No safety concern
1- <i>para</i> -Menthen-9-yl acetate	972	N	No safety concern
<i>para</i> -Mentha-1,8-dien-7-al	973	N	No safety concern
<i>para</i> -Mentha-1,8-dien-7-ol	974	N	No safety concern
<i>para</i> -Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
1,2,5,6-Tetrahydrocuminic acid	976	N	No safety concern
2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	977	N	No safety concern
2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	978	N	No safety concern
2,6,6-Trimethyl-1&2-cyclohexen-1-carboxaldehyde	979	N	No safety concern
2-Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (Myrtenal)	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	N	No safety concern
6,6-Myrtenyl formate	983	N	No safety concern
Santalol (α and β)	984	N	No safety concern
Santalyl acetate (α and β)	985	N	No safety concern
10-Hydroxymethylene-2-pinene	986	N	No safety concern

^aN, new specifications prepared

B. Phenethyl alcohol, aldehyde, acid and related acetals and esters

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
Phenethyl alcohol	987	N	No safety concern
Phenethyl formate	988	N	No safety concern
Phenethyl acetate	989	N	No safety concern
Phenethyl propionate	990	N	No safety concern
Phenethyl butyrate	991	N	No safety concern
Phenethyl isobutyrate	992	N	No safety concern
Phenethyl 2-methylbutyrate	993	N	No safety concern
Phenethyl isovalerate	994	N	No safety concern
Phenethyl hexanoate	995	N	No safety concern
Phenethyl octanoate	996	N	No safety concern
Phenethyl tiglate	997	N	No safety concern
Phenethyl senecioate	998	N	No safety concern
Phenethyl phenylacetate	999	N	No safety concern
Acetaldehyde phenethyl propyl acetal	1000	N	No safety concern
Acetaldehyde butyl phenethyl acetal	1001	N	No safety concern
Phenylacetaldehyde	1002	N	No safety concern
Phenylacetaldehyde dimethyl acetal	1003	N	No safety concern
Phenylacetaldehyde glyceryl acetal	1004	N	No safety concern
Phenylacetaldehyde 2,3-butylene glycol acetal	1005	N	No safety concern
Phenylacetaldehyde diisobutyl acetal	1006	N	No safety concern

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
Phenylacetic acid	1007	N	No safety concern
Methyl phenylacetate	1008	N	No safety concern
Ethyl phenylacetate	1009	N	No safety concern
Propyl phenylacetate	1010	N	No safety concern
Isopropyl phenylacetate	1011	N	No safety concern
Butyl phenylacetate	1012	N	No safety concern
Isobutyl phenylacetate	1013	N	No safety concern
Isoamyl phenylacetate	1014	N	No safety concern
Hexyl phenylacetate	1015	N	No safety concern
3-Hexenyl phenylacetate	1016	N	No safety concern
Octyl phenylacetate	1017	N	No safety concern
Rhodinyl phenylacetate	1018	N	No safety concern
Linalyl phenylacetate	1019	N	No safety concern
Geranyl phenylacetate	1020	N	No safety concern
Citronellyl phenylacetate	1021	N	No safety concern
Santalyl phenylacetate (α and β)	1022	N	No safety concern
<i>para</i> -Tolylacetaldehyde	1023	N	No safety concern
<i>para</i> -Isopropylphenylacetaldehyde	1024	N	No safety concern
Methyl <i>para-tert</i> -butylphenylacetate	1025	N	No safety concern
Phenoxyacetic acid	1026	N	No safety concern
Ethyl (<i>para</i> -tolylloxy)acetate	1027	N	No safety concern
2-Phenoxyethyl isobutyrate	1028	N	No safety concern
Sodium 2-(4-methoxyphenoxy)propanoate	1029	N	No safety concern

^aN, new specifications prepared

C. Sulfur-containing heterocyclic compounds

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
Thiamine hydrochloride	1030	N	No safety concern
4-Methyl-5-thiazoleethanol	1031	N	No safety concern
Thiazole	1032	N	No safety concern
2-(1-Methylpropyl)thiazole	1033	N	No safety concern
2-Isobutylthiazole	1034	N	No safety concern
4,5-Dimethylthiazole	1035	N	No safety concern
2,4,5-Trimethylthiazole	1036	N	No safety concern
2-Isopropyl-4-methylthiazole	1037	N	No safety concern
4-Methyl-5-vinylthiazole	1038	N	No safety concern
2,4-Dimethyl-5-vinylthiazole	1039	N	No safety concern
Benzothiazole	1040	N	No safety concern
2-Acetylthiazole	1041	N	No safety concern
2-Propionylthiazole	1042	N	No safety concern
4-Methylthiazole	1043	N	No safety concern
2-Ethyl-4-methylthiazole	1044	N	No safety concern
4,5-Dimethyl-2-isobutyl-3-thiazoline	1045	N	No safety concern
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1046	N	No safety concern
2-Isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1047	N	No safety concern
2,4,6-Triisobutyl-5,6-dihydro-4 <i>H</i> -1,3,5-dithiazine	1048	N	No safety concern
2,4,6-Trimethyldihydro-4 <i>H</i> -1,3,5-dithiazine	1049	N	No safety concern
5-Methyl-2-thiophenecarboxaldehyde	1050	N	No safety concern
3-Acetyl-2,5-dimethylthiophene	1051	N	No safety concern
2-Thienylmercaptan	1052	N	No safety concern
2-Thienyl disulfide	1053	N	No safety concern
4-Methyl-5-thiazoleethanol acetate	1054	N	No safety concern
2,4-Dimethyl-5-acetylthiazole	1055	N	No safety concern

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
2-Ethoxythiazole	1056	N	No safety concern
2-Methyl-5-methoxythiazole	1057	N	No safety concern
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	N	No safety concern
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	N	No safety concern

^aN, new specifications prepared

D. Sulfur-substituted furan derivatives

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
2-Methyl-3-furanthiol	1060	N	No safety concern
2-Methyl-3-(methylthio)furan	1061	N	No safety concern
2-Methyl-5-(methylthio)furan	1062	N	No safety concern
2,5-Dimethyl-3-furanthiol	1063	N	No safety concern
Methyl 2-methyl-3-furyl disulfide	1064	N	No safety concern
Propyl 2-methyl-3-furyl disulfide	1065	N	No safety concern
Bis(2-methyl-3-furyl) disulfide	1066	N	No safety concern
Bis(2,5-dimethyl-3-furyl) disulfide	1067	N	No safety concern
Bis(2-methyl-3-furyl) tetrasulfide	1068	N	No safety concern
2-Ethanethoic acid, S-(2-methyl-3-furanyl) ester	1069	N	No safety concern
2,5-Dimethyl-3-furan thioisovalerate	1070	N	No safety concern
2,5-Dimethyl-3-thiofuroylfuran	1071	N	No safety concern
Furfuryl mercaptan	1072	N	No safety concern
S-Furfuryl thioformate	1073	N	No safety concern
S-Furfuryl thioacetate	1074	N	No safety concern
S-Furfuryl thiopropionate	1075	N	No safety concern
Furfuryl methyl sulfide	1076	N	No safety concern
Furfuryl isopropyl sulfide	1077	N	No safety concern
Methyl furfuryl disulfide	1078	N	No safety concern
Propyl furfuryl disulfide	1079	N	No safety concern
2,2'-(Thiodimethylene)difuran	1080	N	No safety concern
2,2'-(Dithiodimethylene)difuran	1081	N	No safety concern
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082	N	No safety concern
S-Methyl thiofuroate	1083	N	No safety concern
4-[(2-Furanmethyl)thio]-2-pentanone	1084	N	No safety concern
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085	N	No safety concern
2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086	N	No safety concern
4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087	N	No safety concern
Ethyl 3-(furfurylthio)propionate	1088	N	No safety concern
2-Methyl-3-thioacetox-4,5-dihydrofuran	1089	N	No safety concern
2-Methyl-3-tetrahydrofuranthiol	1090	N	No safety concern
2,5-Dimethyl-3-tetrahydrofuranthiol, <i>cis</i> and <i>trans</i> isomers	1091	N	No safety concern
2,5-Dimethyl-3-thioacetox-4,5-dihydrofuran, <i>cis</i> and <i>trans</i> isomers	1092	N	No safety concern

^aN, new specifications prepared

E. Alicyclic ketones, secondary alcohols and related esters

Flavouring agent	No.	Specifications ^a
Cyclohexyl acetate	1093	N
Cyclohexyl butyrate	1094	N
Cyclohexyl formate	1095	N
Cyclohexyl isovalerate	1096	N
Cyclohexyl propionate	1097	N
<i>cis</i> and <i>trans</i> - <i>para</i> -1(7)8-Menthadien-2-yl acetate	1098	N

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
3,3,5-Trimethyl cyclohexanol	1099	N	No safety concern
Cyclohexanone	1100	N	No safety concern
Cyclopentanone	1101	N	No safety concern
2-Methylcyclohexanone	1102	N	No safety concern
3-Methylcyclohexanone	1103	N	No safety concern
4-Methylcyclohexanone	1104	N	No safety concern
1-Methyl-1-cyclopenten-3-one	1105	N	No safety concern
2-Hexylidene cyclopentanone	1106	N	No safety concern
3-Methyl-2-cyclohexen-1-one	1107	N	No safety concern
2,2,6-Trimethylcyclohexanone	1108	N	No safety concern
2-sec-Butylcyclohexanone	1109	N	No safety concern
4-Isopropyl-2-cyclohexenone	1110	N	No safety concern
Tetramethylethylcyclohexenone (mixture of isomers)	1111	N	No safety concern
Isophorone	1112	N	No safety concern
3-Methyl-5-propyl-2-cyclohexen-1-one	1113	N	No safety concern
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114	N	No safety concern
Isojasnone	1115	N	No safety concern
(E)-2-(2-Octenyl)cyclopentanone	1116	N	No safety concern
2-(3,7-Dimethyl-2,6-octadienyl)cyclopentanone	1117	N	No safety concern

^aN, new specifications prepared

F. Aliphatic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
3-Decanone	1118	N	No safety concern
5-Methyl-5-hexen-2-one	1119	N	No safety concern
6-Methyl-5-hepten-2-one	1120	N	No safety concern
3,4,5,6-Tetrahydropseudoionone	1121	N	No safety concern
6,10-Dimethyl-5,9-undecadien-2-one	1122	N	No safety concern
2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one	1123	N	No safety concern
3-Penten-2-one	1124	N	No safety concern
4-Hexen-3-one	1125	N	No safety concern
2-Hepten-4-one	1126	N	No safety concern
3-Hepten-2-one	1127	N	No safety concern
3-Octen-2-one	1128	N	No safety concern
2-Octen-4-one	1129	N	No safety concern
3-Decen-2-one	1130	N	No safety concern
4-Methyl-3-penten-2-one	1131	N	No safety concern
5-Methyl-3-hexen-2-one	1132	N	No safety concern
5-Methyl-2-hepten-4-one	1133	N	No safety concern
6-Methyl-3,5-heptadien-2-one	1134	N	No safety concern
(E)-7-Methyl-3-octen-2-one	1135	N	No safety concern
3-Nonen-2-one	1136	N	No safety concern
(E) and (Z)-4,8-Dimethyl-3,7-nonadien-2-one	1137	N	No safety concern
(E)-6-Methyl-3-hepten-2-one	1138	N	No safety concern
(E,E)-3,5-Octadien-2-one	1139	N	No safety concern
3-Octen-2-ol	1140	N	No safety concern
(E)-2-Octen-4-ol	1141	N	No safety concern
2-Pentyl butyrate	1142	N	No safety concern
(+/-)Heptan-3-yl acetate	1143	N	No safety concern
(+/-)Heptan-2-yl butyrate	1144	N	No safety concern
(+/-)Nonan-3-yl acetate	1145	N	No safety concern
2-Pentyl acetate	1146	N	No safety concern
1-Penten-3-one	1147	N	No safety concern
1-Octen-3-one	1148	N	No safety concern
2-Pentyl-1-buten-3-one	1149	N	No safety concern

Flavouring agent	No.	Specifi- cations ^a	Conclusions based on current intake
1-Penten-3-ol	1150	N	No safety concern
1-Hexen-3-ol	1151	N	No safety concern
1-Octen-3-ol	1152	N	No safety concern
1-Decen-3-ol	1153	N	No safety concern
(E,R)-3,7-Dimethyl-1,5,7-octatrien-3-ol	1154	N	No safety concern
6-Undecanone	1155	N	No safety concern
2-Methylheptan-3-one	1156	N	No safety concern

^aN, new specifications prepared

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications ^a
111	Lauric acid	R
113	Myristic acid	R
115	Palmitic acid	R
116	Stearic acid	R
182	Isoamyl laurate	R
310	Isopropyl isovalerate	R
390	γ-Ionone	R
455	Butyl sulfide	R
476	Ethyl 3-methylthiopropionate	R
483	Ethyl thioacetate	R
490	Allyl thiopropionate	R
492	Methylthio 2-(acetyloxy)propionate	R
493	Methylthio 2-(propionyloxy) propionate	R
540	1,6-Hexanedithiol	R
542	1,9-Nonanedithiol	R
551	2-Mercaptopropionic acid	R
553	Ethyl 3-mercaptopropionate	R
564	Dimethyl disulfide	R
566	Propyl disulfide	R
601	Ethyl 3-hydroxyhexanoate	R
602	Ethyl 3-oxohexanoate	R
606	Laevulinic acid	R
608	Butyl levulinate	R
609	1,4-Nonanediol diacetate	R
614	Diethyl malonate	R
616	Dimethyl succinate	R
617	Diethyl succinate	R
622	Diethyl tartrate	R
624	Diethyl sebacate	R
625	Dibutyl sebacate	R
626	Ethylene brassylate	R
627	Aconitic acid	R
642	3-Phenylpropyl hexanoate	R
678	α-Amylcinnamyl isovalerate	R
729	Dihydroxyacetophenone	R
745	5-Methylfurfural	R
752	2-Phenyl-3-carbethoxyfuran	R
814	α-Methylphenethyl butyrate	R
817	4-(<i>para</i> -Tolyl)-2-butanone	R
847	Benzyl 2,3-dimethylcrotonate	R
861	Glyceryl tribenzoate	R
862	Propylene glycol dibenzoate	S
866	Tolualdehydes (mixed <i>ortho meta</i> and <i>para</i>)	R
870	Butyl <i>para</i> -hydroxybenzoate	S
872	Anisyl formate	R

No.	Flavouring agent	Specifications ^a
910	3-Oxohexanoic acid diglyceride	R
911	3-Oxooctanoic acid glyceride	R
914	3-Oxodecanoic acid glyceride	R
915	3-Oxododecanoic acid glyceride	R
916	3-Oxotetradecanoic acid glyceride	R
917	3-Oxohexadecanoic acid glyceride	R
918	Glyceryl monostearate	R,T
919	Glyceryl monooleate	R,T
921	Glyceryl tripropanoate	S
922	Tributyrin	S
923	Glycerol 5-hydroxydecanoate	S,T
924	Glycerol 5-hydroxydodecanoate	S,T
926	Propylene glycol stearate	R
937	Pyruvaldehyde	S,T
943	Acetaldehyde ethyl cis-3-hexenyl acetal	R
954	Ethyl vanillin propylene glycol acetal	R
955	4-Hydroxybenzyl alcohol	N
956	4-Hydroxybenzaldehyde	N
957	4-Hydroxybenzoic acid	N
958	2-Hydroxybenzoic acid	N
959	4-Hydroxy-3-methoxy benzoic acid	N
960	Vanillin erythro- and threo-butan-2,3-diol acetal	N

^aN, new specifications prepared; R, existing specifications revised; S, existing specifications were maintained; T, the existing, new, or revised specifications are tentative and new information is required.

Flavouring agents for which confirmation of flavour use was required

The Committee could not complete its evaluation of 18 substances at its fifty-seventh meeting, pending confirmation that they were in current use as flavouring agents. The Committee received information from the flavour industry on these substances, and concluded that flavour uses have been established for 16 of them. The conclusions of the Committee are summarized in the following table.

No.	Flavouring agent	Evaluation based on current intake as a flavouring agent
850	Benzoic acid ^a	No safety concern
861	Glyceryl tribenzoate	No safety concern
862	Propylene glycol dibenzoate	No safety concern
870	Butyl- <i>para</i> -hydroxybenzoate	No safety concern
909	Glycerol ^b	Evaluation not finalized, pending definition of 'flavouring agent'
914	3-Oxodecanoic acid glyceride	No safety concern
915	3-Oxododecanoic acid glyceride	No safety concern
916	3-Oxotetradecanoic acid glyceride	No safety concern
917	3-Oxohexadecanoic acid glyceride	No safety concern
918	Glyceryl monostearate ^c	No safety concern
919	Glyceryl monooleate ^c	No safety concern
920	Triacetin ^d	No safety concern
921	Glyceryl tripropionate (Tripropionin)	No safety concern
922	Tributyrin	No safety concern
923	Glycerol 5-hydroxydecanoate	No safety concern
924	Glycerol 5-hydroxydodecanoate	No safety concern
925	Propylene glycol ^e	Evaluation not finalized, pending definition of 'flavouring agent'
926	Propylene glycol stearate ^f	No safety concern

Annex 3

Further information required or desired

Mineral oils (low- and medium-viscosity), class II and class III

Information on the relevance to humans of the response of Fischer 344 and Sprague-Dawley rats to these materials is required for evaluation in 2006. In order that the data be applicable to as wide a range of mineral oils as possible, the Committee suggested that commercial mineral oils of the lowest viscosity be used in such studies. Additional studies might be required, depending on the outcome of these studies.

Aluminium powder, iron oxides and titanium dioxide

The high levels of heavy metals set in the present specifications for these inorganic colours should be reconsidered. The Committee maintained the existing limits but decided to call for data on the raw materials and manufacturing methods and analytical data on impurities for review at a future meeting.

Annex 4

Summary of conclusions on flavouring agents with minimum assay values of 95% or less

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
42	Isoamyl formate	92	4–8% isoamyl alcohol	Isoamyl alcohol (No. 52) has been evaluated by JECFA.	Secondary components do not raise safety concern.
53	Citronellyl formate	86	5–10% citronellol	NOEL for citronellol is > 50 mg/kg bw per day in rats (Trubek Laboratories, Inc., 1958); oral LD ₅₀ in rats: 3400 mg/kg bw (Moreno, 1973)	Secondary components do not raise safety concern.
54	Geranyl formate	85 (total esters)	8–10% geraniol; 2–4% nerol	Data for geraniol in rats: 28-week NOEL: 50 mg/kg bw per day (Hagan et al., 1967) 13-week NOEL: 500 mg/kg bw per day (Hagan et al., 1967) 16-week NOEL: 50 mg/kg bw per day (Food and Drug Administration, 1954) Oral LD ₅₀ : 3600 mg/kg bw (Jenner et al., 1964) Geranyl and citronellyl acetate had no effect at 2000 mg/kg bw per day for 2 years in F344 rats or B6C3F ₁ mice (National Toxicology Program, 1987). Nerol is metabolized by oxidation of the primary alcohol and ω-oxidation to yield polar, excretable metabolites. Oral LD ₅₀ in rats: 4500 mg/kg bw (Moreno, 1972) For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
55	Neryl formate	85	4–6% geraniol; 1–3% nerol		Secondary components do not raise safety concern.
56	Rhodinyll formate	85 (total esters)	10–13% rhodinol	Rhodinol is metabolized by oxidation of the primary alcohol and ω-oxidation to yield polar, excretable metabolites. Acute toxicity in rats treated orally: 3/10 deaths at 5000 mg/kg bw (Moreno, 1973)	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
57	Citronellyl acetate	92 (total esters)	4–6% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
60	Rhodinyl acetate	87 (total esters)	9–12% rhodinol	For data on rhodinol, see No. 56 above.	Secondary components do not raise safety concern.
61	Citronellyl propionate	90 (total esters)	5–8% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
62	Geranyl propionate	92 (total esters)	3–4% geraniol; 1–2% nerol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
65	Citronellyl butyrate	90 (total esters)	6–8% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
66	Geranyl butyrate	92 (total esters)	3–5% geraniol; 1% nerol	For data on geraniol, and nerol, see No. 54 above.	Secondary components do not raise safety concern.
68	Rhodinyl butyrate	90	3–5% rhodinol	For data on rhodinol, see No. 56 above.	Secondary components do not raise safety concern.
71	Citronellyl isobutyrate	92 (total esters)	3–5% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
73	Neryl isobutyrate	92	2–5% nerol; 1–2% geraniol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
95	Heptanal	92	4–7% 2-methylhexanal	2-Methylhexanal is oxidized to 2-methylhexanoic acid, which is oxidized in the fatty acid pathway similarly to 2-methyldecanoic acid (see No. 107)	Secondary components do not raise safety concern.
98	Octanal	92	4–7% 2-methylheptanal	2-Methylheptanal is oxidized to 2-methylheptanoic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
101	Nonanal	92	4–8% 2-methyloctanal	2-Methyloctanal (No. 270) has been evaluated by JECFA.	Secondary components do not raise safety concern.
104	Decanal	92	4–7% 2-methylnonanal	2-Methylnonanal is oxidized to 2-methylnonanoic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
107	Undecanal	92	4–8% 2-methyldecanal	2-Methyldecanal is oxidized to the corresponding acid, which undergoes β -oxidation to yield a propionic acid fragment and octanoyl coenzyme A, which is metabolized to carbon dioxide and water. Acute toxicity Rats: 0/10 deaths at 5000 mg/kg bw (Moreno, 1975) Rabbits: 1/10 deaths at 5000 mg/kg bw (Moreno, 1975)	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
110	Lauric aldehyde	90	3–6% tetradecanal; 2–5% decanal; 1–2% hexadecanal	Tetradecanal (No. 112) has been evaluated by JECFA. Decanal (No. 104) has been evaluated by JECFA. Hexadecanal is oxidized to palmitic acid and enters the fatty acid pathway, where it is completely metabolized.	Secondary components do not raise safety concern.
112	Myristaldehyde	85	10–12% dodecanal, hexadecanal and octadecanal	Dodecanal (No. 110) has been evaluated by JECFA. Hexadecanal and octadecanal are oxidized to palmitic and stearic acid, respectively, and metabolized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
117	Propyl formate	94	4–6% propyl alcohol	Propyl alcohol (No. 82) has been evaluated by JECFA.	Secondary components do not raise safety concern.
119	n-Amyl formate	92	4–8% n-amyl alcohol	Amyl alcohol (No. 88) has been evaluated by JECFA.	Secondary components do not raise safety concern.
124	Isobutyl formate	94	4–6% isobutyl alcohol	Isobutyl alcohol (No. 251) has been evaluated by JECFA.	Secondary components do not raise safety concern.
170	n-Amyl heptanoate	93	4–7% n-amyl 2-methylhexanoate	n-Amyl-2-methylhexanoate is hydrolysed to amyl alcohol (No. 88) and 2-methylhexanoic acid (No. 265), which have been evaluated by JECFA.	Secondary components do not raise safety concern.
180	Methyl laurate	90	3–6% methyl tetradecanoate; 2–5% methyl decanoate; 1–2% methyl hexadecanoate	Methyl tetradecanoate (No. 183) has been evaluated by JECFA. A 90-day study of a mixture of methyl tetradecanoate, methyl decanoate and methyl hexadecanoate in rats showed no effects with any of the materials at 1% of the diet, equivalent to 500 mg/kg bw per day (Alfin-Slater et al., 1965).	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
205	Methyl 2-methylbutyrate	92	5–7% methyl isovalerate	Methyl isovalerate (No. 195) has been evaluated by JECFA.	Secondary components do not raise safety concern.
212	2-Methylbutyl 2-methylbutyrate	90	5–7% 2-methylbutyl 3-methylbutyrate	2-Methylbutyl 3-methylbutyrate (No. 204) has been evaluated by JECFA.	Secondary components do not raise safety concern.
237	6-Hydroxy-3,7-dimethyloctanoic acid lactone	90	5–6% 6-hydroxy-3,7-dimethyl-2-octenoic acid lactone	The secondary component differs from the primary component only in the presence of a double bond at the 2-position of the secondary component. The NOEL for a similar 2-alkenoic acid lactone (5-hydroxy-2,4-decadienoic acid- δ -lactone) in a 90-day study in rats was 12 mg/kg bw per day (Cox et al., 1974a).	Secondary components do not raise safety concern.
272	3,7-Dimethyl-1-octanol	90	5–7% geraniol and citronellol	For data on geraniol, see No. 54 above; for data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
302	2,6-Dimethyl-4-heptanone	80	15–17% 4,6-dimethyl-2-heptanone	4,6-Dimethyl-2-heptanone is reduced in vivo, and the resulting alcohol is conjugated with glucuronic acid and excreted in the urine. Oral LD ₅₀ in rats: 5800 mg/kg bw (Bar & Griepentrog, 1967)	Secondary components do not raise safety concern.
303	2,6-Dimethyl-4-heptanol	90	8–9% 2-heptanol	2-Heptanol (No. 284) has been evaluated by JECFA.	Secondary components do not raise safety concern.
322	cis-5-Octen-1-ol	90	7–9% trans-5-octen-1-ol	trans-5-Octen-1-ol is oxidized to trans-5-octenoic acid, which is oxidized in the fatty acid pathway and the citric acid cycle.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
323	<i>cis</i> -5-Octenal	85	10–15% <i>trans</i> -5-octenal	<i>trans</i> -5-Octenal is oxidized to the corresponding carboxylic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
325	<i>cis</i> -6-Nonenal	90	6–9% <i>trans</i> -6-nonenal	<i>trans</i> -6-Nonenal is oxidized to 6-nonenic acid, which is completely metabolized in the fatty acid pathway.	Secondary components do not raise safety concern.
332	Linoleic acid	62	44–46% linolenic acid; 18–20% linoleic acid; 22–25% stearic and oleic acid; 7–8% palmitic acid	Stearic acid (No. 116), oleic acid (No. 333) and palmitic acid (No. 115) have all been evaluated by JECFA.	Secondary components do not raise safety concern.
346	Methyl linoleate and methyl linolenate	60	44–46% methyl linolenate; 18–20% methyl linoleate; 22–25% methyl stearate and methyl oleate; 7–8% methyl palmitate	The NOELs for methyl stearate, methyl oleate and methyl palmitate fed to rats for 90 days at 1% of the diet was 500 mg/kg bw per day (Alfin-Slater et al., 1965).	Secondary components do not raise safety concern.
348	2,6-Dimethyl-6-hepten-1-ol	90	5–10% 6-methyl-5-hepten-2-one	6-Methyl-5-hepten-2-one (No. 1120) was evaluated at the present meeting.	Secondary components do not raise safety concern.
349	2,6-Dimethyl-5-heptenal	85	9–10% 6-methyl-5-hepten-2-one; 1–2% 2,6-dimethyl-6-heptenal	For data on 6-methyl-5-hepten-2-one, see No. 348 above. 2,6-Dimethyl-6-heptenal is oxidized to the corresponding acid, which undergoes β -oxidation.	Secondary components do not raise safety concern.
358	Linalyl formate	90	6–8% linalool	Linalool (No. 356) has been evaluated by JECFA.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
360	Linyl/ propionate	92	2–5% linolool	For data on linolool, see No. 358 above.	Secondary components do not raise safety concern.
384	β -Damascone	90 (sum of <i>cis</i> and <i>trans</i> isomers)	5–8% α - and δ -damascone	α -Damascone (No. 385) and δ -damascone (No. 386) have been evaluated by JECFA.	Secondary components do not raise safety concern.
385	α -Damascone	98 (sum of <i>cis</i> and <i>trans</i> isomers)	92–96% <i>trans</i> , 4–8% <i>cis</i> isomers	The NOEL in a 90-day study with α -damascone containing a <i>trans</i> - and <i>cis</i> -isomer mixture listed in specifications was 2 mg/kg bw per day (Posternak & Vodoz, 1975). The position of the double bond and the presence of <i>cis</i> and <i>trans</i> isomers is not expected to alter metabolic detoxication pathways. α -Damascone is metabolized by reduction of the side-chain ketone group to yield the corresponding alcohol, which is excreted as the glucuronic acid conjugate.	Secondary components do not raise safety concern.
399	Methyl- β -ionone	88 (sum of <i>cis</i> and <i>trans</i> isomers of α -, β - and γ -methylionone)	7–10% α - and β -isomethylionone	α - and β -isomethylionone and α - and β -methylionone are prepared by methylation of ionone. Metabolic detoxication proceeds by reduction of the ketone followed by conjugation of the resulting alcohol with glucuronic acid; the presence of a methyl group is not anticipated to alter the metabolism significantly.	Secondary components do not raise safety concern.
410	2,3-Pentadione	93	2–3% 2,5-diethylcyclohexadien-1,4-dione	2,3-Pentadione dimerizes to 2,5-diethylcyclohexadien-1,4-dione, which is expected to be reduced to the corresponding alcohol and excreted or conjugated with glutathione.	Secondary components do not raise safety concern.
419	Ethyl cyclopentenolone	90	5–10% 3-ethylcyclopentan-1,2-dione (enolic form)	The secondary component is the enol form of the principal substance, ethyl cyclopentenolone (keto form), which is a diketone in equilibrium with the corresponding enol.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
435	<i>para</i> -Menth-1-en-3-one	94	2-3% menthol and menthone	Menthol (No. 427) and menthone (No. 429) have been evaluated by JECFA.	Secondary components do not raise safety concern.
592	Citronellyloxy-acetaldehyde	75	20-21% geranyloxyacetaldehyde; 1-2% citronellool	The structures of geranyloxyacetaldehyde and citronellyloxyaldehyde differ only in the presence of a second double-bond at the 2-position of geranyloxyaldehyde. The safety evaluation of the named flavouring agent also applies to the secondary component. Acute toxicity of oral citronellyloxyaldehyde in rats: no deaths at 5000 mg/kg bw (Moreno, 1976). For data on citronellool, see No. 53 above.	Secondary components do not raise safety concern.
604	3-(Hydroxymethyl)-2-heptanone	90	5-8% 1-hydroxy-3-octanone	The secondary component, 1-hydroxy-3-octanone, is oxidized to 3-oxooctanoic acid, which is predicted to be metabolized in the fatty acid pathway.	Secondary components do not raise safety concern.
625	Dibutyl sebacate	93	2-4% butyl esters of C14, C16 and C18 fatty acids	As the acid is prepared by oxidative cleavage of unsaturated fatty acids, small amounts of fatty acids are secondary components of sebacate esters. The butyl esters of C14, C16 and C18 fatty acids are hydrolysed and metabolized as endogenous fatty acids.	Secondary components do not raise safety concern.
668	Linyl cinnamate	94	3-5% linalool	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
978	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	92	2.6% β -cyclocitral; 0.5% β -ionone; 3% methyl β -homocyclogeraniol; 0.6% ethyl β -homocyclogeraniol	β -Cyclocitral is a mixture of predominantly 2,6,6-trimethyl-1-cyclohexene-1-aldehyde (No. 979) and 2,6,6-trimethyl-2-cyclohexene-1-aldehyde. The NOEL for the structurally related 2,2,3-trimethylcyclopent-3-ene-1-aldehyde (No. 967) in a 90-day study in rats was 12 mg/kg bw per day (BIBRA, 1976). β -Ionone (No. 392) has been evaluated by JECFA. Methyl β -homocyclogeraniol and ethyl β -homocyclogeraniol are esters of (2,6,6-trimethyl-2-cyclohexen-1-yl)acetic acid (CAS No. 24739-72-8), a simple cyclohexylacetic acid derivative. The esters are expected to be hydrolysed to the corresponding acid in vivo. The acid may be excreted directly as a glucuronic acid conjugate or undergo β -oxidation of the side-chain.	Secondary components do not raise safety concern.
1005	Phenylacetaldehyde 2,3-butylene glycol acetal	93	2-3% butane-2,3-diol	Butanediol is the diol formed by hydrolysis of phenylacetaldehyde 2,3-butanediol acetal. The diol is excreted as the glucuronide conjugate.	Secondary components do not raise safety concern.
1046	A mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	64% 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine; 18% 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	12% 2,4,6-trimethyldihydro-1,3,5-dithiazine; 4% 2,4-diisobutyl-6-methyl-1,3,5-dithiazine; 2% 2,6-dimethyl-4-butyldihydro-1,3,5-dithiazine; < 1% substituted 1,3,5-thiadiazine	The cyclic compound 2-methyl-1,3-dithiolane has a similar structure to these heterocyclic compounds, and the NOEL in a 90-day study in rats was 7 mg/kg bw per day (Griffiths et al., 1979). The NOELs in dietary 14-day studies on the structural analogues 2-isobutyl-4,6-dimethyldihydrothiazine (Rush, 1989a) and 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989b) were 11 mg/kg bw per day and 10 mg/kg bw per day, respectively. The secondary components are all structural isomers or homologues of the principal components and were present in the 14-day studies on the flavouring agent. Metabolic pathways for these substances include S-oxidation to polar sulfoxides and sulfones.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
1047	Mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine	44% 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine; 8% 6-methyl-2,4-diisopropyl-1,3,5-dithiazine; 4% 4-methyl-2,6-diisopropyl-1,3,5-dithiazine; 27% 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine	13% 2,4,6-trimethyldihydro-1,3,5-dithiazine; 8% 6-methyl-2,4-diisopropyl-1,3,5-dithiazine; 4% 4-methyl-2,6-diisopropyl-1,3,5-dithiazine; 2% 2,4,6-trisopropyl-dihydro-1,3,5-dithiazine	See No. 1046 above.	Secondary components do not raise safety concern.
1069	2-Ethanoic acid, S-(2-methyl-3-furanyl) ester	92	< 7% <i>cis</i> - and <i>trans</i> -2-methyl-3-tetrahydrofuranthiol acetate	This secondary component is an ester that is hydrolysed to 2-methyl-3-tetrahydrofuranthiol (No. 1090), which has been evaluated by JECFA.	Secondary components do not raise safety concern.
1086	2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	94	5.5% 2-[(2-methyl-3-furyl)thio]-4-heptanone isomer	The NOEL for the structural analogue 3-[(2-methyl-3-furyl)thio]-4-heptanone in a 90-day study in rats was 3.8 mg/kg bw per day (Gallo et al., 1976).	Secondary components do not raise safety concern.
1092	2,5-Dimethyl-3-thioacetoxy-tetrahydrofuran, <i>cis</i> and <i>trans</i> isomers	90% as mixture of 4 stereoisomers (2 pairs of diastereomers)	< 4% 2,5-dimethyltetrahydrofuran-3-thiol; < 2% dimethyltetrahydro-3-furyl dithioacetate	The structural analogue 2-methyl-3-tetrahydrofuranthiol (No. 1090) has been evaluated by JECFA.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
1109	2-sec-Butylcyclohexanone	94	2-2.5% 2-isobutylcyclohexanone	In a 90-day study in rats, the gas chromatography data for the 2-sec-butylcyclohexanone tested showed the presence of 2-isobutylcyclohexanone as a secondary component. As the secondary component was present in the test material, this would not be a safety concern (Hummler, 1969). The NOEL in a 90-day study on a structurally related 2-alkyl-substituted cyclopentanone, 2-hexylidene-cyclopentanone, was 2.9 mg/kg bw per day (Posternak et al., 1969). The NOEL for 2-octen-4-one (No. 1129) and 1-octene-3-one in a 90-day oral study in rats was 7.5 mg/kg bw per day (Cox, 1974a,b).	Secondary components do not raise safety concern.
1128	3-Octen-2-one	94	4-6% 4-octen-2-one	The secondary components are all aliphatic α,β -unsaturated ketones and are metabolized by reaction with glutathione to a mercapturic acid derivative and reduction to the corresponding alcohol. The NOELs in 90-day studies in rats with three structurally related α,β -unsaturated ketones were 6 mg/kg bw per day with 4-hexene-3-one (Shellenberger, 1970), 7.5 mg/kg bw per day with 2-octen-4-one (Cox et al., 1974a) and 7.5 mg/kg bw per day with 1-octen-3-one (Cox et al., 1974b). 3-Nonen-2-one is a straight-chain analogue of these unsaturated ketones.	Secondary components do not raise safety concern.
1135	(E)-7-Methyl-3-octen-2-one	94	2-4% 7-methyl-4-octen-2-one; 5,6-dimethyl-3-hepten-2-one; 3-nonen-2-one	As the secondary component is an alcohol which is interconvertible in vivo with the ketone, the safety evaluation applies to both.	Secondary components do not raise safety concern.
1137	(E) & (Z)-4,8-Dimethyl-3,7-nonadien-2-one	94	3-4% 4,8-dimethyl-3,7-nonadien-2-ol	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.
1154	(E,R)-3,7-Dimethyl-1,5,7-octatrien-3-ol	93	3-5% linalool and lesser quantities of linalool oxide and nerol oxide		Secondary components do not raise safety concern.

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I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 56-81-5
CAS No.	56-81-5
EINECS Name	glycerol
EINECS No.	200-289-5
Molecular Formula	C3H8O3

Dataset created by: EUROPEAN COMMISSION - European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non-confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

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European Chemicals Bureau

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1. General Information

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Substance ID: 56-81-5

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1. General Information

date: 18-FEB-2000
Substance ID: 56-81-5

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Substance ID: 56-81-5

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Country: Germany

Name: Wolff Walsrode AG
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Town: 29655 Walsrode
Country: Germany

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

Substance type: natural substance
Physical status: liquid

Substance type: organic
Physical status: liquid

Substance type: organic
Physical status:

1.1.1 Spectra

-

1.2 Synonyms

1,2,3 trihydroxy propane

Source: Fina Chemicals Brussels

1,2,3-Propanetriol

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside
Akzo Nobel Chemicals GmbH Dueren
Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

1,2,3-propanetriol

Source: Quest International Zwijndrecht
Huels AG Marl
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

1,2,3-Propantriol

Source: Pulcra S.A. Barcelona
Bayer AG Leverkusen

1,2,3-Trihydroxypropan

Source: Pulcra S.A. Barcelona
Bayer AG Leverkusen

1,2,3-Trihydroxypropane

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside

Alcohol Glicilico

Source: KAO Corporation S.A. Barbera del Valles

Crude glycerine

Source: Albright & Wilson UK Limited Oldbury

Crude glycerol

Source: Albright & Wilson UK Limited Oldbury

E422

Source: Croda Universal Ltd Goole, North Humberside

Glicerina

Source: KAO Corporation S.A. Barbera del Valles

GLICERINA, GLICEROLO, TRIIDROSSIPROPANO,

Source: FACI Srl CARASCO (GE)

Glicerol

Source: KAO Corporation S.A. Barbera del Valles

Glycerin

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
WITCO Surfactants GmbH Steinau a. d. Straße
Witco Espana S.L. Granollers
Croda Universal Ltd Goole, North Humberside
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

GLYCERIN

Source: Kays (Ramsbottom) Ltd Ramsbottom, Bury, Lancs.
CUSSONS GROUP STOCKPORT

Glycerin (INCI)

Source: Pulcra S.A. Barcelona

Glycerin Mist

Source: Pulcra S.A. Barcelona

Glycerin USP

Source: Croda Universal Ltd Goole, North Humberside

Glycerin, Propantriol, Protol, 1,2,3-Trihydroxypropan

Source: NEUBER GES.M.B.H. WIEN

Glycerine

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside
DOW Deutschland Inc., Werk Stade Stade 5
Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

glycerine

Source: DSM Resins BV Zwolle
IFF Haverhill, Suffolk

Glycerine.

Source: Courtaulds Chemicals(L) Leek,Staffs.

Glyceritol

Source: Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside

Glycerol

Source: VOS B.V. Alphen aan den Rijn
Fina Chemicals Brussels
Akzo Nobel Chemicals GmbH Dueren
DOW Deutschland Inc., Werk Stade Stade 5

glycerol (INN)

Source: Pulcra S.A. Barcelona

Glycerol / Glycerin / 1,2,3-Propanetriol

Source: Wolff Walsrode AG Walsrode

Glycerol, Glycerin

Source: Pronova Oleochemicals a.s. Sandefjord

GLYCEROL, GLYCERINE, 1,2,3, TRIHYDROXYPROPANE

Source: COLGATE-PALMOLIVE SpA ANZIO (ROMA)
COLGATE-PALMOLIVE LTD SALFORD

Glycerol; Glycerine; Glycerin; Glycyl alcohol, Trihydroxypropane

Source: ATOCHEM PARIS LA DEFENSE 10

Glycerol; Glycerine; Glycerin; Glycyl alcohol; Trihydroxypropane

Source: Unichema Chemie B.V. Gouda
Unichema International B.V. Gouda
Simel S.p.A. Cremona
Simel S.p.A. Industria Chimica Cremona
PROCTER & GAMBLE (CHEMICALS EUROPE) LTD. HAYES MIDDLESEX
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemicals Ltd Bebington
Karlshamns Tefac AB Karlshamn
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Glycerol;Glycerine;Glycerin;Glycyl alcohol;Trihydroxypropane

Source: SPIGA nord S.p.A. CARASCO (GE)

Glycerolum

Source: Croda Universal Ltd Goole, North Humberside

glycerolum (INN)

Source: Pulcra S.A. Barcelona

Glycyl alcohol

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside
DOW Deutschland Inc., Werk Stade Stade 5
Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

Glyrol

Source: Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside

Glyzerin

Source: Pulcra S.A. Barcelona
Akzo Nobel Chemicals GmbH Dueren

Gycerol

Source: Courtaulds Chemicals(L) Leek,Staffs.
Bayer AG Leverkusen

1. General Information

date: 18-FEB-2000
Substance ID: 56-81-5

HOCH₂COHHCH₂OH

Source: Amway Europe Zaventem

PROPANE-1,2,3,-DIOL

Source: CUSSONS GROUP STOCKPORT

Propanetriol

Source: Pulcra S.A. Barcelona

Propantriol, 1,2,3-

Source: Pulcra S.A. Barcelona

Trihidroxipropano

Source: KAO Corporation S.A. Barbera del Valles

Trihydroxypropane

Source: Akzo Nobel Chemicals b.v. Amersfoort
Pulcra S.A. Barcelona
Croda Universal Ltd Goole, North Humberside
DOW Deutschland Inc., Werk Stade Stade 5
Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

trihydroxypropane

Source: IFF Haverhill, Suffolk

Source: Witco Espana S.L. Granollers

Remark: glicerina

Source: UNION DERIVAN S.A. VILADECANS

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

Quantity 500 000 - 1 000 000 tonnes

1.6.1 Labelling

-

1.6.2 Classification

-

1.7 Use Pattern

Type:	type
Category:	Non dispersive use
Type:	type
Category:	Use in closed system
Type:	type
Category:	Use resulting in inclusion into or onto matrix
Type:	type
Category:	Wide dispersive use
Type:	industrial
Category:	Agricultural industry
Type:	industrial
Category:	Basic industry: basic chemicals
Type:	industrial
Category:	Chemical industry: used in synthesis
Type:	industrial
Category:	Paints, lacquers and varnishes industry
Type:	industrial
Category:	Personal and domestic use
Type:	industrial
Category:	Polymers industry
Type:	industrial
Category:	Public domain
Type:	industrial
Category:	other: food industry
Type:	industrial
Category:	other: pharmaceutical industry
Type:	industrial
Category:	other: tobacco industry
Type:	industrial
Category:	
Type:	industrial
Category:	other
Type:	use
Category:	Absorbents and adsorbents
Type:	use
Category:	Adhesive, binding agents

Type:	use
Category:	Anti-freezing agents
Type:	use
Category:	Cleaning/washing agents and disinfectants
Type:	use
Category:	Cosmetics
Type:	use
Category:	Dustbinding agents
Type:	use
Category:	Explosives
Type:	use
Category:	Food/foodstuff additives
Type:	use
Category:	Heat transferring agents
Type:	use
Category:	Impregnation agents
Type:	use
Category:	Intermediates
Type:	use
Category:	Laboratory chemicals
Type:	use
Category:	Pesticides
Type:	use
Category:	Pharmaceuticals
Type:	use
Category:	Softeners
Type:	use
Category:	Solvents
Type:	use
Category:	other: many applications in many industries
Type:	use
Category:	other: reactant
Type:	use
Category:	other

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit: MAC (NL)
Limit value: 10 mg/m³
Remark: Glycerin mist
Source: Akzo Nobel Chemicals b.v. Amersfoort

(1)

Type of limit: MAK (DE)
Limit value:
Remark: No occupational exposure limit has been established.
Source: Akzo Nobel Chemicals b.v. Amersfoort

(2)

Type of limit: MEL (UK)
Limit value: 10 mg/m³
Short term expos.
Limit value: 10 mg/m³
Schedule: 10 minute(s)
Remark: When handling, skin and eye protection should be worn.
Source: CUSSONS GROUP STOCKPORT

Type of limit: OES (UK)
Limit value: 10 mg/m³
Remark: Note: Occupational exposure standard applies to Glycerol, mist
Source: Croda Universal Ltd Goole, North Humberside

(3)

Type of limit: OES (UK)
Limit value: 10 mg/m³
Source: Kays (Ramsbottom) Ltd Ramsbottom, Bury, Lancs.

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: Glycerin mist; Total dust/particulate.
Source: Akzo Nobel Chemicals b.v. Amersfoort

(4)

Type of limit: TLV (US)
Limit value: 10 mg/m³
Source: ATOCHEM PARIS LA DEFENSE 10

(5)

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: ACGIH Threshold Limit (TLV) and OSHA Permissible Exposure Level (PEL) is 10 mg/m3 (aerosol).
Source: DOW Deutschland Inc., Werk Stade Stade 5

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: 10 mg/m3 "Glycerin mist" (TLV) inhalable (total) dust/particulate
Source: Wolff Walsrode AG Walsrode

Type of limit: other: VME
Limit value: 10 mg/m3
Country: France
Source: ATOCHEM PARIS LA DEFENSE 10

(6)

Type of limit:
Limit value:
Remark: No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m3). In a dossier that was prepared on the behalf at the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m3.
A recent subchronic inhalation study from Renne et al (1992) determined a NOEL of 167 mg/m3 and LOEL of 662 mg/m3 for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m3 is still open to discussion.
Source: Unichema Chemie B.V. Gouda
Unichema International B.V. Gouda
Simel S.p.A. Cremona
Unichema Chemicals Ltd Bebington

Type of limit:
Limit value:
Remark: No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m3). In a dossier that was prepared on behalf of the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m3.
A recent subchronic inhalation study from Renne et al. (1992) determined a NOEL of 167 mg/m3 and LOEL of 662 mg/m3 for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m3 is still open to discussion.
Source: Akzo Nobel Chemicals b.v. Amersfoort

Type of limit:**Limit value:****Remark:**

No se han establecido límites de exposición en cuanto a la utilización profesional de la glicerina por parte de las autoridades de la UE. De todas maneras, algunos países recomiendan en el caso de polvo respirable, unos valores límite de exposición profesional de 10 mg/m³. En un dossier que se preparó a cuenta de la comisión de la UE, el Grupo Científico de Expertos en límites de exposición profesional, sugirió el mismo valor de 10 mg/m³.

Un estudio reciente de inhalación subcrónica de Renne et al (1992) determina un NOEL de 167 mg/m³ y un LOEL de 662 mg/m³ para la glicerina anhidra. Está todavía en discusión si este estudio y los efectos en el indicados justificarian un límite de exposición profesional por debajo de 10 mg/m³.

Source:

KAO Corporation S.A. Barbera del Valles

Type of limit:**Limit value:****Remark:**

no disponible

Source:

UNION DERIVAN S.A. VILADECANS

Type of limit:**Limit value:****Remark:**

NESSUN LIMITE DI ESPOSIZIONE NELL'AMBIENTE DI LAVORO E' FISSATO DALLE AUTORITA' UE.

ALCUNI PAESI COMUNQUE RACCOMANDANO LIMITI DI ESPOSIZIONE PARI A QUELLI DELLE POLVERI RESPIRABILI (10 mg/m³)

IL GRUPPO DEGLI ESPERTI SCIENTIFICI SUI LIMITI DI ESPOSIZIONE NELL'AMBIENTE DI LAVORO ALLA COMMISSIONE EU HA SUGGERITO LO STESSO VALORE DI 10 mg/m³.

UN RECENTE STUDIO DI RENNE E ALLIEVI HA DETERMINATO UN NOEL DI 167 mg/m³ E UN LOEL DI 662 mg/m³ PER IL GLICEROLO ANIDRO. SE QUESTO E GLI EFFETTI OSSERVATI GIUSTIFICANO UN LIMITE DI ES[POSIZIONE IN AMBIENTE DI LAVORO BASSO COME 10 mg/m³ E' ANCORA IN DISCUSSIONE.

¿

Source:

FACI Srl CARASCO (GE)

Type of limit:**Limit value:****Remark:**

No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m³). In a dossier that was prepared on the behalf at the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m³.

A recent subchronic inhalation study from Renne et al (1992) determined a NOEL of 167 mg/m³ and LOEL of 662 mg/m³ for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m³ is still open to discussion.

Source:

Simel S.p.A. Industria Chimica Cremona

Type of limit:**Limit value:****Remark:** No occupational exposure limit is set by the EU authorities for glycerol. See full HEDSET by Unichema Chemie GmbH for further remarks.**Source:** PROCTER & GAMBLE (CHEMICALS EUROPE) LTD. HAYES MIDDLESEX**Type of limit:****Limit value:****Remark:** No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m³). In a dossier that was prepared on the behalf at the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m³.A recent subchronic inhalation study from Renne et al (1992) determined a NOEL of 167 mg/m³ and LOEL of 662 mg/m³ for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m³ is still open to discussion.**Source:** Lever Brother Ltd. Kingston Upon Thames, Surrey**Type of limit:****Limit value:****Remark:** Low hazard material with very low vapour pressure. No inhalation limits found and the submitting company does not have an internal standard. However the UK regulator, the Health and Safety Executive (HSE) publish (in EH40) a standard for airborne droplets or mist which must not exceed 10mg/m³**Source:** Courtaulds Chemicals(L) Leek, Staffs.**Type of limit:****Limit value:****Remark:** No occupational exposure limit is set by the EU authorities for glycerol**Source:** Karlshamns Tefac AB Karlshamn**Type of limit:****Limit value:****Remark:** Not Applicable**Source:** Pronova Oleochemicals a.s. Sandefjord**Type of limit:****Limit value:****Remark:** Not Applicable**Source:** Pronova Oleochemicals a.s. Sandefjord

Type of limit:**Limit value:****Remark:**

No Occupational Exposure Limit (OEL) value is set by the EU-authorities for glycerol. Some countries, however, recommend Occupational Exposure Limit values as for respirable dust (i.e. 10 mg/m³). In a dossier that was prepared on behalf of the EU-commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m³.

Source:

A recent subchronic inhalation study from Renne et al. (1992) determined a NOEL of 167 mg/m³, and a LOEL of 662 mg/m³ for anhydrous glycerol. If this study and the effects observed therein justify an Occupational Exposure Limit value as low as 10 mg/m³ is still open to discussion.

Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

Type of limit:**Limit value:****Remark:**

No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m³). In a dossier that was prepared on the behalf at the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m³.

Source:

A recent subchronic inhalation study from Renne et al. (1992) determined a NOEL of 167 mg/m³ and LOEL of 662 mg/m³ for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m³ is still open to discussion.

Unichema Chemie GmbH Emmerich

Type of limit:**Limit value:****Remark:**

No occupational exposure limit is set by the EU authorities for glycerol. Some countries, however, recommend occupational exposure limit values as for respirable dust (10 mg/m³). In a dossier that was prepared on the behalf at the EU commission, the Scientific Expert Group on Occupational Exposure Limits suggested the same value of 10 mg/m³.

Source:

A recent subchronic inhalation study from Renne et al. (1992) determined a NOEL of 167 mg/m³ and LOEL of 662 mg/m³ for anhydrous glycerol. If this study and the observed effects there in justify an occupational exposure limit as low as 10 mg/m³ is still open to discussion.

Lever GmbH Hamburg

1.9 Source of Exposure

Remark: Substance is used as a starting material in the manufacturing of various food emulsifiers.

Source: Quest International Zwijndrecht

Remark: a) Glycerine is obtained by hydrolysis under pressure and high temp rature of vegetable oils (rapeseed, soya, sunflower, etc...) or animal fats (tallow)

b) Number of production sites: one

Source: Fina Chemicals Brussels

Remark: Process: Transesterification of ricin oil by methanol in esters, in presence of sodium hydroxide. After neutralisation, the chemical is purified by distillation. Effluents when changes of distillator. These waste waters are treated in the water treatment plant of the site, after storage in inland drainage basins. The basins are analysed each day.

Source: ATOCHEM PARIS LA DEFENSE 10

Remark: durante el envasado

Source: UNION DERIVAN S.A. VILADECANS

Remark: Direct exposure by ingestion due to ubiquitous use as food additive (E422), Humectant, solvent, cake icing component, confectionary component, bodying agent, plasticiser for foods.

Source: Croda Universal Ltd Goole, North Humberside

Remark: LOW ORAL TOXICITY. UNLIKELY TO CAUSE IRRITATION IN HUMANS. ENVIRONMENTAL EFFECTS - BIODEGRADABLE.

Source: Kays (Ramsbottom) Ltd Ramsbottom, Bury, Lancs.

Remark: Environmental emissions: negligible
One manufacturing site in the UK, no other sites in the EU
Production process: soap is produced (in a continuous process) by saponification of oils with caustic. Glycerol is a by product and is extracted by washing with brine. Thewashings are then treated with ferric chloride and sodium hydroxide to remove any soap, and the water is then evaporated off.
Sources of exposure: spillages

Source: CUSSONS GROUP STOCKPORT

Remark: Exposure of the general population due to use in consumer products which are flavoured. Such products may contain low levels of this substance.

Source: IFF Haverhill, Suffolk

Remark: Food additive E422

Source: IFF Haverhill, Suffolk

- Remark:** This submitter carries out Ester manufacture with this substance and the only opportunity for contact is following some type of accident during the transport,unloading or charging of glycerine
- Source:** Courtaulds Chemicals(L) Leek,Staffs.
- Remark:** Not applicable
- Source:** Pronova Oleochemicals a.s. Sandefjord
- Remark:** Not applicable
- Source:** Pronova Oleochemicals a.s. Sandefjord
- Remark:** Production process: Epichlorohydrin is reacted with sodium carbonate to glycerine, sodium chloride, sodium hydrogencarbonate and water. In a parallel reaction epichlorohydrin is processed with sodium hydrogencarbonate to glycerine, sodium chloride and CO₂. (One manufacturing site for Dow in Europe: Stade (G))
- Environmental emissions: appr. 18 mt of glycerine are released per annum via cooling tower to the air (calculation).
- Human exposure: due to the absence of toxicity glycerine canbe safely used in cosmetics, pharmaceuticals and as food additive.
- Source:** DOW Deutschland Inc., Werk Stade Stade 5
- Remark:** Consumer and professional exposures related to the use pattern are described in section 1.7
Possibility of a natural background exposure.
Glycerine is found in natural oils.
- Country where Solvay production plant is located in EU:
- Germany
- Source:** Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen
- Remark:** IUCLID DATA SHEET:
Direct exposure by ingestion due to ubiquitous use as food additive (E422), humectant, solvent, cake icing component, confectionary component, bodying agent, plasticizer for foods.
- Source:** Wolff Walsrode AG Walsrode

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

Classified by: KBWS (DE)
Labelled by: KBWS (DE)
Class of danger: 0 (generally not water polluting)
Source: Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

(7)

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

Remark: Wassergefährdungsklasse (German water pollution classification): 0 (not hazardous to the aquatic environment)

Source: Akzo Nobel Chemicals b.v. Amersfoort

Remark: Recommended mode of disposal is via incineration under controlled conditions

Source: DOW Deutschland Inc., Werk Stade Stade 5

Remark: Glycerol is permitted food additive; ist EEC number is E 442.
The Joint FAO/WHO Expert Committee on Food Additives considered that glycerol was of such low toxicity that it is acceptable for use without a formal Acceptable Daily Intake (ADI) figure being specified.

Source: Henkel KGaA Duesseldorf
Henkel KGaA Duesseldorf

(8)

Remark: CONVERSION FACTORS (20 deg C, 101 kPa):

1 mg/m³ = 0.26 ppm

1 ppm = 3.83 mg/m³

Source: Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Remark: WGK: 0 (generally not water polluting)
WGK: 0 (im allgemeinen nicht wassergefährdend)
Source: Wolff Walsrode AG Walsrode

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2.1 Melting Point

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Method: other
GLP: yes
Source: UNION DERIVAN S.A. VILADECANS

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Method: other: Unknown (literature)
GLP: no data
Remark: Glycerol is seldom seen in its crystallized state, because of its tendency to supercool, and the pronounced effect of small amounts of water in depressing the melting (freezing) point.
Source: Simel S.p.A. Industria Chimica Cremona (9)

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Method: other: Unknown (literature)
GLP: no data
Remark: Glycerol is seldom seen in its crystallized state, because of its tendency to supercool, and the pronounced effect of small amounts of water in depressing the melting (freezing) point.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (9)

Value: ca. 18 degree C
Decomposition: ambiguous
Sublimation: no
Source: Pronova Oleochemicals a.s. Sandefjord

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Method: other: Unknown (literature)
GLP: no data
Remark: Glycerol is seldom seen in its crystallized state, because of its tendency to supercool, and the pronounced effect of small amounts of water in depressing the melting (freezing) point.
Source: Unichema Chemie GmbH Emmerich (9)

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Method: other: Unknown (literature)
GLP: no data
Remark: Glycerol is seldom seen in its crystallized state, because of its tendency to supercool, and the pronounced effect of small amounts of water in depressing the melting (freezing) point.
Source: Lever GmbH Hamburg (9)

Value: ca. 18 degree C
Decomposition: no
Sublimation: no
Source: Wolff Walsrode AG Walsrode

Value: = 18.2 degree C
Source: Simel S.p.A. Industria Chimica Cremona (10)

Value: = 18.2 degree C
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value: = 18.2 degree C
Source: Unichema Chemie GmbH Emmerich (11)

Value: = 18.2 degree C
Source: Lever GmbH Hamburg (11)

Value: = 20 degree C
Decomposition: no
Sublimation: no
Method: other
Year: 1993
GLP: no data
Source: Croda Universal Ltd Goole, North Humberside (12)

2.2 Boiling Point

Value: = 149 degree C at 5.3 hPa
Source: Simel S.p.A. Industria Chimica Cremona (10)

Value: = 149 degree C at 5.3 hPa
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value: = 149 degree C at 5.3 hPa
Source: Unichema Chemie GmbH Emmerich (11)

Value:	= 149 degree C at 5.3 hPa	
Source:	Lever GmbH Hamburg	(11)
Value:	= 166.1 degree C at 13.3 hPa	
Source:	Simel S.p.A. Industria Chimica Cremona	(10)
Value:	= 166.1 degree C at 13.3 hPa	
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
Value:	= 166.1 degree C at 13.3 hPa	
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	= 166.1 degree C at 13.3 hPa	
Source:	Lever GmbH Hamburg	(11)
Value:	< 180 degree C	
Decomposition:	yes	
GLP:	no data	
Remark:	In temperatures higher than 180 °C glycerol decomposes to Di/Polyglycolethers and acrolein.	
Source:	Simel S.p.A. Industria Chimica Cremona Lever Brother Ltd. Kingston Upon Thames, Surrey Unichema Chemie GmbH Emmerich Lever GmbH Hamburg	(13)
Value:	= 222.4 degree C at 133.3 hPa	
Source:	Simel S.p.A. Industria Chimica Cremona	(10)
Value:	= 222.4 degree C at 133.3 hPa	
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
Value:	= 222.4 degree C at 133.3 hPa	
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	= 222.4 degree C at 133.3 hPa	
Source:	Lever GmbH Hamburg	(11)
Value:	ca. 290 degree C	
Method:	other	
GLP:	yes	
Source:	UNION DERIVAN S.A. VILADECANS	
Value:	= 290 degree C at 1013 hPa	
Source:	Simel S.p.A. Industria Chimica Cremona	(10)

Value: = 290 degree C at 1013.25 hPa
Decomposition: yes
Method: other
Year: 1993
GLP: no data
Source: Croda Universal Ltd Goole, North Humberside (14)

Value: = 290 degree C at 1013 hPa
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value: = 290 degree C at 1013 hPa
Source: Unichema Chemie GmbH Emmerich (11)

Value: = 290 degree C at 1013 hPa
Source: Lever GmbH Hamburg (11)

Value: ca. 290 degree C at 1013.25 hPa
Decomposition: no
Source: Wolff Walsrode AG Walsrode

2.3 Density

Type: density
Value: ca. 1.261 g/cm³ at 20 degree C
Method: other
GLP: yes
Source: UNION DERIVAN S.A. VILADECANS

Type: relative density
Value: = 1.2613 g/cm³ at 20 degree C
Method: other
Year: 1993
GLP: no data
Source: Croda Universal Ltd Goole, North Humberside (14)

Type: relative density
Value: ca. 1.2613 g/cm³ at 20 degree C
Source: Wolff Walsrode AG Walsrode

Type: density
Value: = 1.262 g/cm³ at 25 degree C
Source: Simel S.p.A. Industria Chimica Cremona (10)

Type: density
Value: = 1.262 g/cm³ at 25 degree C
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Type: density
Value: = 1.262 g/cm³ at 25 degree C
Source: Unichema Chemie GmbH Emmerich (11)

Type: density
Value: = 1.262 g/cm³ at 25 degree C
Source: Lever GmbH Hamburg (11)

Type: density
Value: ca. 1.2 g/cm³ at 75 degree C
Source: Pronova Oleochemicals a.s. Sandefjord

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value: = .0033 hPa at 50 degree C
Source: Simel S.p.A. Industria Chimica Cremona (10)

Value: = .0033 hPa at 50 degree C
Method: other (measured)
Year: 1979
GLP: no data
Remark: (Quotes as 2.5E-03 mm Hg at 50 Deg C).
Source: Croda Universal Ltd Goole, North Humberside (15)

Value: = .0033 hPa at 50 degree C
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value: = .0033 hPa at 50 degree C
Source: Unichema Chemie GmbH Emmerich (11)

Value: = .0033 hPa at 50 degree C
Source: Lever GmbH Hamburg (11)

Value: ca. .0033 hPa at 50 degree C
Source: Wolff Walsrode AG Walsrode

Value: = .26 hPa at 100 degree C
Source: Simel S.p.A. Industria Chimica Cremona (10)

Value: = .26 hPa at 100 degree C
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value:	= .26 hPa at 100 degree C	
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	= .26 hPa at 100 degree C	
Source:	Lever GmbH Hamburg	(11)
Value:	= 5.73 hPa at 150 degree C	
Source:	Simel S.p.A. Industria Chimica Cremona	(10)
Value:	= 5.73 hPa at 150 degree C	
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
Value:	= 5.73 hPa at 150 degree C	
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	= 5.73 hPa at 150 degree C	
Source:	Lever GmbH Hamburg	(11)
Value:	= 61 hPa at 200 degree C	
Source:	Simel S.p.A. Industria Chimica Cremona	(10)
Value:	= 61 hPa at 200 degree C	
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
Value:	= 61 hPa at 200 degree C	
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	= 61 hPa at 200 degree C	
Source:	Lever GmbH Hamburg	(11)
Value:		
Remark:	no disponible	
Source:	UNION DERIVAN S.A. VILADECANS	

2.5 Partition Coefficient

log Pow:	-2.66 - -2.47	
Method:	other (calculated)	
Year:		
Source:	Simel S.p.A. Industria Chimica Cremona	(10)

log Pow:	-2.66 - -2.47	
Method:	other (calculated)	
Year:		
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
log Pow:	-2.66 - -2.47	
Method:	other (calculated)	
Year:		
Source:	Unichema Chemie GmbH Emmerich	(11)
log Pow:	-2.66 - -2.47	
Method:	other (calculated)	
Year:		
Source:	Lever GmbH Hamburg	(11)
log Pow:	ca. -2.6 - -1.8	
Method:		
Year:		
Source:	Wolff Walsrode AG Walsrode	
log Pow:	= -2.56	
Method:	other (calculated)	
Year:		
Source:	Simel S.p.A. Industria Chimica Cremona	(10)
log Pow:	= -2.56	
Method:	other (calculated)	
Year:		
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
log Pow:	= -2.56	
Method:	other (calculated)	
Year:		
Source:	Unichema Chemie GmbH Emmerich	(11)
log Pow:	= -2.56	
Method:	other (calculated)	
Year:		
Source:	Lever GmbH Hamburg	(11)
log Pow:	= -1.76	
Method:	other (measured)	
Year:		
Source:	Simel S.p.A. Industria Chimica Cremona Lever Brother Ltd. Kingston Upon Thames, Surrey Unichema Chemie GmbH Emmerich Lever GmbH Hamburg	(16)

log Pow:
Method:
Year:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

2.6.1 Water Solubility

Remark: soluble en agua
Source: UNION DERIVAN S.A. VILADECANS

Remark: Completely miscible with water.
Source: Simel S.p.A. Industria Chimica Cremona

(17)

pKa: 14.4 at 25 degree C
Method: other: not mentioned
GLP: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(18)

Value: =
Qualitative: of very high solubility
pH: ca. 7
Method: other
Year: 1993
GLP: no data
Remark: Soluble in all proportions.
Source: Croda Universal Ltd Goole, North Humberside

(19) (14)

Remark: Completely miscible with water.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(17)

Qualitative: soluble
Source: Pronova Oleochemicals a.s. Sandefjord

Remark: Completely miscible with water.
Source: Unichema Chemie GmbH Emmerich

(17)

Remark: Completely miscible with water.
Source: Lever GmbH Hamburg

(17)

pKa: 14.4 at 25 degree C
pH: ca. 7 - 8.5
Remark: In jedem Verhältnis mit Wasser mischbar.
Source: Wolff Walsrode AG Walsrode

2.6.2 Surface Tension

-

2.7 Flash Point**Value:** ca. 160 degree C**Type:** open cup**Method:****Year:****Source:** Pronova Oleochemicals a.s. Sandefjord**Value:** ca. 177 degree C**Type:** open cup**Method:** other: Cleveland Open Cup (ASTM D92-85)**Year:** 1985**GLP:** no**Remark:** When aqueous glycerol is tested it will not flash until enough water has evaporated to bring the glycerol concentration to about 97.5% by weight. It will then flash at 190 °C.**Source:** Simel S.p.A. Industria Chimica Cremona

(20)

Value: = 177 degree C**Type:** open cup**Method:****Year:****Source:** Simel S.p.A. Industria Chimica Cremona

(10)

Value: = 177 degree C**Type:** open cup**Method:** other**Year:** 1971**GLP:** no data**Source:** Croda Universal Ltd Goole, North Humberside

(21)

Value: ca. 177 degree C**Type:** open cup**Method:** other: Cleveland Open Cup (ASTM D92-85)**Year:** 1985**GLP:** no**Remark:** When aqueous glycerol is tested it will not flash until enough water has evaporated to bring the glycerol concentration to about 97.5% by weight. It will then flash at 190 °C.**Source:** Lever Brother Ltd. Kingston Upon Thames, Surrey

(22)

Value:	= 177 degree C	
Type:	open cup	
Method:		
Year:		
Source:	Lever Brother Ltd. Kingston Upon Thames, Surrey	(11)
Value:	ca. 177 degree C	
Type:	open cup	
Method:	other: Cleveland Open Cup (ASTM D92-85)	
Year:	1985	
GLP:	no	
Remark:	When aqueous glycerol is tested it will not flash until enough water has evaporated to bring the glycerol concentration to about 97.5% by weight. It will then flash at 190 °C.	
Source:	Unichema Chemie GmbH Emmerich	(22)
Value:	= 177 degree C	
Type:	open cup	
Method:		
Year:		
Source:	Unichema Chemie GmbH Emmerich	(11)
Value:	ca. 177 degree C	
Type:	open cup	
Method:	other: Cleveland Open Cup (ASTM D92-85)	
Year:	1985	
GLP:	no	
Remark:	When aqueous glycerol is tested it will not flash until enough water has evaporated to bring the glycerol concentration to about 97.5% by weight. It will then flash at 190 °C.	
Source:	Lever GmbH Hamburg	(22)
Value:	= 177 degree C	
Type:	open cup	
Method:		
Year:		
Source:	Lever GmbH Hamburg	(11)
Value:	> 180 degree C	
Type:	open cup	
Method:	other	
Year:		
GLP:	yes	
Source:	UNION DERIVAN S.A. VILADECANS	

Value: ca. 180 degree C
Type: open cup
Method:
Year:
Source: Wolff Walsrode AG Walsrode

Value: = 199 degree C
Type: closed cup
Method:
Year:
Source: Simel S.p.A. Industria Chimica Cremona (10)

Value: = 199 degree C
Type: closed cup
Method:
Year:
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Value: = 199 degree C
Type: closed cup
Method:
Year:
Source: Unichema Chemie GmbH Emmerich (11)

Value: = 199 degree C
Type: closed cup
Method:
Year:
Source: Lever GmbH Hamburg (11)

2.8 Auto Flammability

Value: > 350 degree C
Method: other
GLP: yes
Source: UNION DERIVAN S.A. VILADECANS

Value: ca. 370 - 422 degree C at 1013.25 hPa
Source: Wolff Walsrode AG Walsrode

Value: 370 degree C
Source: Simel S.p.A. Industria Chimica Cremona (23)

Value: 370 degree C
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (24)

Value: 370 degree C
Source: Unichema Chemie GmbH Emmerich (24)

Value: 370 degree C
Source: Lever GmbH Hamburg (24)

Value: = 422 degree C at 1013.25 hPa
Method: other
Year: 1977
Remark: Value give refers to Autoignition temperature (739 Deg F)
Source: Croda Universal Ltd Goole, North Humberside (25)

Value:
Remark: The auto ignition temperature of glycerol is 523 °C on platinum, 429 °C on glass, and 412 °C in oxygen at 1 atm.
Source: Simel S.p.A. Industria Chimica Cremona (26)

Value:
Remark: The auto ignition temperature of glycerol is 523 °C on platinum, 429 °C on glass, and 412 °C in oxygen at 1 atm.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (27)

Value:
Remark: The auto ignition temperature of glycerol is 523 °C on platinum, 429 °C on glass, and 412 °C in oxygen at 1 atm.
Source: Unichema Chemie GmbH Emmerich (27)

Value:
Remark: The auto ignition temperature of glycerol is 523 °C on platinum, 429 °C on glass, and 412 °C in oxygen at 1 atm.
Source: Lever GmbH Hamburg (27)

2.9 Flammability

Result: non flammable
Remark: It is not to be expected that glycerol:
- will produce flammable gasses if in contact with water;-
will show spontaneous ignition in contact with inert material and intense contact with air (i.e. pyrophoric properties).
Source: Simel S.p.A. Industria Chimica Cremona

Result: non flammable
Method: other
GLP: no data
Remark: See flash point data.
Source: Croda Universal Ltd Goole, North Humberside

Result: non flammable
Remark: It is not to be expected that glycerol:
- will produce flammable gasses if in contact with water;-
will show spontaneous ignition in contact with inert material and intense contact with air (i.e. pyrophoric properties).
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

Result: non flammable
Remark: It is not to be expected that glycerol:
- will produce flammable gasses if in contact with water;-
will show spontaneous ignition in contact with inert material and intense contact with air (i.e. pyrophoric properties).
Source: Unichema Chemie GmbH Emmerich

Result: non flammable
Remark: It is not to be expected that glycerol:
- will produce flammable gasses if in contact with water;-
will show spontaneous ignition in contact with inert material and intense contact with air (i.e. pyrophoric properties).
Source: Lever GmbH Hamburg

Result: non flammable
Source: Wolff Walsrode AG Walsrode

Result:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

2.10 Explosive Properties

Result: not explosive
Remark: No explosive properties are to be expected.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Result: not explosive
Source: Croda Universal Ltd Goole, North Humberside

Result: not explosive
Source: Wolff Walsrode AG Walsrode

Result:
Remark: no presenta riesgo de explosión
Source: UNION DERIVAN S.A. VILADECANS

2.11 Oxidizing Properties

Result: no oxidizing properties
Remark: No oxidizing properties are to be expected.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Result: no oxidizing properties
Source: Croda Universal Ltd Goole, North Humberside

Result: no oxidizing properties
Source: Wolff Walsrode AG Walsrode

Result:
Remark: no es comburente
Source: UNION DERIVAN S.A. VILADECANS

2.12 Additional Remarks

Remark: Viscosity (20 °C) : 1410 mPa.s
Surface Tension (20 °C) : 63.4 mN/m
Solubility : Glycerol will dissolve a large number of organic and inorganic compounds and will miscible with many other substances. Thus, glycerol will be completely miscible with most of the lower aliphatic alcohols, phenol, ethylene, propylene, and trialkyl glycols, some glycol ethers, but only partially, or not at all, with others.
Source: Simel S.p.A. Industria Chimica Cremona (28)

Remark: Viscosity (20 °C) : 1410 mPa.s
Surface Tension (20 °C) : 63.4 mN/m
Solubility : Glycerol will dissolve a large number of organic and inorganic compounds and will miscible with many other substances. Thus, glycerol will be completely miscible with most of the lower aliphatic alcohols, phenol, ethylene, propylene, and trialkyl glycols, some glycol ethers, but only partially, or not at all, with others.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (29)

Remark: Viscosity (20 °C) : 1410 mPa.s
Surface Tension (20 °C) : 63.4 mN/m
Solubility : Glycerol will dissolve a large number of organic and inorganic compounds and will miscible with many other substances. Thus, glycerol will be

completely miscible with most of the lower aliphatic alcohols, phenol, ethylene, propylene, and trialkyl glycols, some glycol ethers, but only partially, or not at all, with others.

Source: Unichema Chemie GmbH Emmerich

(29)

Remark: Viscosity (20 °C) : 1410 mPa.s

Surface Tension (20 °C) : 63.4 mN/m

Solubility : Glycerol will dissolve a large number of organic and inorganic compounds and will miscible with many other substances. Thus, glycerol will be completely miscible with most of the lower aliphatic alcohols, phenol, ethylene, propylene, and trialkyl glycols, some glycol ethers, but only partially, or not at all, with others.

Source: Lever GmbH Hamburg

(29)

Remark: Zu vermeidende Stoffe: Oxidationsmittel

Source: Wolff Walsrode AG Walsrode

3.1.1 Photodegradation**Type:** air**INDIRECT PHOTOLYSIS****Sensitizer:** OH**Method:** other (measured)**Year:** **GLP:** no data**Test substance:****Result:** Rate constant: 0.95×10^9 l/mol.sec.**Source:** Simel S.p.A. Industria Chimica Cremona**Test condition:** Sensitizer (OH radical) concentration in water: 10×10^{-17} mol/l.

(30)

Type: air**INDIRECT PHOTOLYSIS****Sensitizer:** OH**Method:** other (measured)**Year:** **GLP:** no data**Test substance:****Result:** Rate constant: 0.95×10^9 l/mol.sec.**Source:** Lever Brother Ltd. Kingston Upon Thames, Surrey**Test condition:** Sensitizer (OH radical) concentration in water: 10×10^{-17} mol/l.

(31)

Type: air**INDIRECT PHOTOLYSIS****Sensitizer:** OH**Method:** other (measured)**Year:** **GLP:** no data**Test substance:****Result:** Rate constant: 0.95×10^9 l/mol.sec.**Source:** Unichema Chemie GmbH Emmerich**Test condition:** Sensitizer (OH radical) concentration in water: 10×10^{-17} mol/l.

(31)

Type: air**INDIRECT PHOTOLYSIS****Sensitizer:** OH**Method:** other (measured)**Year:** **GLP:** no data**Test substance:****Result:** Rate constant: 0.95×10^9 l/mol.sec.**Source:** Lever GmbH Hamburg**Test condition:** Sensitizer (OH radical) concentration in water: 10×10^{-17} mol/l.

(31)

Type: water

INDIRECT PHOTOLYSIS

Sensitizer: OH

Method: other (measured)

Year: GLP: no data

Test substance:

Result: Rate constant: $0.19 \times 10^{\exp 10}$ l/mol.sec.

Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Test condition: - OH formed by pulsed radiolysis;
- Neutral pH;
- Oxidation by H abstraction reaction.

(32)

Type:

Method:

Year: GLP:

Test substance:

Remark: no disponible

Source: UNION DERIVAN S.A. VILADECANS

Type:

Method:

Year: GLP:

Test substance:

Remark: No data.

Source: Croda Universal Ltd Goole, North Humberside

Type:

Method:

Year: GLP:

Test substance:

Source: Wolff Walsrode AG Walsrode

(33)

3.1.2 Stability in Water

Type:

Method:

Year: GLP:

Test substance:

Remark: no disponible

Source: UNION DERIVAN S.A. VILADECANS

Type:

Method:

Year: 1989 GLP: no data

Test substance: no data

Remark: No data.

Source: Croda Universal Ltd Goole, North Humberside

Type:
Method:
Year:
Test substance:
Source: Wolff Walsrode AG Walsrode

GLP:

(34)

3.1.3 Stability in Soil

Type:
Concentration:
Cation exch.
capac.
Microbial
biomass:
Method:
Year:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Radiolabel:

GLP:

Type:
Concentration:
Cation exch.
capac.
Microbial
biomass:
Method:
Year:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Radiolabel:

GLP:

Type:
Concentration:
Cation exch.
capac.
Microbial
biomass:
Method:
Year:
Test substance:
Source: Wolff Walsrode AG Walsrode

GLP:

(34)

3.2 Monitoring Data (Environment)

Type of measurement: background concentration
Medium: biota
Remark:
- Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellularly as lipids.
- It is an important intermediary in the physiology of all forms of life.
- Glycerol is widely distributed in our food, both as natural constituent and as an additive; fat or oil molecules of either animal or vegetable origin contain about 10% glycerol by weight.
- Glycerin may be formed from sugars by microbial fermentation.
Source: Simel S.p.A. Industria Chimica Cremona (35)

Type of measurement: background concentration
Medium: biota
Remark:
- Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellularly as lipids.
- It is an important intermediary in the physiology of all forms of life.
- Glycerol is widely distributed in our food, both as natural constituent and as an additive; fat or oil molecules of either animal or vegetable origin contain about 10% glycerol by weight.
- Glycerin may be formed from sugars by microbial fermentation.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (35)

Type of measurement: background concentration
Medium: biota
Remark:
- Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellularly as lipids.
- It is an important intermediary in the physiology of all forms of life.
- Glycerol is widely distributed in our food, both as natural constituent and as an additive; fat or oil molecules of either animal or vegetable origin contain about 10% glycerol by weight.
- Glycerin may be formed from sugars by microbial fermentation.
Source: Unichema Chemie GmbH Emmerich (35)

- Type of measurement:** background concentration
Medium: biota
Remark:
- Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellularly as lipids.
- It is an important intermediary in the physiology of all forms of life.
- Glycerol is widely distributed in our food, both as natural constituent and as an additive; fat or oil molecules of either animal or vegetable origin contain about 10% glycerol by weight.
- Glycerin may be formed from sugars by microbial fermentation.
Source: Lever GmbH Hamburg (35)
- Type of measurement:**
Medium:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS
- Type of measurement:**
Medium: air
Remark: A validated method for the determination of glycerol in air is not available.
Source: Simel S.p.A. Industria Chimica Cremona (10)
- Type of measurement:**
Medium: air
Remark: A validated method for the determination of glycerol in air is not available.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)
- Type of measurement:**
Medium: air
Remark: A validated method for the determination of glycerol in air is not available.
Source: Unichema Chemie GmbH Emmerich (11)
- Type of measurement:**
Medium: air
Remark: A validated method for the determination of glycerol in air is not available.
Source: Lever GmbH Hamburg (11)

Type of measurement:**Medium:****Source:** Wolff Walsrode AG Walsrode

(36)

Type of measurement: other**Medium:****Remark:** No data.**Source:** Croda Universal Ltd Goole, North Humberside**3.3.1 Transport between Environmental Compartments****Type:** other: soil permeability**Media:** water - soil**Method:** other: soil column transport**Year:****Result:**

- Permeability is inversely correlated to logP and not to solvent density of viscosity;
- Increase of permeability as bulk density of soil decreases;
- Soil movement: 0.9×10^{-9} cm/sec.

Source: Simel S.p.A. Industria Chimica Cremona**Test substance:** Soil column:

- 1000-2000 g compacted clay;
- optimum moisture content;
- 35 days experiment;
- temperature of 22°C;
- permeability rates determined at equilibrium.

(37)

Type: other: soil permeability**Media:** water - soil**Method:** other: soil column transport**Year:****Result:**

- Permeability is inversely correlated to logP and not to solvent density of viscosity;
- Increase of permeability as bulk density of soil decreases;
- Soil movement: 0.9×10^{-9} cm/sec.

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey**Test substance:** Soil column:

- 1000-2000 g compacted clay;
- optimum moisture content;
- 35 days experiment;
- temperature of 22°C;
- permeability rates determined at equilibrium.

(38)

Type: other: soil permeability
Media: water - soil
Method: other: soil column transport
Year:
Result: - Permeability is inversely correlated to logP and not to solvent density of viscosity;
- Increase of permeability as bulk density of soil decreases;
- Soil movement: 0.9×10^{-9} cm/sec.
Source: Unichema Chemie GmbH Emmerich
Test substance: Soil column:
- 1000-2000 g compacted clay;
- optimum moisture content;
- 35 days experiment;
- temperature of 22°C;
- permeability rates determined at equilibrium.

(38)

Type: other: soil permeability
Media: water - soil
Method: other: soil column transport
Year:
Result: - Permeability is inversely correlated to logP and not to solvent density of viscosity;
- Increase of permeability as bulk density of soil decreases;
- Soil movement: 0.9×10^{-9} cm/sec.
Source: Lever GmbH Hamburg
Test substance: Soil column:
- 1000-2000 g compacted clay;
- optimum moisture content;
- 35 days experiment;
- temperature of 22°C;
- permeability rates determined at equilibrium.

(38)

Type:
Media:
Method:
Year:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type:
Media:
Method:
Year:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Type:
Media:
Method:
Year:
Source: Wolff Walsrode AG Walsrode

(39)

3.3.2 Distribution

Media:

Method:

Year:

Remark: no disponible

Source: UNION DERIVAN S.A. VILADECANS

Media:

Method:

Year:

Remark: Keine Daten

Source: Wolff Walsrode AG Walsrode

3.4 Mode of Degradation in Actual Use

Remark: no disponible

Source: UNION DERIVAN S.A. VILADECANS

Remark: When used in foods or injected as part of pharmaceutical preparations Glycerol is metabolized by glycerokinase in the liver to Carbon Dioxide and water, or used in glucose or glycogen synthesis. Most of ingested material has been metabolised within 2.5 hours.

Source: Croda Universal Ltd Goole, North Humberside

(40)

Source: Wolff Walsrode AG Walsrode

(41)

3.5 Biodegradation

Type: aerobic

Inoculum: activated sludge

Concentration: 100 mg/l related to Test substance

Degradation: = 63 % after 14 day

Result: readily biodegradable

Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"

Year: 1981

GLP: no data

Test substance:

Remark: Degree of biodegradation expressed as BOD.

Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Test condition: Concentration of activated sludge: 30 mg/l.

(42)

Type: aerobic
Inoculum: other: effluent from domestic sewage treatment plant
Concentration: 2 mg/l related to Test substance
Degradation: 93 % after 30 day
Result: readily biodegradable
Method: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"

Year: **GLP:** no
Test substance: other TS: as prescribed by 1.1-1.4 (Henkel KGaA).
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(43)

Type: aerobic
Inoculum: other: effluent from biological sewage treatment plant
Degradation: 95 % after 5 day
Method: other: COD-test according to potassium dichromate method.
Annual Book of ASTM Standards No. D 1252-67; Am. Soc. Test.
Mat., Philadelphia

Year: 1974 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Parameter: COD/ThOD
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Test condition: No data on concentration of test substance.

(44)

Type: aerobic
Inoculum: other: effluent from biological sewage treatment plant
Degradation: 82 % after 5 day
Method: other: Standard dilution method according to Standard Methods
for Examination of Water and Wastewater; Am. Publ. Health
Assoc. Inc., New York, Method No. 219

Year: 1971 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Parameter: BOD/ThOD
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Test condition: No data on concentration of test substance.

(44)

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Concentration: 220 mg/l related to Test substance
Degradation: 97 % after 14 day
Method: other: activated sludge degradability test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: 94% TOC removal.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: Aeration, neutral pH, 1 day adaptation, parameter: COD removal.

(45)

Type: aerobic
Inoculum: activated sludge
Concentration: 238 mg/l related to Test substance
Degradation: = 97 % after 24 hour(s)
Result: other: biodegradable under test conditions.
Method: other: not mentioned
Year: **GLP:** no data
Test substance:
Remark: % COD removal was 50 and 92% after 2 and 4 hours respectively.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: - Activated sludge, aerated 5 l/min at 25 °C;
- Microbial population: 952 ppm;
- % removal expressed as COD.

(46)

Type: aerobic
Inoculum: activated sludge, adapted
Concentration: 200 mg/l related to Test substance
Degradation: = 98.7 %
Result: other: biodegradable under test conditions
Method: other: not mentioned
Year: **GLP:** no data
Test substance:
Remark: Rate of degradation: 85 mg COD/g.hour.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: - Medium stirred, containing mineral salts;
- 20 °C, pH 7.2;
- % removal expressed as COD.

(47)

Type: aerobic
Inoculum: Pseudomonas aeruginosa (Bacteria)
Degradation: = 100 % after 7 day
Result: other: biodegradable under test conditions
Method: other: not mentioned
Year: **GLP:** no data
Test substance:
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: - Substance concentration: 2% as sole carbon source;
- Mineral salt medium;
- Shaking at 30 °C for 7 days incubation.

(48)

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Degradation: 75.2 % after 5 day
Method: other: radiorespirometric test
Year: **GLP:** no data
Test substance: other TS: Glycerol-U-14C (uniformly labelled) from NEN Corp., Boston
Remark: Inoculum was activated sludge from laboratory unit fed continuously with photographic processing effluent.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: Parameter: % 14CO₂ recovery.
pH 7.3, dark, room temperature, sealed serum bottle.

(49)

Type: aerobic
Inoculum: other: sewage
Concentration: 6000 mg/l related to Test substance
Degradation: = 75.3 % after 10 day
Result: other: biodegradable under test conditions
Method: other: sewage die-away test
Year: **GLP:**
Test substance:
Remark: Degradation half life: $t_{1/2} = 0.89$ day.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: % degradation expressed as BOD.

(50)

Type: aerobic
Inoculum: activated sludge
Concentration: 220 mg/l related to Test substance
Degradation: = 94 - 97 % after 24 hour(s)
Result: readily biodegradable
Method:
Year: **GLP:**
Test substance:
Remark: Biodegradable.
Source: Croda Universal Ltd Goole, North Humberside

(51)

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Concentration: 100 mg/l
Degradation: ca. 63 % after 14 day
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: anaerobic
Inoculum: anaerobic microorganisms
Concentration: 500 mg/l related to Test substance
Degradation: 90 % after 8 day
Method: other: anaerobic degradation
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Inoculum was anaerobic acetate-enriched culture from digested domestic sludge.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: Acetate-grown culture was cross-fed with glycerol; 8 days required for acetate culture to start metabolizing the cross-fed compound. Parameter: total gas production/theoretical gas production.
100 mg acetate added in culture medium.

(52)

Type: anaerobic
Inoculum: anaerobic microorganisms
Concentration: 5 mmol/l related to Test substance
Degradation: 100 % after 2 day
Method: other: anaerobic degradation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Microbial culture taken from anaerobic lagoon of alcohol distillery waste waters.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: incubation in minimal medium under 100% nitrogen gas at 37 degr. C, pH 7.2, enzymatic determination of glycerol concentration.

(53)

Type:
Inoculum:
Method:
Year: **GLP:**
Test substance:
Remark: producto 100% biodegradable
Source: UNION DERIVAN S.A. VILADECANS

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: other
Concentration: 100 g/l related to Test substance
BOD5: ca. 700 mgO₂/l

C O D

Method: other
COD: ca. 1160 mg/g substance
Source: Wolff Walsrode AG Walsrode

Method: other: see remark**Year:****GLP:** no data**Remark:**

RS: BOD5: 0.65 g/g
TC: Standard dilution method using sewage as seed.
RE: Gellmann, I. Masters Thesis, Rutgers University (1950).

RS: BOD5: 0.80 g/g
TC: Standard dilution method using sewage as seed.
RE: Gellmann, I. PhD Thesis, Rutgers University (1952).

RS: BOD5: 0.617 g/g
TC: Standard dilution method using sewage as seed.
RE: Meissner, B. Wasserwirtsch.-Wassertechn. 4 (1954), 166.

RS: BOD5: 0.78 g/g
TC: Sier method using sewage (10%) as seed.
RE: Gellmann, I. Masters Thesis, Rutgers University (1950).

RS: BOD5: 0.83 g/g
TC: Warburg method using sewage (10%) as seed.
RE: Gellmann, I. Masters Thesis, Rutgers University (1950).

RS: BOD5: 0.81 g/g
TC: Standard dilution method using sp. cult as seed.
RE: Zobell, C.E., Biol. Bull., vol. 78 (1940), 388.

RS: BOD5: 0.64 g/g
TC: Standard dilution method using sewage as seed.
RE: Burford, M.G. et al. Rpt New Engl Intern. Water Poll. Con. Comm. (1953).

Source:

Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Method: other: standard dilution method**Year:****GLP:** no data**C O D****Method:** other: standard potassium dichromate method**Year:****GLP:** no data**COD:** 1160 mg/g substance**Remark:** BOD5: 1.00 g/g

Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(54)

Remark: no disponible**Source:** UNION DERIVAN S.A. VILADECANS

Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: Glycerin is not bioaccumulable as its log Pow = -2.6 (see section 2.5).
Source: Simel S.p.A. Industria Chimica Cremona

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: Glycerin is not bioaccumulable as its log Pow = -2.6 (see section 2.5).
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

Species:**Exposure period:****Concentration:****BCF:****Elimination:****Method:****Year:****GLP:****Test substance:****Remark:** Glycerin is not bioaccumulable as its log Pow = -2.6 (see section 2.5).**Source:** Unichema Chemie GmbH Emmerich**Species:****Exposure period:****Concentration:****BCF:****Elimination:****Method:****Year:****GLP:****Test substance:****Remark:** Glycerin is not bioaccumulable as its log Pow = -2.6 (see section 2.5).**Source:** Lever GmbH Hamburg**Species:****Exposure period:****Concentration:****BCF:****Elimination:****Method:****Year:****GLP:****Test substance:****Remark:** Bioakkumulation nicht gegeben wegen log Pow -1.8 - -2.6**Source:** Wolff Walsrode AG Walsrode

3.8 Additional Remarks

-

AQUATIC ORGANISMS**4.1 Acute/Prolonged Toxicity to Fish**

Type: static
Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: > 5000
Method: other: Standard Methods for Examination of Water and Wastewater. Am. Publ. Health Assoc. Inc., New York, Method No. 231
Year: 1971 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: 20+/-1 degr. C, pH 7.8, aeration, concentration of test substance measured at beginning and end of test by TOC analysis or GC (not stated which method). Dissolved oxygen >4 mg/l.

(55)

Type: static
Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: > 5000
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: > 250
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: **GLP:** no
Test substance: other TS: as prescribed by 1.1-1.4 (Henkel KGaA).
Remark: 250 mg/l was highest concentration tested.
Test method conforms with OECD Guideline 203.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(43)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 10000
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1976 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Ring test in two laboratories: data from Juhnke
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(56)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 10000
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1976 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Ring test in two laboratories: data from Luedemann
Source: Simel S.p.A. Industria Chimica Cremona

(56)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 10000
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1976 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Ring test in two laboratories: data from Luedemann
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(56)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 10000
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1976 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Ring test in two laboratories: data from Luedemann
Source: Unichema Chemie GmbH Emmerich

(56)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 10000
Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1976 **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Ring test in two laboratories: data from Luedemann
Source: Lever GmbH Hamburg

(56)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC100: = 51000 - 57000
Method: other: not mentioned
Year: **GLP:** no data
Test substance:
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: - Rainbow trout of 0.9 g;
- 12 °C, hardness 40-50 mg/l CaCO₃, alkalinity 30-35 mg/l CaCO₃, pH 7.2-7.5;
- Reconstituted dilution water;
- Unmeasured concentration.

(57)

Type:
Species: Idus idus (Fish, fresh water)
Exposure period:
Unit: **Analytical monitoring:**
LC50: > 10000
Method:
Year: 1978 **GLP:** no data
Test substance: no data
Remark: Golden Orfe.
Units are mg/dm³.
Source: Croda Universal Ltd Goole, North Humberside

(58)

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: > 500
Method: other: Daphnien-Kurzzeitest, DIN 38412 Teil 11, Bestimmung der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse
Year: **GLP:** no
Test substance: other TS: as prescribed by 1.1-1.4 (Henkel KGaA).
Remark: 500 mg/l was highest concentration tested.
Test method conforms with OECD Guideline 202 A.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(43)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 10000
Method: other: static immobilisation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: Tested Daphnia strain: D. magna collected from small pond, 20 - 22 degr. C, pH before addition of test substance: 7.6 - 7.7, no adjustment of pH after addition of test substance.

(59)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 10000
Method: other: static immobilisation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Simel S.p.A. Industria Chimica Cremona
Test condition: Tested Daphnia strain: D. magna Straus IRCHA, 20 degr. C, pH before addition of test substance: 8.0, no adjustment of pH after addition of test substance. Hardness: 70 mg/l CaCO3.

(60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 10000
Method: other: static immobilisation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test condition: Tested Daphnia strain: D. magna Straus IRCHA, 20 degr. C, pH before addition of test substance: 8.0, no adjustment of pH after addition of test substance. Hardness: 70 mg/l CaCO3.
(60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 10000
Method: other: static immobilisation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Unichema Chemie GmbH Emmerich
Test condition: Tested Daphnia strain: D. magna Straus IRCHA, 20 degr. C, pH before addition of test substance: 8.0, no adjustment of pH after addition of test substance. Hardness: 70 mg/l CaCO3.
(60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 10000
Method: other: static immobilisation test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Source: Lever GmbH Hamburg
Test condition: Tested Daphnia strain: D. magna Straus IRCHA, 20 degr. C, pH before addition of test substance: 8.0, no adjustment of pH after addition of test substance. Hardness: 70 mg/l CaCO3.
(60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 500
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Species: other: Chilomonas paramecium (saprozoic flagellate)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TGK 5% : > 10000
Method: other: cell multiplication inhibition test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which growth of culture is reduced by 5% compared to untreated control.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: static test at 20 degr. C; pH 6.9; glycine as sole C- and N-source; parameter: growth measured with Coulter counter.
(61) (62)

Species: other: Entosiphon sulcatum (bacteriovorous flagellate)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TGK 5% : 3200
Method: other: cell multiplication inhibition test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which growth of culture is reduced by 5% compared to untreated control.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: static test at 25 degr. C; parameter: growth measured with Coulter counter.
(61) (63) (64)

Species: other: Uronema parduczi (bacteriovorous flagellate)
Exposure period: 20 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TGK 5% : > 10000
Method: other: cell multiplication inhibition test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which growth of culture is reduced by 5% compared to untreated control.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: static test at 25 degr. C; pH 6.9; parameter: growth measured with Coulter counter.
(61) (65)

Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: growth rate
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
TGK 3% : 2900
Method: other: cell multiplication inhibition test **GLP:** no data
Year:
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which optical density of culture is >1% below control value.
Source: Simel S.p.A. Industria Chimica Cremona
 Lever Brother Ltd. Kingston Upon Thames, Surrey
 Unichema Chemie GmbH Emmerich
 Lever GmbH Hamburg
Test condition: Static test; 27 degr. C; cell growth determined photometrically (OD 578nm). pH 7.

(66) (67) (68) (69)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: growth rate
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:**
TGK 3% : = 2900
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
TGK 3% : > 10000
Method: other: cell multiplication inhibition test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which optical density of culture is >3% below control value.
Source: Simel S.p.A. Industria Chimica Cremona
 Lever Brother Ltd. Kingston Upon Thames, Surrey
 Unichema Chemie GmbH Emmerich
 Lever GmbH Hamburg
Test condition: static test, 25 degr. C, parameter: Growth (optical density at 436 nm). pH 7.
 (66) (68) (63) (70)

Species: other aquatic plant
Endpoint: growth rate
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
EC : = 46000
Method: other: not mentioned
Year: **GLP:** no data
Test substance:
Remark: RESULTS:

Alga species	Effect endpoint
Braciomonas submarina	50% decrease in growth rate
Dunaliella tertiolecta	10% increase in growth rate
Phaeodactylum tricornutum	578% increase in growth rate
Skeletonema costatum	increased growth rate
Anacystis marina	190% increase in growth rate
Agmenellum quadruplicatum	124% increase in growth rate

Source: Other species were tested and gave similar results.
 Simel S.p.A. Industria Chimica Cremona
 Lever Brother Ltd. Kingston Upon Thames, Surrey
 Unichema Chemie GmbH Emmerich
 Lever GmbH Hamburg
Test condition:

- Age/life stage: 10exp6 cells/ml;
- Static test;
- 20 °C, pH 7.6-7.8, salinity 33 ppt;
- 2700-3200 Lux, 24 hour photoperiod;
- Unmeasured concentration.

(71)

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: > 10000
Method: other: DIN 38412, Teil 8
Year: 1991 **GLP:** yes
Test substance: other TS: as prescribed by 1.1-1.4 (Henkel KGaA).
Remark: 10000 mg/l was highest concentration tested.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(72)

Type: aquatic
Species: *Pseudomonas putida* (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TGK 3% : > 10000
Method: other: cell multiplication inhibition test
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: TGK = toxische Grenzkonzentration (toxic threshold concentration); concentration at which optical density of culture is >3% below control value.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: static test, 25 degr. C, minimal medium with glucose as sole carbon source, parameter: growth (optical density at 436 nm).
(67) (63) (70)

Type: aquatic
Species: *Pseudomonas putida* (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:**
EC0: > 10000
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: aquatic
Species: other bacteria: *Clostridium pasteurianum*
Exposure period:
Unit: **Analytical monitoring:**
Method: other: growth inhibition test
Year: **GLP:**
Test substance: other TS: glycerol. No indication about purity.
Remark: *C. pasteurianum* can tolerate high concentrations of glycerol: 17% (w/v) = 170 g/l was tolerated without impairment of growth.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test condition: Growth on minimal medium with glycerol as sole carbon source, anaerobic atmosphere, parameter: optical density at 578 nm.
(73)

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

TERRESTRIAL ORGANISMS**4.6.1 Toxicity to Soil Dwelling Organisms**

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: no dispònible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

4.7 Biological Effects Monitoring

Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Remark: A validated method for biological monitoring of glycerol is not available.
Source: Simel S.p.A. Industria Chimica Cremona (10)

Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Remark: A validated method for biological monitoring of glycerol is not available.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (11)

Remark: A validated method for biological monitoring of glycerol is not available.
Source: Unichema Chemie GmbH Emmerich (11)

Remark: A validated method for biological monitoring of glycerol is not available.
Source: Lever GmbH Hamburg (11)

Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

4.8 Biotransformation and Kinetics

Type:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type:
Remark: Glycerol is readily absorbed into the gastrointestinal tract, metabolised by standard pathways in mammals and its products used to produce glucose, glycogen and fats.
Source: Croda Universal Ltd Goole, North Humberside (74) (75)

Type:
Source: Wolff Walsrode AG Walsrode (76)

4.9 Additional Remarks

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5.1 Acute Toxicity**5.1.1 Acute Oral Toxicity**

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method: other
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 12600 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona

(77)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: 27200 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 27.2 g/kg
LD50 (synthetic glycerin): 27.2 g/kg
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%.

(78)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 12600 mg/kg bw
Method: other
Year: 1987 GLP: no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(79)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 12600 mg/kg bw
Method: other
Year: GLP: no data
Test substance: no data
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(80)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 12600 mg/kg bw
Method: other
Year: GLP: no data
Test substance: no data
Source: Unichema Chemie GmbH Emmerich

(80)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 12600 mg/kg bw
Method: other
Year: GLP: no data
Test substance: no data
Source: Lever GmbH Hamburg

(80)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: ca. 12600 - 27200 mg/kg bw
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 4090 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(81)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: ca. 26000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance:
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 20.65 cc/kg
LD50 (synthetic glycerin): 20.81 cc/kg
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(82)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 38000 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Glycerol, DAB 7 purity.

(83)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: ca. 23000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 23.0 g/kg
LD50 (synthetic glycerin): 23.0 g/kg
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%.

(78)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 4250 mg/kg bw
Method:
Year: 1977 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(84)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: ca. 26000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance:
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 20.65 cc/kg
LD50 (synthetic glycerin): 20.81 cc/kg
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(85)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 38000 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Glycerol, DAB 7 purity.

(86)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: ca. 26000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance:
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 20.65 cc/kg
LD50 (synthetic glycerin): 20.81 cc/kg
Source: Unichema Chemie GmbH Emmerich
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(85)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 38000 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Unichema Chemie GmbH Emmerich
Test substance: Glycerol, DAB 7 purity.

(86)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: ca. 26000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance:
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 20.65 cc/kg
LD50 (synthetic glycerin): 20.81 cc/kg
Source: Lever GmbH Hamburg
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(85)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 38000 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Lever GmbH Hamburg
Test substance: Glycerol, DAB 7 purity.

(86)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: = 27000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(87)

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: = 27000 mg/kg bw
Method:
Year: 1959 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(88)

Type: LD50
Species: guinea pig
Sex:
Number of Animals:
Vehicle:
Value: = 7750 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(89)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: guinea pig
Sex:
Number of Animals:
Vehicle:
Value: 10000 - 11500 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 10.0 g/kg
LD50 (synthetic glycerin): 11.5 g/kg
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%.

(78)

Type: LD50
Species: guinea pig
Sex:
Number of Animals:
Vehicle:
Value: = 7750 mg/kg bw
Method:
Year: 1941 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(90)

Type: LDLo
Species: human
Sex:
Number of Animals:
Vehicle:
Value: = 1428 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(91)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: Keine Daten
Source: Wolff Walsrode AG Walsrode

5.1.3 Acute Dermal Toxicity

Type: other
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 21900 mg/kg bw
Method: other
Year: 1960 **GLP:** no data
Test substance: no data
Remark: Skin contact with about 2.52g of the neat liquid (21900 mg/kg bw) for more than 20 minutes produced excretion of haemoglobin in the urine of male rats, indicating red blood cell damage. (No deaths).
Source: Croda Universal Ltd Goole, North Humberside (92)

Type: other
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 21900 mg/kg bw
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: LD0
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: > 18700 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%. (93)

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: 7500 - 10100 mg/kg bw
Method:
Year: 1976 GLP: no data
Test substance: as prescribed by 1.1 - 1.4
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol, DAB 7 purity

(94)

Type: LD50
Species: rat
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: GLP: no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona

(95)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: 1984 GLP: no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(96)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: GLP: no data
Test substance: no data
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(97)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: GLP: no data
Test substance: no data
Source: Unichema Chemie GmbH Emmerich

(97)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: GLP: no data
Test substance: no data
Source: Lever GmbH Hamburg

(97)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rat
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: = 4420 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: LD50
Species: mouse
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: 8600 - 9500 mg/kg bw
Method:
Year: 1976 GLP: no data
Test substance: as prescribed by 1.1 - 1.4
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol, DAB 7 purity

(94)

Type: LD50
Species: mouse
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: = 8700 mg/kg bw
Method:
Year: 1978 GLP: no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(98)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: = 100 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(99)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: = 100 mg/kg bw
Method:
Year: **GLP:**
Test substance:
Source: Croda Universal Ltd Goole, North Humberside

(100)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: = 91 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(99)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: = 91 mg/kg bw
Method:
Year: 1982 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(101)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 5200 - 6600 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol, DAB 7 purity

(94)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: = 5566 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(102)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 5700 - 6700 mg/kg bw
Method:
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol, DAB 7 purity

(94)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 4250 - 4370 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 4.37 g/kg
LD50 (synthetic glycerin): 4.25 g/kg
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(82)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: = 4250 mg/kg bw
Method:
Year: 1950 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(103)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 4250 - 4370 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 4.37 g/kg
LD50 (synthetic glycerin): 4.25 g/kg
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(85)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 4250 - 4370 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 4.37 g/kg
LD50 (synthetic glycerin): 4.25 g/kg
Source: Unichema Chemie GmbH Emmerich
Test substance: Synthetic glycerol: Purity 99.8%
Natural glycerol: no data

(85)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: 4250 - 4370 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
LD50 (natural glycerin) : 4.37 g/kg
LD50 (synthetic glycerin): 4.25 g/kg
Source: Lever GmbH Hamburg
Test substance: Synthetic glycerol: Purity 99.8%

Natural glycerol: no data

(85)

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: = 53000 mg/kg bw
Method:
Year: **GLP:** no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(99)

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.v.
Value: = 53000 mg/kg bw
Method:
Year: 1982 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(101)

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Route of admin.:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.2 Corrosiveness and Irritation**5.2.1 Skin Irritation****Species:** rabbit**Concentration:****Exposure:****Exposure Time:****Number of
Animals:****PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:****Year:** 1979**GLP:** no data**Test substance:** other TS**Remark:** rabbit strain: New Zealand; sex: female**Source:** Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg**Test substance:** Glycerol/Water mixture of unknown composition. Source:
Solargard G from Daystar Corp. (Heat Transfer Fluid).

(104)

Species: rabbit**Concentration:****Exposure:****Exposure Time:****Number of
Animals:****PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:****Year:** 1971**GLP:** no data**Test substance:** other TS**Remark:** rabbit strain: albino; sex: male**Source:** Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg**Test substance:** Glycerol of unspecified purity was used.

(105)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5**Species:** rabbit
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:** Draize Test**Year:** 1944**GLP:** no data**Test substance:** other TS**Remark:** Comparative acute toxicity of synthetic and natural glycerin.

No signs of local irritation after 90 days of application.

Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg**Test substance:** Natural and synthetic glycerin, purity 99.5%.

(93)

Species: rabbit
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** slightly irritating**EC classificat.:** not irritating**Method:** other**Year:** 1986**GLP:** no data**Test substance:** no data**Source:** Croda Universal Ltd Goole, North Humberside

(106)

Species: rabbit
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:** Draize Test**Year:****GLP:****Test substance:****Source:** Wolff Walsrode AG Walsrode

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: not irritating
EC classificat.: not irritating
Method: other

Year: **GLP:** no data

Test substance: no data

Remark: In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.

Source: Simel S.p.A. Industria Chimica Cremona

(107)

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: slightly irritating
EC classificat.:
Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Slightly irritating after 48 hours application of 0.05 ml on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin.

Source: Simel S.p.A. Industria Chimica Cremona

(108)

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: not irritating
EC classificat.: not irritating
Method: other

Year: 1979 **GLP:** no data

Test substance: no data

Remark: In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.

Source: Croda Universal Ltd Goole, North Humberside

(109) (110)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5**Species:** human
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:** other**Year:****GLP:** no data**Test substance:** no data**Remark:** In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.**Source:** Lever Brother Ltd. Kingston Upon Thames, Surrey

(111)

Species: human
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** slightly irritating**EC classificat.:****Method:** other**Year:****GLP:** no data**Test substance:** other TS**Remark:** Slightly irritating after 48 hours application of 0.05 ml on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin.**Source:** Lever Brother Ltd. Kingston Upon Thames, Surrey

(108)

Species: human
Concentration:**Exposure:**
Exposure Time:
Number of
Animals:**PDII:****Result:** not irritating**EC classificat.:** not irritating**Method:** other**Year:****GLP:** no data**Test substance:** no data**Remark:** In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.**Source:** Unichema Chemie GmbH Emmerich

(111)

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: slightly irritating

EC classificat.:

Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Slightly irritating after 48 hours application of 0.05 ml on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin.

Source: Unichema Chemie GmbH Emmerich

(108)

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: not irritating

EC classificat.: not irritating

Method: other

Year: **GLP:** no data

Test substance: no data

Remark: In "several thousand" dermatitis patients, 20 to 24-hr covered skin contact with a 50% solution was non-irritating.

Source: Lever GmbH Hamburg

(111)

Species: human
Concentration:

Exposure:
Exposure Time:
Number of
Animals:

PDII:
Result: slightly irritating

EC classificat.:

Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Slightly irritating after 48 hours application of 0.05 ml on human skin in a closed patch test. Further the investigators observed a maximum score for irritation of 4 on a scale of 9 at day 14 during a 21 day application of a 10% solution on human skin.

Source: Lever GmbH Hamburg

(108)

Species:

Concentration:

Exposure:

Exposure Time:

**Number of
Animals:**

PDII:

Result:

EC classificat.:

Method:

Year:

GLP:

Test substance:

Remark: no disponible

Source: UNION DERIVAN S.A. VILADECANS

5.2.2 Eye Irritation

Species: rabbit

Concentration:

Dose:

Exposure Time:

Comment:

**Number of
Animals:**

Result: not irritating

EC classificat.: not irritating

Method:

Year: 1979

GLP: no data

Test substance: other TS

Remark: rabbit strain: New Zealand; sex: female

Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

Test substance: Glycerol/Water mixture of unknown composition. Source:
Solargard G from Daystar Corp. (Heat Transfer Fluid).

(104)

Species: rabbit

Concentration:

Dose:

Exposure Time:

Comment:

**Number of
Animals:**

Result: not irritating

EC classificat.: not irritating

Method:

Year: 1971

GLP: no data

Test substance: other TS

Remark: rabbit strain: albino; sex: male

Source: Simel S.p.A. Industria Chimica Cremona

Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol of unspecified purity was used. (112)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: **GLP:** yes
Test substance: no data
Remark: Rabbit strain: New Zealand White; sex: male.
The conjunctiva was slightly to moderately irritated in all rabbits one hour after treatment. Thereafter the irritation diminished and had disappeared at 48 hours after treatment.
Source: Simel S.p.A. Industria Chimica Cremona (113)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classificat.: not irritating
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%. (93)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: other
Year: 1986 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(106)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: other
Year: 1986 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(114)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: **GLP:** yes
Test substance: no data
Remark: Rabbit strain: New Zealand White; sex: male.
The conjunctiva was slightly to moderately irritated in all rabbits one hour after treatment. Thereafter the irritation diminished and had disappeared at 48 hours after treatment.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(113)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: **GLP:** yes
Test substance: no data
Remark: Rabbit strain: New Zealand White; sex: male.
The conjunctiva was slightly to moderately irritated in all rabbits one hour after treatment. Thereafter the irritation diminished and had disappeared at 48 hours after treatment.
Source: Unichema Chemie GmbH Emmerich (113)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: **GLP:** yes
Test substance: no data
Remark: Rabbit strain: New Zealand White; sex: male.
The conjunctiva was slightly to moderately irritated in all rabbits one hour after treatment. Thereafter the irritation diminished and had disappeared at 48 hours after treatment.
Source: Lever GmbH Hamburg (113)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Species: human
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classificat.: not irritating
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A strong burning and stinging sensation, with tear production but no injury apparently from contact with the neat chemical.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(115)

Species:
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.3 Sensitization

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Because the purity of the glycerol tested was not given, it is not excluded that the observed effects could have been caused by an impurity or a contamination of the sample (e.g. propylene glycol or butanetriol). With respect to the very high number of individuals tested and the concentration of the test compound (50%) one would expect to see more incidences, if glycerol had a relevant allergenic potential (Lit. Henkel, 1992).

Out of "several thousand" dermatitis patients who were tested, only two showed reactions in 20 to 24-hr covered patch tests with 50% glycerol.

Source: Simel S.p.A. Industria Chimica Cremona (116)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: 1971 **GLP:** no
Test substance: other TS: glycerol. No indication about purity.
Remark: Medicolegal aspects of occupational dermatitis survey in a foam rubber factory.
Skin patch tests for 48 hours.

Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Glycerol in water. (117)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification: not sensitizing
Method: other
Year: 1979 **GLP:** no data
Test substance: no data
Remark: Out of "several thousand" dermatitis patients tested, two showed skin reactions in 20 to 24-hr covered patch tests with 50% glycerol and were thus diagnosed as glycerol sensitized. One also reacted with 1% glycerol. Both had regularly used a skin cream containing 10% glycerol.

Source: Croda Universal Ltd Goole, North Humberside (109) (110)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Because the purity of the glycerol tested was not given, it is not excluded that the observed effects could have been caused by an impurity or a contamination of the sample (e.g. propylene glycol or butanetriol). With respect to the very high number of individuals tested and the concentration of the test compound (50%) one would expect to see more

incidences, if glycerol had a relevant allergenic potential (Lit. Henkel, 1992).
Out of "several thousand" dermatitis patients who were tested, only two showed reactions in 20 to 24-hr covered patch tests with 50% glycerol.

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (116)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: 1971 **GLP:** no
Test substance: other TS: glycerol. No indication about purity.
Remark: Medicolegal aspects of occupational dermatitis survey in a foam rubber factory.
Skin patch tests for 48 hours.

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Glycerol in water. (117)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Because the purity of the glycerol tested was not given, it is not excluded that the observed effects could have been caused by an impurity or a contamination of the sample (e.g. propylene glycol or butanetriol). With respect to the very high number of individuals tested and the concentration of the test compound (50%) one would expect to see more incidences, if glycerol had a relevant allergenic potential (Lit. Henkel, 1992).
Out of "several thousand" dermatitis patients who were tested, only two showed reactions in 20 to 24-hr covered patch tests with 50% glycerol.

Source: Unichema Chemie GmbH Emmerich (116)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: 1971 **GLP:** no
Test substance: other TS: glycerol. No indication about purity.
Remark: Medicolegal aspects of occupational dermatitis survey in a foam rubber factory.
Skin patch tests for 48 hours.
Source: Unichema Chemie GmbH Emmerich
Test substance: Glycerol in water. (117)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS: glycerol. No indication about purity.
Remark: Because the purity of the glycerol tested was not given, it is not excluded that the observed effects could have been caused by an impurity or a contamination of the sample (e.g. propylene glycol or butanetriol). With respect to the very high number of individuals tested and the concentration of the test compound (50%) one would expect to see more incidences, if glycerol had a relevant allergenic potential (Lit. Henkel, 1992).
Out of "several thousand" dermatitis patients who were tested, only two showed reactions in 20 to 24-hr covered patch tests with 50% glycerol.
Source: Lever GmbH Hamburg (116)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: 1971 **GLP:** no
Test substance: other TS: glycerol. No indication about purity.
Remark: Medicolegal aspects of occupational dermatitis survey in a foam rubber factory.
Skin patch tests for 48 hours.
Source: Lever GmbH Hamburg
Test substance: Glycerol in water. (117)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: not sensitizing
Classification: not sensitizing
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: other
Species: guinea pig
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Following 10 injections of 0.1 ml of a 0.1% solution of natural or synthetic glycerin, the investigators noted that a further injection of 0.05 ml of the solutions gave no indications of sensitization to glycerin in any of the 24 adult white male guinea pigs.
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Natural and synthetic glycerin, purity 99.5%.

(93)

Type: other
Species: guinea pig
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Following 10 injections of 0.1 ml of a 0.1% solution of natural or synthetic glycerin, the investigators noted that a further injection of 0.05 ml of the solutions gave no indications of sensitization to glycerin in any of the 24 adult white male guinea pigs.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Natural and synthetic glycerin, purity 99.5%.

(93)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: other
Species: guinea pig
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Following 10 injections of 0.1 ml of a 0.1% solution of natural or synthetic glycerin, the investigators noted that a further injection of 0.05 ml of the solutions gave no indications of sensitization to glycerin in any of the 24 adult white male guinea pigs.
Source: Unichema Chemie GmbH Emmerich
Test substance: Natural and synthetic glycerin, purity 99.5%. (93)

Type: other
Species: guinea pig
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Following 10 injections of 0.1 ml of a 0.1% solution of natural or synthetic glycerin, the investigators noted that a further injection of 0.05 ml of the solutions gave no indications of sensitization to glycerin in any of the 24 adult white male guinea pigs.
Source: Lever GmbH Hamburg
Test substance: Natural and synthetic glycerin, purity 99.5%. (93)

Type:
Species:
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.4 Repeated Dose Toxicity

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 h/d, 5d/week
Post. obs. period: no
Doses: 0.033, 0.167, 0.662 mg/l of air
Control Group: yes
NOAEL: = .167 mg/l
Method: Directive 87/302/EEC, part B, p. 20 "Sub-chronic inhalation toxicity study: 90-day repeated dose study using rodent species"
Year: 1992 **GLP:** no data
Test substance: other TS
Remark: Nose-only study. Effect: minimal to mild squamous metaplasia of the epithelium lining the base of the epiglottis.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: USP, purity >99.8%

(118)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 2 weeks
Frequency of treatment: 6h/d, 5d/week
Post. obs. period: no
Doses: 1; 2; and 4 mg/l of air
Control Group: yes
LOAEL: 1 - 4 mg/l
Method:
Year: 1992 **GLP:** yes
Test substance: other TS
Remark: Nose only study. Result: only minimal to mild squamous metaplasia of the epithelium of the epiglottis.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: USP, purity >99.8%

(118)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:**
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 Wochen
Frequency of treatment: 6 h/d, 5d/week
Post. obs. period:
Doses: 0.03, 0.167, 0.662 mg/l of air
Control Group:
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Species: rat **Sex:** male
Strain: other: Carworth
Route of admin.: oral feed
Exposure period: 4 weeks
Frequency of treatment:
Post. obs. period:
Doses: diets containing 20% glycerin
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: no data
Remark: At the end of 4 weeks, the rats were killed and both epididymus fat pads were quantitatively excised, dried, and weighed. Liver total lipids and cholesterol were determined.
Result: Mentioned criteria, as well as weight gain, food intake, and calorie intake, revealed no adverse effects attributable to glycerin consumption.
Source: Simel S.p.A. Industria Chimica Cremona

(119)

Species: rat **Sex:** male/female
Strain: Long-Evans
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Result: It was concluded that neither synthetic nor natural glycerine, when fed at levels up to 20% of the diet, cause any untoward effects in rats.
Natural and synthetic glycerin, purity 99.5%.
Source: Simel S.p.A. Industria Chimica Cremona

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

(93)

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10, or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: No deleterious or toxicological effects were noted among the groups of rats fed for two years on diets containing 5, 10 or 20% of either Atlas synthetic glycerin or natural glycerin. or among either of these experimental groups and the controls.
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: ATLAS synthetic glycerin and natural glycerin.

(120)

Species: rat **Sex:** male
Strain: other: Carworth
Route of admin.: oral feed
Exposure period: 4 weeks
Frequency of treatment:
Post. obs. period:
Doses: diets containing 20% glycerin
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: no data
Remark: At the end of 4 weeks, the rats were killed and both epididymus fat pads were quantitatively excised, dried, and weighed. Liver total lipids and cholesterol were determined.
Result: Mentioned criteria, as well as weight gain, food intake, and calorie intake, revealed no adverse effects attributable to glycerin consumption.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(121)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain: Long-Evans
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Result: It was concluded that neither synthetic nor natural glycerine, when fed at levels up to 20% of the diet, cause any untoward effects in rats.
Natural and synthetic glycerin, purity 99.5%.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(93)

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10, or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: No deleterious or toxicological effects were noted among the groups of rats fed for two years on diets containing 5, 10 or 20% of either Atlas synthetic glycerin or natural glycerin. or among either of these experimental groups and the controls.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: ATLAS synthetic glycerin and natural glycerin.

(120)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male
Strain: other: Carworth
Route of admin.: oral feed
Exposure period: 4 weeks
Frequency of treatment:
Post. obs. period:
Doses: diets containing 20% glycerin
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: no data
Remark: At the end of 4 weeks, the rats were killed and both epididymus fat pads were quantitatively excised, dried, and weighed. Liver total lipids and cholesterol were determined.
Result: Mentioned criteria, as well as weight gain, food intake, and calorie intake, revealed no adverse effects attributable to glycerin consumption.
Source: Unichema Chemie GmbH Emmerich (121)

Species: rat **Sex:** male/female
Strain: Long-Evans
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Result: It was concluded that neither synthetic nor natural glycerine, when fed at levels up to 20% of the diet, cause any untoward effects in rats.
Natural and synthetic glycerin, purity 99.5%.
Source: Unichema Chemie GmbH Emmerich (93)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10, or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: No deleterious or toxicological effects were noted among the groups of rats fed for two years on diets containing 5, 10 or 20% of either Atlas synthetic glycerin or natural glycerin. or among either of these experimental groups and the controls.
Source: Unichema Chemie GmbH Emmerich
Test substance: ATLAS synthetic glycerin and natural glycerin. (120)

Species: rat **Sex:** male
Strain: other: Carworth
Route of admin.: oral feed
Exposure period: 4 weeks
Frequency of treatment:
Post. obs. period:
Doses: diets containing 20% glycerin
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: no data
Remark: At the end of 4 weeks, the rats were killed and both epididymus fat pads were quantitatively excised, dried, and weighed. Liver total lipids and cholesterol were determined.
Result: Mentioned criteria, as well as weight gain, food intake, and calorie intake, revealed no adverse effects attributable to glycerin consumption.
Source: Lever GmbH Hamburg (121)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain: Long-Evans
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Comparative acute toxicity of synthetic and natural glycerin.
Result: It was concluded that neither synthetic nor natural glycerine, when fed at levels up to 20% of the diet, cause any untoward effects in rats.
Natural and synthetic glycerin, purity 99.5%.
Source: Lever GmbH Hamburg

(93)

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10, or 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: No deleterious or toxicological effects were noted among the groups of rats fed for two years on diets containing 5, 10 or 20% of either Atlas synthetic glycerin or natural glycerin. or among either of these experimental groups and the controls.
Source: Lever GmbH Hamburg
Test substance: ATLAS synthetic glycerin and natural glycerin.

(120)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 3 months
Frequency of treatment:
Post. obs. period:
Doses: 1, 2, 5, 10 or 20% solution of glycerin.
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Two of the 12 rats receiving 20% glycerin died during the 6th week of the test; the others showed, at this point, disturbance of the development and growth but recovered and grew normally in the further course of the experiment such that at the end of the test period no indication of injury attributable to glycerin ingestion was perceivable.
Source: Simel S.p.A. Industria Chimica Cremona (122)

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 96 days
Frequency of treatment:
Post. obs. period:
Doses: 0, 1, 2, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth of the rats receiving 10% or less of glycerin was normal. The animals receiving 20% glycerin exhibited normal growth for the first few weeks, followed by a temporary impairment. Growth of these rats then returned to normal.
Source: Simel S.p.A. Industria Chimica Cremona (123)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 3 months
Frequency of treatment:
Post. obs. period:
Doses: 1, 2, 5, 10 or 20% solution of glycerin.
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Two of the 12 rats receiving 20% glycerin died during the 6th week of the test; the others showed, at this point, disturbance of the deveoplment and growth but recovered and grew normally in the further course of the experiment such that at the end of the test period no indication of injury attributable to glycerin ingestion was perceivable.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (122)

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 96 days
Frequency of treatment:
Post. obs. period:
Doses: 0, 1, 2, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth of the rats receiving 10% or less of glycerin was normal. The animals receiving 20% glycerin exhibited normal growth for the first few weeks, followed by a temporary impairment. Growth of these rats then returned to normal.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (123)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 3 months
Frequency of treatment:
Post. obs. period:
Doses: 1, 2, 5, 10 or 20% solution of glycerin.
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Two of the 12 rats receiving 20% glycerin died during the 6th week of the test; the others showed, at this point, disturbance of the development and growth but recovered and grew normally in the further course of the experiment such that at the end of the test period no indication of injury attributable to glycerin ingestion was perceivable.
Source: Unichema Chemie GmbH Emmerich (122)

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 96 days
Frequency of treatment:
Post. obs. period:
Doses: 0, 1, 2, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth of the rats receiving 10% or less of glycerin was normal. The animals receiving 20% glycerin exhibited normal growth for the first few weeks, followed by a temporary impairment. Growth of these rats then returned to normal.
Source: Unichema Chemie GmbH Emmerich (123)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 3 months
Frequency of treatment:
Post. obs. period:
Doses: 1, 2, 5, 10 or 20% solution of glycerin.
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Two of the 12 rats receiving 20% glycerin died during the 6th week of the test; the others showed, at this point, disturbance of the deveoplment and growth but recovered and grew normally in the further course of the experiment such that at the end of the test period no indication of injury attributable to glycerin ingestion was perceivable.
Source: Lever GmbH Hamburg (122)

Species: rat **Sex:** male/female
Strain:
Route of admin.: drinking water
Exposure period: 96 days
Frequency of treatment:
Post. obs. period:
Doses: 0, 1, 2, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth of the rats receiving 10% or less of glycerin was normal. The animals receiving 20% glycerin exhibited normal growth for the first few weeks, followed by a temporary impairment. Growth of these rats then returned to normal.
Source: Lever GmbH Hamburg (123)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** female
Strain: other: Charles River
Route of admin.: other: stomach tube
Exposure period: 3 d
Frequency of treatment: 3/d
Post. obs. period: no
Doses: 0.75;1,5; and 3.0 mg undiluted Glycerol/kg
Control Group: yes
LOAEL: <= .75 mg/kg
Method:
Year: 1967 **GLP:** no data
Test substance: no data
Remark: The irritating effects of undiluted vs. diluted Glycerol on the gastrointestinal mucosa of rats were investigated.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(124)

Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach tube
Exposure period: 44 days
Frequency of treatment: daily
Post. obs. period:
Doses: 1 ml of a 0, 1, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth curves, lethality, and histological examination of the kidneys, livers, and bladders of the rats disclosed no untoward effects.
Source: Simel S.p.A. Industria Chimica Cremona

(125)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: other: stomach tube
Exposure period: approx. 1/2 year
Frequency of treatment:
Post. obs. period:
Doses: 1.0 ml of a 50% aqueous glycerine solution/100 g bw
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: All of the rats developed normally and the mortality of all groups were comparable. After 6, 12 and 18 weeks, rats from each group were sacrificed, dissected and their organs removed for histological examination. No pathological changes were disclosed and determination of liver glycogen revealed normal levels. X-ray examinations carried out on animals of each group at the beginning as well as in intervals of 6 weeks, remained without abnormal findings.
Source: Simel S.p.A. Industria Chimica Cremona (126)

Species: rat **Sex:** no data
Strain: no data
Route of admin.: oral unspecified
Exposure period: 2 Years
Frequency of treatment: with each meal
Post. obs. period: no data
Doses: 5000 mg/kg bw/d
Control Group: no data specified
NOAEL: = 5000 mg/kg bw
Method: other
Year: 1933 **GLP:** no data
Test substance: no data
Remark: No overt signs of toxicity or tissue damage when animals received 10% in the diet for 2 Years.
Source: Croda Universal Ltd Goole, North Humberside (127)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** no data
Strain: no data
Route of admin.: oral unspecified
Exposure period: 25 Weeks
Frequency of treatment: with each meal
Post. obs. period: no data
Doses: Approximately 20000 mg/kg bw/d
Control Group: no data specified
NOAEL: ca. 2000 mg/kg bw
Method: other
Year: 1933 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach tube
Exposure period: 44 days
Frequency of treatment: daily
Post. obs. period:
Doses: 1 ml of a 0, 1, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth curves, lethality, and histological examination of the kidneys, livers, and bladders of the rats disclosed no untoward effects.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(125)

Species: rat **Sex:** male/female
Strain:
Route of admin.: other: stomach tube
Exposure period: approx. 1/2 year
Frequency of treatment:
Post. obs. period:
Doses: 1.0 ml of a 50% aqueous glycerine solution/100 g bw
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: All of the rats developed normally and the mortality of all groups were comparable.
After 6, 12 and 18 weeks, rats from each group were sacrificed, dissected and their organs removed for histological examination. No pathological changes were disclosed and determination of liver glycogen revealed normal levels. X-ray examinations carried out on animals of each group at the beginning as well as in intervals of 6 weeks, remained without abnormal findings.

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (128)

Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach tube
Exposure period: 44 days
Frequency of treatment: daily
Post. obs. period:
Doses: 1 ml of a 0, 1, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth curves, lethality, and histological examination of the kindneys, livers, and bladders of the rats disclosed no untoward effects.

Source: Unichema Chemie GmbH Emmerich (125)

Species: rat **Sex:** male/female
Strain:
Route of admin.: other: stomach tube
Exposure period: approx. 1/2 year
Frequency of treatment:
Post. obs. period:
Doses: 1.0 ml of a 50% aqueous glycerine solution/100 g bw
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: All of the rats developed normally and the mortality of all groups were comparable.
After 6, 12 and 18 weeks, rats from each group were sacrificed, dissected and their organs removed for histological examination. No pathological changes were disclosed and determination of liver glycogen revealed normal levels. X-ray examinations carried out on animals of each group at the beginning as well as in intervals of 6 weeks, remained without abnormal findings.

Source: Unichema Chemie GmbH Emmerich (128)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach tube
Exposure period: 44 days
Frequency of treatment: daily
Post. obs. period:
Doses: 1 ml of a 0, 1, 5, 10 or 20% aqueous glycerin solution
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Growth curves, lethality, and histological examination of the kindneys, livers, and bladders of the rats disclosed no untoward effects.
Source: Lever GmbH Hamburg

(125)

Species: rat **Sex:** male/female
Strain:
Route of admin.: other: stomach tube
Exposure period: approx. 1/2 year
Frequency of treatment:
Post. obs. period:
Doses: 1.0 ml of a 50% aqueous glycerine solution/100 g bw
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Result: All of the rats developed normally and the mortality of all groups were comparable.
After 6, 12 and 18 weeks, rats from each group were sacrificed, dissected and their organs removed for histological examination. No pathological changes were disclosed and determination of liver glycogen revealed normal levels. X-ray examinations carried out on animals of each group at the beginning as well as in intervals of 6 weeks, remained without abnormal findings.
Source: Lever GmbH Hamburg

(128)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rabbit **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 4 Rabbits were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Doses of 10 ml of the glycerin solution daily were well tolerated by the rabbits. Those animals which were autopsied at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell cholesterol levels showed any consistent changes which could be attributed to the intake of glycerin.
Source: Simel S.p.A. Industria Chimica Cremona (129)

Species: rabbit **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 4 Rabbits were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Doses of 10 ml of the glycerin solution daily were well tolerated by the rabbits. Those animals which were autopsied at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell cholesterol levels showed any consistent changes which could be attributed to the intake of glycerin.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (129)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rabbit **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 4 Rabbits were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Doses of 10 ml of the glycerin solution daily were well tolerated by the rabbits. Those animals which were autopsied at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell chlolesterol levels showed any consistent changes which could be attributed to the intake of glycerin.
Source: Unichema Chemie GmbH Emmerich (129)

Species: rabbit **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 4 Rabbits were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Doses of 10 ml of the glycerin solution daily were well tolerated by the rabbits. Those animals which were autopsied at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell chlolesterol levels showed any consistent changes which could be attributed to the intake of glycerin.
Source: Lever GmbH Hamburg (129)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: dog **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 and 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: These studies were conducted to investigate the oral toxicity of Atlas synthetic glycerin compared to natural glycerin.
Result: The observations failed to reveal any treatment effects from either the Atlas synthetic or the natural glycerin.
Source: Simel S.p.A. Industria Chimica Cremona
Test substance: Atlas synthetic glycerin and natural glycerin.

(120)

Species: dog **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 and 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: These studies were conducted to investigate the oral toxicity of Atlas synthetic glycerin compared to natural glycerin.
Result: The observations failed to reveal any treatment effects from either the Atlas synthetic or the natural glycerin.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test substance: Atlas synthetic glycerin and natural glycerin.

(120)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: dog **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 and 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: These studies were conducted to investigate the oral toxicity of Atlas synthetic glycerin compared to natural glycerin.
Result: The observations failed to reveal any treatment effects from either the Atlas synthetic or the natural glycerin.
Source: Unichema Chemie GmbH Emmerich
Test substance: Atlas synthetic glycerin and natural glycerin. (120)

Species: dog **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 years
Frequency of treatment:
Post. obs. period:
Doses: diets containing 5, 10 and 20% glycerin
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: These studies were conducted to investigate the oral toxicity of Atlas synthetic glycerin compared to natural glycerin.
Result: The observations failed to reveal any treatment effects from either the Atlas synthetic or the natural glycerin.
Source: Lever GmbH Hamburg
Test substance: Atlas synthetic glycerin and natural glycerin. (120)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: dog **Sex:** male/female
Strain: other: Mongrel
Route of admin.: other: stomach tube
Exposure period: 3 d
Frequency of treatment: 3/d
Post. obs. period: no
Doses: 0.75; 1.5; 3.0 mg undiluted Glycerol/kg
Control Group: yes
LOAEL: <=
Method:
Year: **GLP:**
Test substance: no data
Remark: The irritating effects of Glycerol on the gastrointestinal mucosa of dogs were investigated.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(130)

Species: guinea pig **Sex:** no data
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: 10 Guinea pigs were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Guinea pigs receiving more than 5 ml of a 50% glycerin solution daily by stomach tube died with acutely symptoms. Autopsies performed on animals at termination of the experiment revealed no pathological changes. The plasma cholesterol level showed no consistent changes attributable to the intake of glycerin while the red blood count of the guinea pigs (2 exposed via stomach tube, 1 exposed via drinking water) receiving glycerin dropped during the experiment indicating a probable anemic effect of glycerin in the guinea pig.
Source: Simel S.p.A. Industria Chimica Cremona

(129)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: guinea pig **Sex:** no data
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 10 Guinea pigs were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Guinea pigs receiving more than 5 ml of a 50% glycerin solution daily by stomach tube died with acutely symptoms. Autopsies performed on animals at termination of the experiment revealed no pathological changes. The plasma cholesterol level showed no consistent changes attributable to the intake of glycerin while the red blood count of the guinea pigs (2 exposed via stomach tube, 1 exposed via drinking water) receiving glycerin dropped during the experiment indicating a probable anemic effect of glycerin in the guinea pig.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (129)

Species: guinea pig **Sex:** no data
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 10 Guinea pigs were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Guinea pigs receiving more than 5 ml of a 50% glycerin solution daily by stomach tube died with acutely symptoms. Autopsies performed on animals at termination of the experiment revealed no pathological changes. The plasma cholesterol level showed no consistent changes attributable to the intake of glycerin while the red blood count of the guinea pigs (2 exposed via stomach tube, 1 exposed via drinking water) receiving glycerin dropped during the experiment indicating a probable anemic effect of glycerin in the guinea pig.
Source: Unichema Chemie GmbH Emmerich (129)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: guinea pig **Sex:** no data
Strain:
Route of admin.: other: see remark
Exposure period: 30-40 days
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method: other **GLP:** no data
Year:
Test substance: other TS
Remark: 10 Guinea pigs were given either a 50% aqueous solution in saline or saline alone either by stomach tube or from a drinking cup daily.
Result: Guinea pigs receiving more than 5 ml of a 50% glycerin solution daily by stomach tube died with acutely symptoms. Autopsies performed on animals at termination of the experiment revealed no pathological changes. The plasma cholesterol level showed no consistent changes attributable to the intake of glycerin while the red blood count of the guinea pigs (2 exposed via stomach tube, 1 exposed via drinking water) receiving glycerin dropped during the experiment indicating a probable anemic effect of glycerin in the guinea pig.
Source: Lever GmbH Hamburg (129)

Species: human **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 50 days
Frequency of treatment: after each of the 3 daily meals
Post. obs. period:
Doses: about 1.3-2.2 g glycerol/kg bw/day
Control Group: no data specified
Method: other **GLP:** no data
Year:
Test substance: other TS: 95% glycerol
Remark: 14 volunteers (10 men, 4 women) drank orange juice mixed with 30 cc of 95% glycerol after each of the 3 daily meals.
Result: No overt signs of toxicity or effect on blood consumption and urine production.
Source: Simel S.p.A. Industria Chimica Cremona (131)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: human **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 50 days
Frequency of treatment: after each of the 3 daily meals
Post. obs. period:
Doses: about 1.3–2.2 g glycerol/kg bw/day
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: other TS: 95% glycerol
Remark: 14 volunteers (10 men, 4 women) drank orange juice mixed with 30 cc of 95% glycerol after each of the 3 daily meals.
Result: No overt signs of toxicity or effect on blood consumption and urine production.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (132)

Species: human **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 50 days
Frequency of treatment: after each of the 3 daily meals
Post. obs. period:
Doses: about 1.3–2.2 g glycerol/kg bw/day
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: other TS: 95% glycerol
Remark: 14 volunteers (10 men, 4 women) drank orange juice mixed with 30 cc of 95% glycerol after each of the 3 daily meals.
Result: No overt signs of toxicity or effect on blood consumption and urine production.
Source: Unichema Chemie GmbH Emmerich (132)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: human **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 50 days
Frequency of treatment: after each of the 3 daily meals
Post. obs. period:
Doses: about 1.3-2.2 g glycerol/kg bw/day
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: other TS: 95% glycerol
Remark: 14 volunteers (10 men, 4 women) drank orange juice mixed with 30 cc of 95% glycerol after each of the 3 daily meals.
Result: No overt signs of toxicity or effect on blood consumption and urine production.
Source: Lever GmbH Hamburg (132)

Species: human **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period:
Frequency of treatment: daily
Post. obs. period:
Doses: 1.2 - 1.5 g/kg
Control Group:
Method: other
Year: **GLP:**
Test substance: no data
Remark: 37 Cases (human) of cerebral edema caused by acute cerebral infarction, were treated daily with 1.2 g/kg of glycerin i.v. or 1.5 g/kg orally. Seventeen other patient with cases of edema of the central nervous system were similarly treated.
Result: During treatment, mortality of the group with cerebral infarction was 11%, however these deaths were not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of 4 days of treatment. No toxic effects were observed which were attributable to glycerin administration.
Source: Simel S.p.A. Industria Chimica Cremona (133)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: human **Sex:** no data
Strain: other
Route of admin.: other
Exposure period: 50 days
Frequency of treatment: with meals
Post. obs. period: No data, see original reference.
Doses: 1.3 - 2.2 g/kg bw/day
Control Group: no data specified
NOAEL: >= 2200 mg/kg bw
Method: other
Year: 1933 **GLP:** no data
Test substance: no data
Remark: No overt signs of toxicity or effects on blood composition and urine were seen when 14 volunteers drank orange juice providing 1.3 - 2.2 g glycerol/kg bw/day with their meals for 50 days.
Source: Croda Universal Ltd Goole, North Humberside

(134)

Species: human **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period:
Frequency of treatment: daily
Post. obs. period:
Doses: 1.2 - 1.5 g/kg
Control Group:
Method: other
Year: **GLP:**
Test substance: no data
Remark: 37 Cases (human) of cerebral edema caused by acute cerebral infarction, were treated daily with 1.2 g/kg of glycerin i.v. or 1.5 g/kg orally. Seventeen other patient with cases of edema of the central nervous system were similarly treated.
Result: During treatment, mortality of the group with cerebral infarction was 11%, however these deaths were not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of 4 days of treatment. No toxic effects were observed which were attributable to glycerin administration.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(133)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: human **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period:
Frequency of treatment: daily
Post. obs. period:
Doses: 1.2 - 1.5 g/kg
Control Group:
Method: other **GLP:**
Year:
Test substance: no data
Remark: 37 Cases (human) of cerebral edema caused by acute cerebral infarction, were treated daily with 1.2 g/kg of glycerin i.v. or 1.5 g/kg orally. Seventeen other patient with cases of edema of the central nervous system were similarly treated.
Result: During treatment, mortality of the group wth cerebral infarction was 11%, however these deaths were not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of 4 days of treatment. No toxic effects were observed which were attributable to glycerin administration.
Source: Unichema Chemie GmbH Emmerich (133)

Species: human **Sex:**
Strain:
Route of admin.: other: see remark
Exposure period:
Frequency of treatment: daily
Post. obs. period:
Doses: 1.2 - 1.5 g/kg
Control Group:
Method: other **GLP:**
Year:
Test substance: no data
Remark: 37 Cases (human) of cerebral edema caused by acute cerebral infarction, were treated daily with 1.2 g/kg of glycerin i.v. or 1.5 g/kg orally. Seventeen other patient with cases of edema of the central nervous system were similarly treated.
Result: During treatment, mortality of the group wth cerebral infarction was 11%, however these deaths were not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of 4 days of treatment. No toxic effects were observed which were attributable to glycerin administration.
Source: Lever GmbH Hamburg (133)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of
treatment:
Post. obs.
period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of
treatment:
Post. obs.
period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Source: Simel S.p.A. Industria Chimica Cremona

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of
treatment:
Post. obs.
period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Source: Unichema Chemie GmbH Emmerich

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Source: Lever GmbH Hamburg

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium strain TA-100
Concentration: 0.1 and 1 mmol per plate
Metabolic activation: with and without
Result: negative
Method: other
Year: 1979 GLP: no data
Test substance: no data
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(135)

Type: Ames test
System of testing: Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538
Concentration: 0.2 - 0.4 - 0.6 - 0.8 - 1.0 mg/plate
Metabolic activation: with and without
Result: negative
Method: other: Maron, D.M. and B.N. Ames. Mutation Res. 113, 247-
Year: 1983 **GLP:** no data
Test substance: other TS: glycerol, purity >99.5%.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(136)

Type: Ames test
System of testing: Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100
Concentration: 10 mg/plate
Metabolic activation: with and without
Result: negative
Method: other: no data
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Chemicals were tested in three different laboratories. Two laboratories did not detect any signs of mutagenicity. One laboratory got an ambiguous result.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol >99% purity, Lot JC 06087 from Aldrich

(137)

Type: Ames test
System of testing: Salmonella typhimurium strains TA98, TA100, TA1537, TA1538
Concentration: 10 mg/plate
Metabolic activation: with and without
Result: negative
Method:
Year: 1979 **GLP:** no data
Test substance: other TS
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol/Water mixture of unknown composition. Source: Solargard G from Daystar Corp. (Heat Transfer Fluid).

(104)

Type: Ames test
System of testing: Salmonella typhimurium strain TA100
Concentration: 0.5 mg/plate
Metabolic activation: with and without
Result: negative
Method:
Year: 1982 **GLP:** no data
Test substance: other TS
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
Test substance: Glycerol of guaranteed grade

(138)

Type: Ames test
System of testing: Salmonella typhimurium strain TA-100
Concentration: 0.1 and 1 mmol per plate
Metabolic activation: with and without
Result: negative
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

Type: Cytogenetic assay
System of testing: Chinese hamster ovary CHO cell line WBL
Concentration: 0.1 - 0.2 - 0.3 - 0.6 - 0.8 - 1.0 mg/ml
Metabolic activation: with and without
Result: negative
Method: other: Galloway, S.M. et al. Envir. Mutagen. 7, 1-
Year: 1985 **GLP:** no data
Test substance: other TS: glycerol, purity >99.5%.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(136)

Type: DNA damage and repair assay
System of testing: Bacillus subtilis strain H17 and M45
Concentration:
Metabolic activation:
Result: ambiguous
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No negative controls included.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(139)

Type: Escherichia coli reverse mutation assay
System of testing: various
Concentration:
Metabolic activation: with and without
Result: negative
Method: other
Year: 1985 **GLP:** no data
Test substance: no data
Source: Croda Universal Ltd Goole, North Humberside

(140) (141)

Type: HGPRT assay
System of testing: CHO-K1-BH4 cell line
Concentration: 0.1 - 0.2 - 0.4 - 0.6 - 0.8 - 1.0 mg/ml
Metabolic activation: with and without
Result: negative
Method: other: O.Neill, J.P. et al Mutation Res. 45, 91-1977 **GLP:** no data
Year:
Test substance: other TS: glycerol, purity >99.5%
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(136)

Type: Salmonella typhimurium reverse mutation assay
System of testing: (Ames Test)
Concentration:
Metabolic activation: with and without
Result: negative
Method: other
Year: 1985 **GLP:** no data
Test substance: no data
Remark: A number of studies have reported no evidence of mutagenicity in Salmonella typhimurium (Ames tests).
Source: Croda Universal Ltd Goole, North Humberside
(142) (143) (140) (141) (144) (145)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary (CHO) cell line WBL
Concentration: 0.2 - 0.4 - 0.6 - 0.8 - 1.0 mg/ml
Metabolic activation: with and without
Result: negative
Method: other: Perry, J and S. Wolff. Nature, Lond., 251, 156-
Year: 1974 **GLP:** no data
Test substance: other TS: glycerol, purity >99.5%.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
(136)

Type: Unscheduled DNA synthesis
System of testing: Rat hepatocytes
Concentration: 0.1 - 0.25 - 0.5 - 0.75 - 1.0 mg/ml
Metabolic activation: without
Result: negative
Method: other: Mitchell, A.D. and J.C. Mirsalis. Cell Mutation Monitoring Systems. p. 165. Plenum New York.
Year: 1984 **GLP:** no data
Test substance: other TS: glycerol, purity >99.5%.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg
(136)

Type:
System of
testing:
Concentration:
Metabolic
activation:
Result:
Method:
Year: GLP:
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay
Species: rat Sex: male
Strain:
Route of admin.: other: stomach
Exposure period:
Doses: 1 g/kg
Result:
Method:
Year: GLP: no data
Test substance: no data
Result: Ambiguous. The authors state that glycerol induces chromosomal aberrations in rat bone marrow cells. This result is in contrast to all in-vitro mammalian mutagenicity data. Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. An indication for this hypothesis is the observed toxicity on reproduction. Such effects are described for structurally similar three-carbon compounds (See: Stolzenberg, J. and H. Hine. J. of the Toxicol. and Env. Health, 5:1149-1158, 1979.)
Source: Simel S.p.A. Industria Chimica Cremona (146) (147)

Type: Cytogenetic assay
Species: rat Sex: male
Strain:
Route of admin.: other: stomach
Exposure period:
Doses: 1 g/kg
Result:
Method:
Year: GLP: no data
Test substance: no data
Result: Ambiguous. The authors state that glycerol induces chromosomal aberrations in rat bone marrow cells. This result is in contrast to all in-vitro mammalian mutagenicity data. Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. An indication for this hypothesis is the observed toxicity

on reproduction. Such effects are described for structurally similar three-carbon compounds (See: Stolzenberg, J. and H. Hine. J. of the Toxicol. and Env. Health, 5:1149-1158, 1979.)

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (146) (147)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach
Exposure period:
Doses: 1 g/kg
Result:
Method:
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. The authors state that glycerol induces chromosomal aberrations in rat bone marrow cells. This result is in contrast to all in-vitro mammalian mutagenicity data. Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. An indication for this hypothesis is the observed toxicity on reproduction. Such effects are described for structurally similar three-carbon compounds (See: Stolzenberg, J. and H. Hine. J. of the Toxicol. and Env. Health, 5:1149-1158, 1979.)

Source: Unichema Chemie GmbH Emmerich (146) (147)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain:
Route of admin.: other: stomach
Exposure period:
Doses: 1 g/kg
Result:
Method:
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. The authors state that glycerol induces chromosomal aberrations in rat bone marrow cells. This result is in contrast to all in-vitro mammalian mutagenicity data. Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. An indication for this hypothesis is the observed toxicity on reproduction. Such effects are described for structurally similar three-carbon compounds (See: Stolzenberg, J. and H. Hine. J. of the Toxicol. and Env. Health, 5:1149-1158, 1979.)

Source: Lever GmbH Hamburg (146) (147)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Cytogenetic assay
Species: rat **Sex:**
Strain:
Route of admin.: other
Exposure period:
Doses: 1 g/kg
Result:
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode (148)

Type: Inhibition of DNA-Synthesis
Species: human **Sex:**
Strain:
Route of admin.: other: lymphocyte
Exposure period:
Doses: 200 mmol/l = 18.4 g/l
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. Inhibition of DNA synthesis probably due to the osmotic effect of the glycerol solution.
Source: Simel S.p.A. Industria Chimica Cremona (149)

Type: Inhibition of DNA-Synthesis
Species: human **Sex:**
Strain:
Route of admin.: other: lymphocyte
Exposure period:
Doses: 200 mmol/l = 18.4 g/l
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. Inhibition of DNA synthesis probably due to the osmotic effect of the glycerol solution.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (150)

Type: Inhibition of DNA-Synthesis
Species: human **Sex:**
Strain:
Route of admin.: other: lymphocyte
Exposure period:
Doses: 200 mmol/l = 18.4 g/l
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. Inhibition of DNA synthesis probably due to the osmotic effect of the glycerol solution.
Source: Unichema Chemie GmbH Emmerich (150)

Type: Inhibition of DNA-Synthesis
Species: human **Sex:**
Strain:
Route of admin.: other: lymphocyte
Exposure period:
Doses: 200 mmol/l = 18.4 g/l
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: Ambiguous. Inhibition of DNA synthesis probably due to the osmotic effect of the glycerol solution.
Source: Lever GmbH Hamburg (150)

Type:
Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Doses:
Result:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.7 Carcinogenicity

Species: mouse **Sex:**
Strain: other: ddY
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses: 5% solution
Result:
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse= about 10 mg/kg bw; s.c.).
Result: The incidence of pulmonary tumor-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.
Source: Simel S.p.A. Industria Chimica Cremona (151)

Species: mouse **Sex:**
Strain: other: ddY
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses: 5% solution
Result:
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse= about 10 mg/kg bw; s.c.).
Result: The incidence of pulmonary tumor-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (152)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: mouse **Sex:**
Strain: other: ddY
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses: 5% solution
Result:
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse= about 10 mg/kg bw; s.c.).
Result: The incidence of pulmonary tumor-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.
Source: Unichema Chemie GmbH Emmerich (152)

Species: mouse **Sex:**
Strain: other: ddY
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses: 5% solution
Result:
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse= about 10 mg/kg bw; s.c.).
Result: The incidence of pulmonary tumor-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.
Source: Lever GmbH Hamburg (152)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: mouse
Strain: other
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:

Sex:**Doses:** 5% solution**Result:****Control Group:****Method:****Year:****GLP:****Test substance:****Remark:** IUCLID DATA SHEET:

Remark:

The promoting effect of glycerol on the pulmonary tumorigenesis in ddY mice induced by 4-nitroquinoline (4NQO; 0.3 mg/mouse = about 10 mg/kg bw; s.c.).

Result:

The incidence of pulmonary tumors-bearing mice as well as the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol for 4-25 weeks after 4NQO treatment, compared with mice given 4NQO alone.

Source: Wolff Walsrode AG Walsrode

Species: rat
Strain:
Route of admin.: oral feed
Exposure period: 2 yr
Frequency of treatment:
Post. obs. period:

Sex: male/female**Doses:** 5 or 10 g/kg (24 males and 24 females)**Result:****Control Group:****Method:****Year:****GLP:** no data**Test substance:** no data**Result:** No increase in tumour incidence.**Source:** Simel S.p.A. Industria Chimica Cremona

(153)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 yr
Frequency of treatment:
Post. obs. period:
Doses: 5 or 10 g/kg (24 males and 24 females)
Result:
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Result: No increase in tumour incidence.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(154)

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 yr
Frequency of treatment:
Post. obs. period:
Doses: 5 or 10 g/kg (24 males and 24 females)
Result:
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Result: No increase in tumour incidence.
Source: Unichema Chemie GmbH Emmerich

(154)

Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure period: 2 yr
Frequency of treatment:
Post. obs. period:
Doses: 5 or 10 g/kg (24 males and 24 females)
Result:
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Result: No increase in tumour incidence.
Source: Lever GmbH Hamburg

(154)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Species: rat **Sex:** male/female
Strain: no data
Route of admin.: oral unspecified
Exposure period: 2 Years
Frequency of treatment: as part of diet
Post. obs. period: see original
Doses: 5000 or 1000 mg/kg bw/d
Result:
Control Group: no data specified
Method: other
Year: 1969 **GLP:** no data
Test substance: no data
Remark: No increase in tumour incidence was reported in two long-term rat studies, one using groups of 24 males and 24 females, in which animals received up to 5 or 10 g/kg bw/day for 2 years.
Source: Croda Universal Ltd Goole, North Humberside (155) (156)

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Result:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: no dsiponible
Source: UNION DERIVAN S.A. VILADECANS

5.8 Toxicity to Reproduction

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: oral unspecified
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 100 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Effects on fertility (Post-implanation mortality)
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(157)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 280 mg/kg (2 days prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymis, spermduct).
Source: Simel S.p.A. Industria Chimica Cremona

(158)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 1600 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Effects on fertility (Male fertility index).
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg

(158)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 862 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source: Simel S.p.A. Industria Chimica Cremona

(159)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 280 mg/kg (2 days prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymis, sperm duct).
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (158)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 862 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (160)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 280 mg/kg (2 days prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymis, sperm duct).
Source: Unichema Chemie GmbH Emmerich

(158)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 862 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source: Unichema Chemie GmbH Emmerich

(160)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 280 mg/kg (2 days prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymis, sperm duct).
Source: Lever GmbH Hamburg

(158)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 862 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source: Lever GmbH Hamburg

(160)

Type: Fertility
Species: rat **Sex:** male
Strain:
Route of admin.: other
Exposure Period: 862 mg/kg (1 day prior to mating)
Frequency of treatment:
Premating Exposure Period
male: 862 mg/kg (1 day prior to mating)
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: IUCLID DATA SHEET:
Route of administration: intratesticular
Remark: Study type: TDL0
Paternal effects (Spermatogenesis).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without any evidence of toxic or endocrine effects.
Source: Wolff Walsrode AG Walsrode

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Fertility
Species: monkey **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 119 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymus, spermduct).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without evidence of toxic or endocrine side effects.
Source: Simel S.p.A. Industria Chimica Cremona (161)

Type: Fertility
Species: monkey **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 119 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymus, sperm duct).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without evidence of toxic or endocrine side effects.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (162)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Fertility
Species: monkey **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 119 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymus, sperm duct).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without evidence of toxic or endocrine side effects.
Source: Unichema Chemie GmbH Emmerich (162)

Type: Fertility
Species: monkey **Sex:** male
Strain:
Route of admin.: other: intratesticular
Exposure Period:
Frequency of treatment:
Duration of test:
Doses: 119 mg/kg (1 day prior to mating)
Control Group:
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Study type: TDL0
Paternal effects (Spermatogenesis; Testes, epididymus, sperm duct).
Result: Intratesticular injection of glycerol solution suppresses spermatogenesis (meiosis) without evidence of toxic or endocrine side effects.
Source: Lever GmbH Hamburg (162)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Two generation study
Species: rat **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure Period: 8-12 weeks (starting before mating and continuing, in females, until weaning).
Frequency of treatment:
Duration of test:
Doses: about 0.2 g/kg/day
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: other TS: 20% glycerol solution
Result: No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility and reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation.
Source: Simel S.p.A. Industria Chimica Cremona (163)

Type: Two generation study
Species: rat **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure Period: 8-12 weeks (starting before mating and continuing, in females, until weaning).
Frequency of treatment:
Duration of test:
Doses: about 0.2 g/kg/day
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: other TS: 20% glycerol solution
Result: No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility and reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (164)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: Two generation study
Species: rat **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure Period: 8-12 weeks (starting before mating and continuing, in females, until weaning).
Frequency of treatment:
Duration of test:
Doses: about 0.2 g/kg/day
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: other TS: 20% glycerol solution
Result: No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility and reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation.
Source: Unichema Chemie GmbH Emmerich (164)

Type: Two generation study
Species: rat **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure Period: 8-12 weeks (starting before mating and continuing, in females, until weaning).
Frequency of treatment:
Duration of test:
Doses: about 0.2 g/kg/day
Control Group: no data specified
Method:
Year: **GLP:** no data
Test substance: other TS: 20% glycerol solution
Result: No effects were found on the reproductive efficiency of the parents, nor on the growth, fertility and reproductive performance of the untreated F1 generation, and no histological changes occurred in the tissues of both the F1 and F2 generation.
Source: Lever GmbH Hamburg (164)

5. Toxicity

date: 18-FEB-2000
Substance ID: 56-81-5

Type: other
Species: rat **Sex:** male/female
Strain:
Route of admin.: oral feed
Exposure Period: 12 weeks
Frequency of treatment: daily with food
Premating Exposure Period
male: see reference
female: see reference
Duration of test:
Doses: 200 mg/kg bw/day
Control Group: no data specified
Method: other
Year: 1953 **GLP:** no data
Test substance: no data
Remark: 0.2 g/kg bw/day given to male and female rats over a period of 12 weeks (starting before mating and continuing, in females, until weaning) did not affect the reproductive efficiency of the parents or the growth, fertility or reproductive performance of the succeeding [untreated] generation of the tissues of the next two [untreated] generations.
Source: Croda Universal Ltd Goole, North Humberside

(165)

Type:
Species: **Sex:**
Strain:
Route of admin.:
Exposure Period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

5.9 Developmental Toxicity/Teratogenicity

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Result: Teratological evaluation of glycerin revealed that administration of glycerin up to 1,180 mg/kg to pregnant rabbits for 13 consecutive days and up to 1,280 and up to 1,310 mg/kg to pregnant mice and rats resp. for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival.
Source: Simel S.p.A. Industria Chimica Cremona

(166)

Species: **Sex:****Strain:****Route of admin.:****Exposure period:****Frequency of****treatment:****Duration of test:****Doses:****Control Group:****Method:****Year:****GLP:****Test substance:**

Result: Teratological evaluation of glycerin revealed that administration of glycerin up to 1,180 mg/kg to pregnant rabbits for 13 consecutive days and up to 1,280 and up to 1,310 mg/kg to pregnant mice and rats resp. for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival.

Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

(166)

Species: **Sex:****Strain:****Route of admin.:****Exposure period:****Frequency of****treatment:****Duration of test:****Doses:****Control Group:****Method:****Year:****GLP:****Test substance:**

Result: Teratological evaluation of glycerin revealed that administration of glycerin up to 1,180 mg/kg to pregnant rabbits for 13 consecutive days and up to 1,280 and up to 1,310 mg/kg to pregnant mice and rats resp. for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival.

Source: Unichema Chemie GmbH Emmerich

(166)

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Result: Teratological evaluation of glycerin revealed that administration of glycerin up to 1,180 mg/kg to pregnant rabbits for 13 consecutive days and up to 1,280 and up to 1,310 mg/kg to pregnant mice and rats resp. for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival.
Source: Lever GmbH Hamburg (166)

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode (167)

5.10 Other Relevant Information

Type: adsorption
Remark: Glycerol is readily absorption after ingestion.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg (168)

Type: Cytotoxicity
Remark: In concentrations exceeding 20 % glycerol is toxic to bacteria due to dehydration.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg (169)

Type: Metabolism
Remark: Rapid transformation takes place either to carbon dioxide or to esters with free fatty acids.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg (168)

Type: Metabolism
Remark: Glycerine constitutes approx. 10% of the amount of fat present in human food. Aside from furnishing energy by contributing to the general pool of oxidizable organic compounds it has no special role in nutrition. Glycerine is well absorbed from the intestinal tract of rats and dogs. Phosphorylation of glycerine to alpha-glycerophosphate takes primarily place in liver and kidneys. Once glycerine is incorporated into the carbohydrate metabolic pathway it can form glucose and glycogen in addition to being oxidized for energy via the tricarboxylic acid cycle. Further exogenous glycerine also participates in lipogenesis.
Source: Simel S.p.A. Industria Chimica Cremona (170)

Type: Metabolism
Remark: Glycerine constitutes approx. 10% of the amount of fat present in human food. Aside from furnishing energy by contributing to the general pool of oxidizable organic compounds it has no special role in nutrition. Glycerine is well absorbed from the intestinal tract of rats and dogs. Phosphorylation of glycerine to alpha-glycerophosphate takes primarily place in liver and kidneys. Once glycerine is incorporated into the carbohydrate metabolic pathway it can form glucose and glycogen in addition to being oxidized for energy via the tricarboxylic acid cycle. Further exogenous glycerine also participates in lipogenesis.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (171)

Type: Metabolism
Remark: Glycerine constitutes approx. 10% of the amount of fat present in human food. Aside from furnishing energy by contributing to the general pool of oxidizable organic compounds it has no special role in nutrition. Glycerine is well absorbed from the intestinal tract of rats and dogs. Phosphorylation of glycerine to alpha-glycerophosphate takes primarily place in liver and kidneys. Once glycerine is incorporated into the carbohydrate metabolic pathway it can form glucose and glycogen in addition to being oxidized for energy via the tricarboxylic acid cycle. Further exogenous glycerine also participates in lipogenesis.
Source: Unichema Chemie GmbH Emmerich (171)

- Type:** Metabolism
Remark: Glycerine constitutes approx. 10% of the amount of fat present in human food. Aside from furnishing energy by contributing to the general pool of oxidizable organic compounds it has no special role in nutrition. Glycerine is well absorbed from the intestinal tract of rats and dogs. Phosphorylation of glycerine to alpha-glycerophosphate takes primarily place in liver and kidneys. Once glycerine is incorporated into the carbohydrate metabolic pathway it can form glucose and glycogen in addition to being oxidized for energy via the tricarboxylic acid cycle. Further exogenous glycerine also participates in lipogenesis.
Source: Lever GmbH Hamburg (171)
- Type:** other
Remark: Glycerol has a hemolytical potency. With 0.002 ml Glycerol/ml blood hemolysis in a 10% extent was observed for bovine erythrocytes. Pharmacodynamics: glycerol acts as a contact laxative.
Source: Simel S.p.A. Industria Chimica Cremona
Lever Brother Ltd. Kingston Upon Thames, Surrey
Unichema Chemie GmbH Emmerich
Lever GmbH Hamburg (172) (173)
- Type:** other
Source: Simel S.p.A. Industria Chimica Cremona
- Type:** other
Remark: Glycerol may have protection effect against tobacco smoke induced cancer. When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced.
Source: Simel S.p.A. Industria Chimica Cremona (174)
- Type:** other
Remark: IN OUR OPINION GLYCEROL COULD HAVE BEEN LISTED IN ANNEX II TO REGULATION 793/93.
Source: Croda Universal Ltd Goole, North Humberside
- Type:** other
Remark: Generally recognised as safe as a miscellaneous and/or general food additive under US FDA CFR 21 SS 182.1320 (Glycerin).
Source: Croda Universal Ltd Goole, North Humberside
- Type:** other
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey

- Type:** other
Remark: Glycerol may have protection effect against tobacco smoke induced cancer.
When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (174)
- Type:** other
Source: Unichema Chemie GmbH Emmerich
- Type:** other
Remark: Glycerol may have protection effect against tobacco smoke induced cancer.
When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced.
Source: Unichema Chemie GmbH Emmerich (174)
- Type:** other
Source: Lever GmbH Hamburg
- Type:** other
Remark: Glycerol may have protection effect against tobacco smoke induced cancer.
When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced.
Source: Lever GmbH Hamburg (174)

5.11 Experience with Human Exposure

- Remark:** no disponible
Source: UNION DERIVAN S.A. VILADECANS
- Remark:** Fertility study of 64 male employees engaged in the manufacture of glycerol.
Compared with a control group of 63 worker, no significant differences were found in several sperm quality parameters of which sperm counts/cc and percent normal forms are considered to be most reliable.
Source: Simel S.p.A. Industria Chimica Cremona (175)
- Remark:** A total of 179 cases with various intracranial disorders wastreated by intravenous administration of 10-15% solution in an attempt to control cerebral edema.
There was no evidence of significant adverse findings attributable to the administration of glycerol.
Source: Simel S.p.A. Industria Chimica Cremona (176)

- Remark:** Free glycerol is present in human plasma, and its excretion in the urine stops if levels fall below 1 mg/ml plasma.
Source: Croda Universal Ltd Goole, North Humberside (177) (178)
- Remark:** The Joint FAO/WHO Expert Committee on Food Additives considered that glycerol was of such low toxicity that it was acceptable for use without a formal acceptable daily intake figure being specified.
Source: Croda Universal Ltd Goole, North Humberside (179)
- Remark:** Fertility study of 64 male employees engaged in the manufacture of glycerol. Compared with a control group of 63 worker, no significant differences were found in several sperm quality parameters of which sperm counts/cc and percent normal forms are considered to be most reliable.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (180)
- Remark:** A total of 179 cases with various intracranial disorders was treated by intravenous administration of 10-15% solution in an attempt to control cerebral edema. There was no evidence of significant adverse findings attributable to the administration of glycerol.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey (176)
- Remark:** Fertility study of 64 male employees engaged in the manufacture of glycerol. Compared with a control group of 63 worker, no significant differences were found in several sperm quality parameters of which sperm counts/cc and percent normal forms are considered to be most reliable.
Source: Unichema Chemie GmbH Emmerich (180)
- Remark:** A total of 179 cases with various intracranial disorders was treated by intravenous administration of 10-15% solution in an attempt to control cerebral edema. There was no evidence of significant adverse findings attributable to the administration of glycerol.
Source: Unichema Chemie GmbH Emmerich (176)
- Remark:** Fertility study of 64 male employees engaged in the manufacture of glycerol. Compared with a control group of 63 worker, no significant differences were found in several sperm quality parameters of which sperm counts/cc and percent normal forms are considered to be most reliable.
Source: Lever GmbH Hamburg (180)

Remark: A total of 179 cases with various intracranial disorders was treated by intravenous administration of 10-15% solution in an attempt to control cerebral edema. There was no evidence of significant adverse findings attributable to the administration of glycerol.

Source: Lever GmbH Hamburg

(176)

Remark: The Joint FAO/WHO Expert Committee on Food Additives considered that glycerol was of such low toxicity that it was acceptable for use without a formal acceptable daily intake figure being specified.

Source: Wolff Walsrode AG Walsrode

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Photodegradation
Type: air
Sensitizer: OH
Rate constant: 0.95 x 10exp9 l/mol.sec
- (34) IUCLID DATA SHEET:
keine Daten (no data)
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Type: other: soil permeability
Media: water - soil
Method: other: soil column transport

Result:
- Soil movement: $0.9 \times 10^{\text{exp.}-9}$ cm/sec.
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7.1 Risk Assessment

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TOXICOLOGICAL EVALUATION OF CERTAIN FOOD ADDITIVES

WHO FOOD ADDITIVES SERIES 10

The evaluations contained in this document were prepared by the
Joint FAO/WHO Expert Committee on Food Additives*
Rome, 21-29 April 1976

Food and Agriculture Organization of the United Nations

World Health Organization

*Twentieth Report of the Joint FAO/WHO Expert Committee on Food
Additives, Geneva, 1976, WHO Technical Report Series No. 599, FAO Food
and Nutrition Series No. 1.

Glycerol and glycerol di-acetate

(Food Grade)

Explanation

Glycerol occurs naturally in varied combined forms as a simple, mixed or complex glyceride in association with fatty acids, carbohydrates, phosphate or amino acid. It rarely exists in significant amounts in the free form in natural fats or oils of animal, vegetable, marine or biological origin. Glycerol constitutes about 10% of the lipid molecule. Natural glycerol is isolated as a by-product from the hydrolysis of fats and oils during the manufacture of soaps, etc. Trace contaminants include fatty acids, their esters, and processing chemicals including background levels of heavy metals.

Synthetic glycerol is produced by the hydrogenolysis of

carbohydrate material (cane or corn syrup sugar), or from propylene and chlorine by a number of different processes including chlorination, chlorohydrination and hydrolysis. Natural and synthetic glycerine contain trace quantities of low-boiling components. Specific analysis of other components of one synthetic glycerine prepared from the hydrogenolysis of chlorhydrate material showed that it contained no more than 0.1%, 1,2,3-butanetriol and 0.1% of 1,2,4-butanetriol. The 1,2,3-butanetriol consists of erythro and threo isomers in the approximate proportion of 90% of the former to 10% of the latter (Atlas Chemical Ind., Inc., 1961).

Glycerol prepared from propylene was shown to be free of aromatic compounds. The only specific contaminants identified were glycerine polymers and glyceraldehyde (Hine et al., 1953).

Biological Data

Biochemical Aspects

Glycerol is readily metabolized and deposited as glycogen in the liver of young white rats (Catron & Lewis, 1929). Glycerol is metabolized by rats in a similar manner when administered intraperitoneally, intragastrically or intravenously. ¹⁴C-Glycerol administration results in a net synthesis of blood glucose and liver glycogen, as well as resulting in ¹⁴C incorporation into the lipids (neutral fat and phosphatides) of most tissues (Gidez & Karnovsky, 1954). Administration of glycerol to 5 to 10 day-old rats resulted in a hepatic glycogenesis. This response was diminished in weaned and almost absent in animals less than 48 hrs old (Johnson et al., 1971).

Phlorizinized dogs administered glycerol via stomach tube converted it almost completely to glucose which was excreted in the urine (Chambers & Deuel, 1925).

Analogy with triacetin and other di and triglycerides of fatty acids shows that glycerol di-acetates will be rapidly hydrolysed by hydrolytic enzymes excreted into the gastrointestinal tract as well as by esterases present in all tissues. The resulting acetate and glycerol will be rapidly and completely metabolized.

TOXICOLOGICAL STUDIES

Special studies on reproduction

Rat

Groups of 9 males and 18 females were maintained on synthetic diets containing 30% glycerol. Rats receiving this diet were carried through seven successive generations. Young produced from females on

the diet weighed on average 20% less than those from control females. Studies utilizing lower levels of glycerol were not successful because of nutritional inadequacies of the synthetic diet (Guerrant et al., 1947).

In another study rats were maintained on diets containing 61% starch and no glycerol, or 20% starch plus 41% glycerol, or no starch plus 61% glycerol. Reproduction was not impaired by diets containing 41% glycerol. However, no pregnancies occurred on diets containing 61% glycerol, presumably due to the inadequate nutritional quality of the diet (Johnson et al., 1933).

Groups each of 20 rats (equally divided by sex) were dosed daily per os with either 1.0 ml distilled water or 1.0 ml of a 20% aqueous solution of glycerol/100 g body wt for eight weeks. Animals (P) from each group were then bred. The pregnant rats in each group were then divided into two groups of 5, one group continued to be dosed during pregnancy, and for 12 weeks subsequent to giving birth to the pups. The growth of the pups (F1) was monitored for 60 days. At 100 days some of the F1 generation were killed for histological examination of the reproductive organs. Ten males and ten females of the F1 generation were mated on maturity. However, these animals were not administered glycerol. The F2 generation was monitored for growth and onset of estrus cycle. Administration of glycerol to the P rats had no effect on reproductive performance or growth of offsprings and onset of estrus cycle of F1 and F2 generations (Wegener, 1953).

Acute toxicity

(a) Glycerol di-acetate

Reference	LD ₅₀			
	Substance	Species	Route	mg/kg body weight
BW	Glycerol Spector, 1956	Mouse	Oral	8.5 ml/kg
BW	Diacetate "	Mouse	s.c.	2.5 ml/kg
BW	"	Mouse	i.v.	2.3 ml/kg
BW	"	Rat	s.c.	4.0 ml/kg

(b) Glycerol

Animal Reference	Oral LD50 mg/kg			
	Origin of glycerol			
	Natural	Synthetic 1	Synthetic 2	
Rat Smyth et al., 1941	27.5	-	-	
Rat Hine et al., (female)	27.2	27.2	-	1953
Rat Atlas Co., (female)	17.2 - 22.7	-	19.4 - 26.6	1961
Rat Atlas Co., (male)	21.1 - 28.8	-	19.8 - 26.4	1961
Mice Anderson et al., 1950	20.65 ± 0.47 ^a	20.81 ± 0.58 ^a	-	
Mice Hine et al., (male)	23 ± 1.3	23 ± 1.9	-	1953
Guinea pig Smyth et al., 1941	7.75	-	-	
Guinea pig Hine et al., (male)	10 ± 1.3	11.5 ± 2.8	-	1953
Rabbit Deichmann et al., 1941	14 - 18 ^a	-	-	

^a cc/kg.

Synthetic 1 - Prepared from propylene.

Synthetic 2 - Prepared from carbohydrate material.

Short-term Studies

Rat

Groups each of 5 female rats were given tap water to drink containing 0, or 5% by volume natural glycerin, or 5% by volume synthetic glycerin (prepared from propylene) for a period of 16 weeks. Ground rat rations were allowed ad lib. Hematologic studies (hemoglobin, erythrocytes, leucocytes and differentials) were made at monthly intervals. At the end of the test period the animals were sacrificed and submitted to necropsy. Gross and microscopic studies were made of the heart, lungs, spleen, stomach, intestines, kidneys, thymus, thyroid and adrenals. Weight gain was slightly increased in groups receiving glycerol. Hematologic parameters in test and control animals were similar. Histopathologic studies indicated that rats (3/5) in the groups receiving glycerol (both natural and synthetic) showed that several tubules near the junction of the cortex and medulla contained calcified masses. No other compound related effects were observed (Anderson et al., 1959).

In another study groups each of 20 rats were administered daily per os either water, or 10 ml/kg body wt glycerol or at 20% aqueous solution. Dosing was for a period of 40 days. At the end of the test period the animals were autopsied, and histological examination made of the liver, kidneys, bladder and small intestine. The only compound related effect reported was calcium deposits in the urinary canals (Kopf et al., 1951).

Groups each of 18 rats (Sprague Dawley strain), equally divided by sex, were maintained on diets containing 0, or 20%, 10% or 5% natural glycerol (99.8% glycerol, 0.02% low boilers) or 20%, 10% or 5% synthetic glycerol prepared by the hydrogenolysis of carbohydrate products (99.76% glycerol, 0.04% low boilers; and 1,2,3-Butanetriol 0.11%, and 1,2,4-Butanetriol 0.09%) for 50 weeks. The protein in the test diets was balanced with that in the control diet by incorporation of casein. The calorie density of all diets was similar. There were no significant differences in the growth rate of the test and control animals. Gross pathological examination of tissues and organs showed that the only possible compound related effect was pituitary growths which accounted for the largest segment of all of the observed growths. However, there was no pattern or difference with respect to the source of glycerol for any of these growths. Microscopic examination of the tissues and organs showed no significant pathology that could be related to dose or type of glycerin. Frequent changes were associated with the kidney (tubular damage, protein counts, inflammation and glomerular fibrosis). In male rats there was no significant difference in the incidence between test and control. In the female, no pathological findings were reported in the control animals, but the incidence in the test groups was similar to that observed in the test males (Atlas Chemical Co., 1969).

Long-term Studies

Rat

Groups each of 48 rats (Sprague Dawley strain) equally divided by sex were maintained on diets containing 0, 20%, 10% or 5% natural glycerol (99.8% glycerol, 0.02% low boilers), or 20%, 10% or 5% synthetic glycerol prepared by the hydrogenolysis of carbohydrate products (99.76% glycerol, 0.04% low boilers, and 1,2,3-Butanetriol and 1,2,4-Butanetriol, 0.09%) for two years. The protein in the test diets was balanced with that in the control by incorporation of casein. The caloric density of the test and control diets was similar. The following parameters were measured during the study: mortality, growth, efficiency of caloric utilization, water consumption, volume of urine excreted, urinalysis (including specific gravity, sediment, acetone, albumin, sugar and oxalate), hematologic studies (including white blood cells and differential count, red blood cells, hematocrit and hemoglobin) and biochemical studies (including blood glucose, urea nitrogen, plasma cholesterol, serum alkaline phosphatase and transaminases), and bromsulfalein retention. At the termination of the study the animals were killed, and subjected to a complete autopsy, including determination of organ/body weight ratios, as well as gross and microscopic examination of the tissues and organs. Rats fed diets containing glycerol gained weight more rapidly than control rats. Urine analysis, hematologic and biochemical determinations were similar for test and control animals. Male rats fed diets containing synthetic glycerol showed a slight dose related trend of increased water consumption. Organ/body weight ratios of male and female rats showed a significant increase in kidney ratio of rats maintained on diets containing 20% glycerol, and of the heart of rats maintained on diets containing 10% glycerol. Histopathological examination of organs and tissues did not reveal compound or dose related effects (Atlas Chemical Ind., 1969).

Groups each of 22 (Long-Evans strain) rats, equally divided by sex were maintained on diets containing 5, 10 or 20% natural glycine or synthetic glycerol (prepared from propylene). The control group consisted of 26 rats (equally divided by sex). The 20% groups were maintained for one year on the test diets, all other groups for two years. Urinalysis of hematologic studies were made at 3 monthly intervals during the first year of the study, then at 6 month intervals. Urinalysis and hematologic parameters were similar for test and control animals. Liver glycogen and fat content of the livers of rats in the 20% group were similar for the natural and synthetic glycerin groups. Organ/body ratios showed females receiving 20% synthetic glycerin had liver/body ratios significantly higher than controls and females receiving 5% synthetic glycerin had heart/body ratios significantly greater than those on the 5% natural glycerin. Microscopic examination of the liver, spleen, adrenals, kidney, small

intestine, bladder and reproductive organs showed no compound or dose related effects (Hine et al., 1953).

Observations in Man

Fourteen (10 male, 4 female) graduate students ingested 110 g of 95% glycerol in 3 divided doses with their food daily for a period of 50 days. Preceding and following this period were 10 day control periods. Uric acid excretion and basal metabolism were not significantly affected, nor were there changes in red and white blood cell counts or hemoglobin level during the test period. No adverse effects were reported (Johnson, 1933).

Comments

Glycerol occurs naturally in fats and other substances which are in part made up of lipid complexes. Glycerol may be derived from natural sources, primarily triglycerides, or be synthesized by the hydrogenolysis of carbohydrate materials or from products such as propylene. Evidence is available to show that glycerol is metabolized in the body to form glycogen or provide a direct energy source. In addition, long-term studies are available to show that synthetically derived glycerols are biologically similar to naturally derived glycerol.

Because certain butanetriols can be contaminants of glycerol produced by hydrogenolysis of carbohydrates, there is need to specify maximum levels of such contaminants.

EVALUATION

ADI for man not specified.

See Also:

Toxicological Abbreviations



WHO FOOD ADDITIVES SERIES: 48

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

ALIPHATIC ACYCLIC DIOLS, TRIOLS, AND RELATED SUBSTANCES

First draft prepared by Dr P.J. Abbott, Professor A.G. Renwick² and Professor I.G. Sipes³

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1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 31 flavouring agents¹ that included aliphatic acyclic diols, triols, and related substances (see Table 1) using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1, Introduction). All members of this group are aliphatic acyclic primary alcohols, aldehydes, acids, or related esters with one or more additional oxygenated functional groups. The group contains four subgroups: glycerol (No. 909) and 15 related glycerol esters and acetals (Nos 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, and 924); propylene glycol (No. 925) and four related esters, acetals, and ketals (Nos 926, 927, 928, and 929); lactic acid (No. 930) and four lactate esters (Nos 931, 932, 934, and 935); and pyruvic acid (No. 936), its corresponding aldehyde (No. 937), two pyruvate esters (Nos 938 and 939) and one acetal of pyruvic acid (No. 933).

Table 1. Summary of results of safety evaluations of aliphatic acyclic diols, triols, and related substances

Flavouring agent	No.	CAS No. and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Conclusion based on current intake
<i>Structural class I</i>					
Glycerol ^{c,d}	909	56-81-5 	Yes Europe: 17 000 USA: 220 000	Yes Glycerol is endogenous	Evaluation not finalized
1,2,3-Tris[(1'-ethoxy)]-propane	913	67715-82-6 	No Europe: 0 USA: 140	NR	No safety concern
Glyceryl monostearate ^c	918	123-94-4 	No Europe: 0 USA: 230	NR	Evaluation not finalized
Glyceryl monooleate ^c	919	111-03-5 	No Europe: ND USA: 860	NR	Evaluation not finalized
Triacetin ^c	920	102-76-1 	Yes Europe: ND USA: 83 000	Yes Expected to be hydrolysed to glycerol, which is endogenous	Evaluation not finalized
Glyceryl tripropanoate ^c	921	139-45-7 	No Europe: 0.1 USA: 280	NR	Evaluation not finalized
Tributylin ^c	922			NR	

		60-01-5 	No Europe: 31 USA: 2		Evaluation not finalized
Glycerol 5-hydroxy-decanoate ^c	923	26446-31-1 	No Europe: 4 USA: 0	NR	Evaluation not finalized
Glycerol 5-hydroxydo-decanoate ^c	924	26446-32-2 	No Europe: 4 USA: 0	NR	Evaluation not finalized
Propylene glycol ^{c,e}	925	57-55-6 	Yes Europe: ND USA: 2 400 000	Yes Expected to be oxidized to lactic acid, which is endogenous	Evaluation not finalized
Propylene glycol stearate ^c	926	142-75-6 	Yes Europe: ND USA: 66 000	Yes Expected to be oxidized to propylene glycol and subsequently to lactic acid	Evaluation not finalized
1,2-Di[(1-ethoxy)-ethoxy]] propane ^c	927	67715-79-1 	No Europe: 7 USA: 150	NR	No safety concern
Lactic acid	930	598-82-3 	Yes Europe: ND USA: 47 000	Yes Lactic acid is endogenous	No safety concern
Ethyl lactate ^f	931	97-64-3 	Yes Europe: 1900 USA: 760	Yes Expected to be hydrolysed to lactic acid, which is endogenous	No safety concern
Butyl lactate	932	138-22-7 	No Europe: 380 USA: 24	NR	No safety concern
Potassium 2-(1'-ethoxy) ethoxy-propanate	933		No Europe: ND USA: 1400	NR	No safety concern
cis-3-Hexenyl lactate	934	61931-81-5 	No Europe: 38	USA: 5NR	No safety concern
Butyl butyryl lactate	935	7492-70-8 	No Europe: 280 USA: 1400	NR	No safety concern
Pyruvic acid	936	127-17-3 	No Europe: 35 USA: 69	NR	No safety concern
Pyruvaldehyde	937	79-98-8 	No Europe: 120 USA: 3	NR	No safety concern

Ethyl pyruvate	938	617-35-6 	No Europe: 1 USA: 20	NR	No safety concern
Isoamyl pyruvate	939	7779--72-8 	No Europe: 0 USA: 0	NR	No safety concern
<i>Structural class III</i>					
3-Oxohexanoic acid glyceride	910	91052-72-1 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	No safety concern
3-Oxooctanoic acid glyceride	911	91052-68-5 	No Europe: 34 USA: 0	NR	No safety concern
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	1708-35-6 	No Europe: 4 USA: 0	NR	No safety concern
3-Oxodecanoic acid glyceride ^c	914	91052-69-6 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	Evaluation not finalized
3-Oxododecanoic acid glyceride ^c	915	91052-70-9 	No Europe: 73 USA: 0	NR	Evaluation not finalized
3-Oxotetradecanoic acid glyceride ^c	916	91052-73-2 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	Evaluation not finalized
3-Oxohexadecanoic acid glyceride ^c	917	91052-71-0 	No Europe: 43 USA: 0	NR	Evaluation not finalized
4-Methyl-2-pentyl-1,3-dioxolane	928	26563-74-6 	No Europe: 0 v	NR	No safety concern
2,2,4-Trimethyl-1,3-oxocyclopentane	929	1193-11-9 	No Europe: 0.3 USA: 0.2	NR	No safety concern

CAS: Chemical Abstracts Service; ND: no data available; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.

- a *Step 2:* All of the flavouring agents in this group are expected to be metabolized to innocuous products.
- b The thresholds for human intake are 1800 µg/day for structural class I and 90 µg/day for structural class III. All intake values are expressed in µg/day.
- c Further information is required to determine whether this substance is currently used as a flavouring agent.

- d An ADI 'not specified' was established for glycerol by the Committee at its twentieth meeting (Annex 1, reference 41), which was maintained at the present meeting.
- e An ADI of 0–25 mg/kg bw was established for propylene glycol by the Committee at its seventeenth meeting (Annex 1, reference 32), which was maintained at the present meeting.
- f Ethyl lactate was included in the group ADI 'not specified' for lactic acid and its salts that was established by the Committee at its twenty-sixth meeting (Annex 1, reference 59), which was maintained at the present meeting.

The Committee previously evaluated three members of the group. Glycerol (No. 909) was considered at the twentieth meeting, when an ADI "not specified" was established (Annex 1, reference 41). Propylene glycol (No. 925) was considered at the seventh meeting, when an ADI of 0–20 mg/kg bw was established (Annex 1, reference 7); it was further considered at the seventeenth meeting, when the ADI was increased to 0–25 mg/kg bw (Annex 1, reference 32). Ethyl lactate (No. 931) was considered at the eleventh (Annex 1, reference 14), twenty-third (Annex 1, reference 50), twenty-fourth (Annex 1, reference 53), and twenty-sixth meetings (Annex 1, reference 59). At its twenty-sixth meeting, the Committee included ethyl lactate in the group ADI 'not specified'² with lactic acid.

Nine of the 31 substances (Nos 909, 929, 930, 931, 932, 934, 936, 937, and 938) have been detected as natural components of foods in cocoa, milk, cider, cognac, asparagus, tomato, and mushrooms (Maarse et al., 1999).

1.2 Estimated per capita intake

The total annual production of the 31 substances in this group of flavouring agents for use in food was reported to be 140 000 kg in Europe (International Organization of the Flavor Industry, 1995) and 21 000 000 kg in the USA (Lucas et al., 1999). These values are equivalent to a total daily *per capita* intake of 20 000 µg in Europe and 2 800 000 µg in the USA. The large difference in the annual volume of production between Europe and the USA is due to the inclusion in the USA of figures on the use of glycerol, triacetin, and propylene glycol as solvents in the preparation of compound flavour mixtures.

In Europe, three flavouring agents, namely, glycerol (17 000 µg/day), ethyl lactate (1900 µg/day), and butyl lactate (380 µg/day), accounted for approximately 97% of the total *per capita* intake. In the USA, three substances, namely glycerol (220 000 µg/day), triacetin (83 000 µg/day), and propylene glycol (2 400 000 µg/day), accounted for 96% of the total annual daily *per capita* intake. The *per capita* intakes of individual substances are shown in Table 2.

Table 2. Annual volumes of use of aliphatic acyclic diols, triols and related substances used as flavouring agents in Europe and the USA

Substance (No.)	Most recent annual volume (kg)	Intake ^a		Annual volume in naturally occurring foods (kg) ^b	Consumption ratio ^c
		µg/day	µg/kg bw per day		
Glycerol (909)					
Europe	120 000	17 000	280	24 000 000	200
USA	1 700 000	220 000	3700		14
1,2,3-Tris[(1'-ethoxy)ethoxy]propane (913)					

Europe	0	0	0	—	
USA	1000	140	2.3		
3-Oxohexadecanoic acid glyceride (917)					
Europe	300	43	0.7	—	
USA	0	0	0		
Glyceryl monostearate (918)					
Europe	0	0	0	—	
USA	1800	230	4		
Glyceryl monooleate (919)					
Europe	NR	NA	NA	—	
USA	6500	860	14		
Triacetin (920)					
Europe	NR	NA	NA	—	
USA	630 000	83 000	1400		
Glyceryl tripropanoate (921)					
Europe	0.5	0.1	0.002	—	
USA	2100	280	5		
Tributyrin (922)					
Europe	220	31	0.5	—	
USA	15	2	0.03		
Glycerol 5-hydroxydecanoate (923)					
Europe	25	4	0.007	—	
USA	0	0	0		
Glycerol 5-hydroxydodecanoate (924)					
Europe	25	4	0.07	—	
USA	0	0	0		
Propylene glycol (925)					
Europe	NR	NA	NA	—	
USA	18 000 000	2 400 000	40 000		
Propylene glycol stearate (926)					

Europe	NR	NA	NA	—	
USA	500 000	66 000	1100		
1,2-Di[(1-ethoxy)ethoxy]propane (927)					
Europe	49	7	0.1	—	
USA	1200	150	2.5		
Lactic acid (930)					
Europe	NR	NA	NA	11 000 000	
USA	360 000	47 000	780		30
Ethyl lactate (931)					
Europe	13 000	1900	32	250 000	19
USA	5800	760	13		43
Butyl lactate (932)					
Europe	2600	380	6	+	
USA	190	24	0.4		
Potassium 2-(1'-ethoxy)ethoxypropanate (933)					
Europe	NR	NA	NA	—	
USA	10 000	1400	23		
<i>cis</i> -3-Hexenyl lactate (934)					
Europe	270	38	0.6	+	
USA	36	5	0.1		
Butyl butyryllactate (935)					
Europe	1900	280	5	—	
USA	11 000	1400	24		
Pyruvic acid (936)					
Europe	250	35	0.6	3 900 000	16 000
USA	520	69	1.1		7 500
Pyruvaldehyde (937)					
Europe	810	120	2	3100	38
USA	22	3	0.05		140

Ethyl pyruvate (938)					
Europe	5	1	0.02	210	42
USA	150	20	0.3		1.4
Isoamyl pyruvate (939)					
Europe	0	0	0	—	
USA	0	0	0		
3-Oxohexanoic acid glyceride (910)					
Europe	0	0	0	—	
USA	2000	270	4.5		
3-Oxo-octanoic acid glyceride (911)					
Europe	240	34	0.6	—	
USA	0	0	0		
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals) (912)					
Europe	27	4	0.07	—	
USA	0.05	0	0		
3-Oxodecanoic acid glyceride (914)					
Europe	0	0	0	—	
USA	2000	270	4.5		
3-Oxododecanoic acid glyceride (915)					
Europe	510	73	1.2	—	
USA	0	0	0		
3-Oxotetradecanoic acid glyceride (916)					
Europe	0	0	0	—	
USA	2000	270	5		
4-Methyl-2-pentyl-1,3-dioxolane (928)					
Europe	0.1	0	0	—	
USA	1.4	0.2	0.003		
2,2,4-Trimethyl-1,3-oxacyclopentane (929)					
Europe	2	0.3	0.005	2300	1200
USA	1.8	0.2	0.003		1300

NA, not applicable; NR, not reported; +, reported to occur naturally in foods (Maarse et al., 1999), but quantitative data were not available; –, not reported to occur naturally in foods

- a Intake expressed as $\mu\text{g}/\text{person}$ per day calculated as follows: $[(\text{annual volume, kg}) \times (1 \times 10^9 \mu\text{g}/\text{kg}) / (\text{population} \times \text{survey correction factor} \times 365 \text{ days})]$, where population (10%, 'eaters only') = 32×10^6 for Europe and 26×10^6 for the USA. The correction factor = 0.6 for Europe and 0.8 for the USA, representing the assumption that only 60% and 80% of the annual volume of the flavour, respectively, was reported in the poundage surveys (International Organization of the Flavor Industry, 1995; Lucas et al., 1999). Intake expressed as $\mu\text{g}/\text{kg}$ bw per day calculated as follows: $[(\mu\text{g}/\text{person per day})/\text{body weight}]$, where body weight = 60 kg. Slight variations may occur from rounding.
- b Quantitative data from Stoffberg & Grundschober (1987)
- c Calculated as follows: $(\text{annual consumption in food, kg})/(\text{most recently reported volume as a flavouring agent, kg})$

1.3 Metabolic considerations

The aliphatic esters of propylene glycol, lactic acid and pyruvic acid and their parent compounds would all be expected to be readily absorbed from the gastrointestinal tract. Hydrolysis of the aliphatic esters is catalysed largely by hepatic esterases to give the component alcohol and carboxylic acid or aldehyde. After hydrolysis of glycerol esters in the intestine, glycerol is also readily absorbed. Glycerol, pyruvic acid, and lactic acid are endogenous in humans. Glycerol and pyruvic acid are metabolized completely and are not excreted. Lactic acid is also mainly metabolized, although urinary excretion may occur if the blood concentration is high. Propylene glycol can be metabolized, but high doses are likely to be excreted largely unchanged in the urine.

Glycerol is metabolized via the glycolytic pathway after it has been converted in the liver to glycerol-3-phosphate. Glycerol-3-phosphate is then oxidized to yield dihydroxyacetone phosphate, which is isomerized to glyceraldehyde-3-phosphate, eventually yielding pyruvic acid.

Pyruvic acid follows two primary routes of metabolism. Under aerobic conditions, it is converted to acetyl coenzyme A and enters the citric acid cycle. Under anaerobic conditions, primarily in muscles as a result of strenuous physical activity, pyruvic acid is reduced by lactic dehydrogenase to lactic acid.

Lactic acid diffuses through muscle tissue and is transported to the liver in the bloodstream. In the liver, it is converted to glucose by gluconeogenesis. Lactic acid can also be further catabolized in the lactic acid cycle (also known as the Cori cycle).

Propylene glycol can be oxidized to lactic acid via two biochemical pathways. If propylene glycol is phosphorylated, it can be converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate, and then lactic acid. If it is not phosphorylated, propylene glycol is successively oxidized to lactaldehyde, methylglyoxal, and lactic acid.

1.4 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1 Twenty-eight of the 31 flavouring agents in this group are linear, simple branched aliphatic compounds. Twenty-two of these are in structural class I (Cramer et al., 1978) because they contain fewer than three different types of functional group (Nos 909, 913, 918–927, and 930–939). Six of these 28 substances are in structural class III because they contain three or more different types of functional group (Nos 910, 911, 914–917). The three remaining substances in the group are in structural class III because they are cyclic acetals and ketals (Nos 912, 928, and 929).

Step 2 The data on the metabolism of individual members of the group were sufficient to allow conclusions about their probable metabolic fate. The aliphatic esters of propylene glycol (Nos 926, 927, 928, and 929),

lactic acid (Nos 931, 932, 934, and 935), and pyruvic acid (Nos 938 and 939) are expected to be hydrolysed to their component alcohol and carboxylic acid. Glycerol esters (Nos 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, and 924) are expected to be hydrolysed to glycerol and carboxylic acids. Esters of propylene glycol are expected to be hydrolysed to propylene glycol and component acid. Esters of lactic acid and pyruvic acid are expected to be hydrolysed to lactic acid and pyruvic acid, respectively, and the corresponding alcohol. Acetals (Nos 927 and 933) are expected to be hydrolysed to their component alcohols and aldehydes, while ketals (Nos 928 and 929) are expected to be hydrolysed to their component ketones and alcohols. Glycerol (No. 909), lactic acid (No. 930), and pyruvic acid (No. 936) are endogenous and are metabolized through the glycolytic and citric acid pathways. Propylene glycol (No. 925) is oxidized to lactic acid. For all substances in this group, therefore, the evaluation proceeded via the A side of the scheme.

Step A3 The daily *per capita* intakes of 22 of the substances in this group are below the threshold of concern for their respective structural classes (class I, 1800 µg; class III, 90 µg). These substances would not be expected to be of safety concern. The daily *per capita* intakes of the remaining nine substances (Nos 909, 910, 914, 916, 920, 925, 926, 930, and 931) exceed the threshold of concern for their respective structural classes. Evaluation of these substances therefore proceeds to step A4.

Step A4 Glycerol (No. 909), lactic acid (No. 930), and ethyl lactate (No. 931) are endogenous in humans and are therefore not expected to be a safety concern. Triacetin (No. 920), 3-oxohexanoic acid glyceride (No. 910), 3-oxodecanoic acid glyceride (No. 914), and 3-oxotetradecanoic acid glyceride (No. 916) are glycerol esters and are hydrolysed to glycerol. Propylene glycol (No. 925) and propylene glycol stearate (No. 926) are not endogenous in humans; however, the ester is expected to be hydrolysed to propylene glycol and stearic acid. Propylene glycol is known to be oxidized to lactic acid in mammals. These substances would therefore not be expected to be a safety concern.

1.5 Consideration of combined intakes

In the unlikely event that all 23 substances in structural class I were to be consumed concurrently on a daily basis, the estimated *per capita* consumption in Europe and the USA would exceed the human intake threshold for class I. The estimated *per capita* consumption in Europe and the USA for combined intake of the eight flavouring agents in structural class III would also exceed the human intake threshold for class III. Given that the substances are expected to be efficiently metabolized by known metabolic pathways, the Committee considered that the combined intake would not give rise to concerns about safety.

1.6 Conclusions

On the basis of the predicted metabolism, the Committee concluded that the 31 aliphatic acyclic diols, triols, and related substances in this group would not raise safety concerns at the current levels of intake when used as flavouring agents. In applying the Procedure, the Committee noted that all of the available data on toxicity are consistent with the results of the safety evaluation.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the key data relevant to the evaluation of the 31 flavouring agents in this group. All members of this group are aliphatic acyclic primary alcohols, aldehydes, acids, or related esters with one or more additional oxygenated functional groups. The group consists of four subgroups: glycerol (No. 909) and 15 related glycerol esters and acetals (Nos 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, and 924); propylene glycol (No. 925) and four related esters, acetals, and ketals (Nos 926, 927, 928, and 929); lactic acid (No. 930) and four lactate esters (Nos 931, 932, 934, and 935); and pyruvic acid (No. 936), its corresponding aldehyde (No. 937), two pyruvate esters (Nos 938 and 939) and one acetal of pyruvic acid (No. 933).

2.2 Additional considerations on intake

Quantitative data on natural occurrence and consumption ratios have been reported for seven flavouring agents in the group, which indicate that they are consumed predominantly from traditional foods (i.e., consumption ratio > 1) (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987) (Table 2).

The daily *per capita* intake of this group of flavouring substances is 20 000 µg/day in Europe (330 µg/kg bw per day) and 2 500 000 µg/day in the USA (14 000 µg/kg bw per day). Glycerol accounted for approximately 85% (17 000 µg/day) of the total *per capita* intake in Europe; in the USA, propylene glycol accounted for 96% (2 400 000 µg/day) of the total *per capita* intake.

2.3 Biological data

2.3.1 Biochemical data

(a) Absorption and transformation

Little specific information was available on the absorption and transformation of individual members of this group of flavouring substances. The esters, acetals, and ketals of glycerol, lactic acid, and pyruvic acid would be expected to be readily absorbed, as would the parent compounds. After hydrolysis of glycerol esters in the intestine, glycerol is readily absorbed. Glycerol and pyruvic acid are metabolized completely and are not excreted. Lactic acid is also largely metabolized, although urinary excretion may occur if the blood concentration is high. Propylene glycol can be metabolized, but at high concentrations is likely to be largely excreted unchanged in the urine.

Propylene glycol given orally to three persons at a dose of 1038 mg (0.017 g/kg bw) was rapidly absorbed and eliminated in the urine and saliva (Hanzlik et al., 1939). In a study of the pharmacokinetics of propylene glycol in humans, multiple oral doses were rapidly absorbed, and its rate of clearance from blood was dose-dependent (Yu et al., 1985).

In studies of the minor pathways of metabolism of propylene glycol, administration to rats in drinking-water resulted in excretion unchanged in the urine (Van Winkle, 1941). Propylene glycol given orally to rabbits was conjugated with glucuronic acid and excreted in the urine (Miura, 1911; Fellows et al., 1947).

(b) Metabolism

(i) Hydrolysis of linear and branched-chain esters

In general, aliphatic esters of propylene glycol, lactic acid, and pyruvic acid are expected to be hydrolysed to their component alcohol and carboxylic acids. The hydrolysis is catalysed by classes of enzymes recognized as carboxylesterases or esterases (Heymann, 1980), the most important of which are the B-esterases, which, in mammals, predominate in hepatocytes (Heymann, 1980; Anders, 1989). The rates of hydrolysis follow first-order kinetics, with hydrolysis of the straight-chain esters occurring approximately 100 times more rapidly than that of branched-chain esters (Butterworth et al., 1975; Longland et al., 1977; Grundschober, 1977; Leegwater & van Straten, 1979).

Glycerol esters are hydrolysed to glycerol and the corresponding carboxylic acids (see Figure 1). The hydrolysis is catalysed by intestinal lipase (Tietz, 1986), which attacks the ester bonds at carbons 1 and 3. The ester bond at carbon 2 is more resistant to hydrolysis, possibly because of its stereochemistry and steric hindrance. The *beta*-monoglyceride can, however, spontaneously isomerise to the *alpha*-form (3-acylglycerol), permitting further hydrolysis to yield glycerol.



Figure 1. Hydrolysis of glycerol esters in humans

The rate of hydrolysis of glycerol esters depends on the surface area of the lipid–water interfaces, which increases greatly with the churning peristaltic movements of the intestine and the emulsifying action of bile acids. Lipase is rapidly denatured at these interfaces; however, colipase, a pancreatic protein that forms a 1:1 complex with lipase, inhibits the surface denaturation of lipase and anchors it to the lipid–water interface (Voet & Voet, 1990).

Studies of the hydrolysis of the glycerol fatty acid esters (tributyrin (No. 922) (Pilz, 1959; Pilz & Johann, 1967), glycerol 5-hydroxydecanoate (No. 923) (Als, 1975), and glycerol 5-hydroxydodecanoate (No. 924) (Als, 1975) showed complete hydrolysis to glycerol and the corresponding fatty acids, butyric acid, 5-hydroxy-decanoic acid, and 5-hydroxydodecanoic acid, respectively.

Hydroxylated and keto acids formed by hydrolysis of glycerol esters such as 5-hydroxydecanoic acid and 5-hydroxydodecanoic acid may form lactones by acid-catalysed intramolecular cyclization to yield five-member rings (see Figure 2). In aqueous media, equilibrium is established between the open-chain hydroxycarboxylic acid and the lactone. At basic pH, the equilibrium favours the open-chain hydroxycarboxylate anion, but the lactone predominates at acidic pH. 5-Hydroxy-decanoic acid and 5-hydroxydodecanoic acid may form the *delta*-lactones *delta*-decalactone and *delta*-dodecalactone, respectively. Their metabolic fate can be predicted on the basis of an analogy with the known biotransformation of structurally related aliphatic lactones previously considered by the Committee (Annex 1, reference 132). Linear saturated 5-hydroxycarboxylic acids, which are formed from *delta*-lactones, are converted, via acetyl coenzyme A (CoA) to hydroxythioesters, which then undergo *beta*-oxidation and cleavage to yield an acetyl CoA fragment and a new *beta*-hydroxy-thioester reduced by two carbons. Even-numbered carbon acids continue to be oxidized and cleaved to yield acetyl CoA, while odd-numbered carbon acids yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly, while propionyl CoA is transformed into succinyl CoA, which then enters the citric acid cycle.



Figure 2. Equilibrium between *delta*-lactones and their corresponding hydroxycarboxylic acids

Esters of propylene glycol are hydrolysed to propylene glycol and their component acid. In the presence of pancreatic lipase, propylene glycol stearate (No. 926) was hydrolysed to propylene glycol and stearic acid (Balls & Matlock, 1938).

Esters of lactic acid are hydrolysed to lactic acid and the corresponding alcohol. In rat plasma, ethyl lactate (No. 931) was hydrolysed to ethyl alcohol and lactic acid (Falke et al., 1981).

Esters of pyruvic acid are expected to be hydrolysed to pyruvic acid and the corresponding alcohol.

(ii) Hydrolysis of acetals and ketals

In general, acetals are hydrolysed to their component alcohols and aldehydes. Studies on the hydrolysis of 1,2,3-tris[(1'-ethoxy)ethoxy] propane (No. 913), which is readily hydrolysed to yield acetaldehyde and glycerol (DeSimone, 1976), support this conclusion.

Acetals of propylene glycol have also been shown to be hydrolysed to their component alcohol and aldehyde. *In vitro*, 1,2-di[(1'-ethoxy)ethoxy]propane (No. 927) was completely hydrolysed to acetaldehyde and propylene glycol (DeSimone, 1976). Potassium 2-(1'-ethoxy) ethoxypropanoate (No. 933), an acetal of lactic acid, was completely hydrolysed to lactic acid, acetaldehyde, and ethanol in simulated stomach fluids (Moreno et al., 1984). Aldehydes are oxidized to their corresponding carboxylic acids, which are subsequently metabolized through known biochemical pathways (Voet & Voet, 1990). For example, pyruvaldehyde (No. 937), the aldehyde of pyruvic acid, was metabolized to pyruvic acid when incubated with rat liver homogenate (Bonsignore et al., 1968).

Ketals are hydrolysed to their component ketones and alcohols. The related compound, benzaldehyde propylene glycol acetal, was hydrolysed in simulated gastric fluid and, to a lesser extent, in intestinal fluid

(Morgareidge, 1962). Similarly, 2,2,4-trimethyl-1,3-oxacyclopentane (No. 929) would be expected to be hydrolysed in humans to yield acetone and propylene glycol.

(iii) Metabolism of glycerol (No. 909)

Glycerol is endogenous in the human body. It enters the glycolytic pathway after its conversion in the liver to glycerol-3-phosphate by glycerol kinase. Glycerol-3-phosphate is then oxidized by glycerol-3-phosphate dehydrogenase to yield dihydroxyacetone phosphate (see Figure 3), which is then isomerized to glyceraldehyde-3-phosphate, eventually yielding pyruvic acid.

 FIGURE 3

Figure 3. Metabolism of glycerol in humans

(iv) Metabolism of pyruvic acid (No. 936) and lactic acid (930)

Pyruvic acid is endogenous in the human body. It is a critical metabolic intermediate, and its fate depends on the oxidation state of the cell (see Figure 4). Under aerobic conditions, pyruvic acid is converted to acetyl CoA and enters the citric acid cycle, where it is completely metabolized. Under anaerobic conditions, lactate dehydrogenase catalyses the reduction of pyruvic acid to lactic acid and the oxidation of NADH to NAD⁺, primarily in muscles.

 FIGURE 4

CoA, coenzyme A

Figure 4. Metabolism of pyruvic acid in humans

Lactic acid diffuses from the muscles and is transported through the bloodstream to oxygen-rich tissues such as the heart and liver, where it is catabolized further through the lactic acid cycle (also known as the Cori cycle) (see Figure 5), or converted to glucose *via* gluconeogenesis. Even in fully oxygenated muscle tissue, as much as 50% of the metabolized glucose is converted to lactic acid by way of pyruvic acid (Voet & Voet, 1990).

 FIGURE 5

Figure 5. Lactic acid cycle (Cori cycle)

In resting women who received intravenous injections of [2-¹⁴C]pyruvate, analysis of blood glucose 1 h later showed 96% conversion of pyruvic acid to glucose (Hostetler et al., 1969). When [2-¹⁴C]pyruvate was incubated with liver slices from fasted normal rats, 86% had been used after 90 min of incubation. Of the radiolabel associated with metabolized pyruvic acid, 23% was associated with glycogen and glucose, 16% with CO₂, and 16% with lactic acid. In the presence of glycerol, the use of pyruvic acid was increased to 95%, accompanied by a decrease in conversion to glycogen (16%) and CO₂ (8.6%) and an increase in the production of lactic acid (Teng et al., 1953).

(v) Metabolism of propylene glycol (No. 925)

Propylene glycol can be oxidized to lactic acid *via* one of two pathways, depending on whether the glycol is phosphorylated (Rudney, 1954; Miller & Bazzano, 1965). In studies *in vitro* with rat liver, the free glycol was successively oxidized to lactaldehyde, methylglyoxal (pyruvaldehyde), and lactic acid (see pathway 1, Figure 6) (Ting et al., 1964; Miller & Bazzano, 1965), while the phosphorylated glycol followed the pathway of acetyl phosphate, lactaldehyde phosphate, lactyl phosphate, and lactic acid (Ruddick, 1972; see pathway 2, Figure 6). Lactate is subsequently converted to pyruvate, which enters the citric acid cycle and/or the gluconeogenesis pathway (Ruddick, 1972; Wittman & Bawin, 1974).



Figure 6. Metabolism of propylene glycol in mammals

2.3.2 Toxicological studies

(a) Acute toxicity

LD₅₀ values after oral administration were available for 12 of the 31 substances in this group. In rats, the values ranged from 2000 to 31 000 mg/kg bw (Nos 909, 920, 922, 925, 930–933, 912, and 929), indicating little acute toxicity of this group by the oral route (Smyth et al., 1941; Fassett & Roudabush, 1952; Dominguez-Gil & Cadorniga, 1971; deGroot et al., 1974; Bailey, 1976; Bartsch et al., 1976; Moreno, 1976, 1977, 1978; Moreno et al., 1984; Clary et al., 1998). The available values for mice ranged from 1100 to 5000 mg/kg bw (Nos 922, 935, and 928) (Gast, 1963; Moran et al., 1980; Moreno, 1980).

(b) Short-term studies of toxicity

The results of short-term and long-term studies of toxicity conducted with substances in this group are summarized in Table 3.

Table 3. Results of short-term studies of toxicity with aliphatic acyclic diols, triols and related substances

Flavouring agent (No.)	Species, sex	No. of test groups ^a /no. per group ^b	Route	Length (days)	NOEL (mg/kg bw per day)	Reference
Glycerol (909)	Rat, M,F	10/10	Diet	140	5000	Guerrant et al. (1947)
	Mouse, M,F	1/81	Oral	365	No tumours at 5000 mg/kg bw	Witschi et al. (1989)
	Human, M,F	1/14	Oral	50	No adverse effects reported at 24 000 mg/kg bw	Johnson et al. (1933)
	Rat, M,F	3/18	Diet	350	20 000	Annex 1, reference 41
	Rat, M,F	3/48	Diet	730	10 000	Annex 1, reference 41
	Rat	3/22	Diet	730	10 000	Annex 1, reference 41
3-Oxo-octanoic acid glyceride (911)	Rat, M,F	5/10	Diet	14	10	Gill & van Miller (1987)
3-Oxotetradecanoic acid glyceride (916)	Rat, M,F	5/10	Diet	14	10	Gill & van Miller (1987)
Tributyrin (922)	Rat,	NR1/66	Diet	245	Marked hyperplasia and papillomatous growth in fore stomach at 7500 mg/kg bw	Salmon & Copeland (1949)

Glycerol 5-hydroxydecanoate ^c (923)	Rat, M,F	2/8-17	Diet	343	150	Wilson (1961)
Glycerol 5-hydroxydodecanoate ^c (924)	Rat, M,F	2/8-17	Diet	343	300	Wilson (1961)
Propylene glycol (925)	Rat, M,F	2/10	Diet	730	1800	Morris et al. (1942)
	Rat, M,F	4/60	Diet	730	1300	Gaunt et al. (1972)
	Dog, M,F	2/10	Diet	730	2000	Weil et al. (1971)
2,2,4-Trimethyl-1,3-oxacyclopentane (929)	Rat, M,F	2/5	Gavage	14	38	de Groot et al. (1974)
Lactic acid (930)	Rat, M,F	2/?	Diet	730	No increase in tumour incidence at 5000 mg/kg bw	Maekawa et al. (1991)
Pyruvaldehyde (937)	Rat, M	3/30 2/10	Oral	224	No increase in tumour incidence at 2500 mg/kg bw	Takahashi et al. (1989)

M, male; F, female; NR, not reported

- a Does not include control animals
- b Both male and female animals
- c *delta*-Decalactone and *delta*-dodecalactone are metabolites of glycerol 5-hydroxydecanoate and glycerol 5-hydroxydodecanoate, respectively.

Glycerol (No. 909)

Groups of five young rats of each sex were fed a diet containing glycerol at a concentration of 0, 1, 3, 6, 10, 15, 20, 30, 40, 50, or 60% (equivalent to 0, 1000, 3000, 6000, 10 000, 15 000, 20 000, 30 000, 40 000, 50 000, or 60 000 mg/kg bw per day) for 20 weeks. There was no significant difference in the body-weight gain at concentrations of glycerol 30%, but reduced body-weight gain was observed at $\geq 40\%$. Histological examination revealed no treatment-related changes at $< 10\%$. The pathological changes observed at concentrations $\geq 10\%$ were marked hydropic and fatty degeneration of liver parenchymal cells. The NOEL was 5% glycerol in the diet, equivalent to 5000 mg/kg bw per day (Guerrant et al., 1947).

Ten men and four women were given glycerol orally at a dose calculated to result in an average daily intake of 24 000 mg/kg bw per day, for 50 days. No toxic effects were reported. The only effect was a slight tendency towards an increase in body weight (Johnson et al., 1933).

3-Oxo-octanoic acid glyceride (No. 911) and 3-oxotetradecanoic acid glyceride (No. 916)

Groups of five male and five female Fischer 344 rats were given diets containing either 3-oxooctanoic acid or 3-oxotetradecanoic acid as esters of hydrogenated palm oil at a concentration calculated to provide a dose of 10 mg/kg bw per day, for 14 days. Detailed clinical examinations were conducted daily, and food consumption was measured on days 7 and 14. No physical signs of toxicity, abnormal body-weight gain, abnormal food consumption, or treatment-related effects were observed at necropsy. The absolute and relative weights of the liver and kidney were increased by 10% in female rats, but this effect was not considered to be biologically significant as no histological changes were found in these tissues and there were no other observed toxic effects (Gill & van Miller, 1987).

Glycerol 5-hydroxydecanoic acid (No. 923) and glycerol 5-hydroxydodecanoic acid (No. 924)

Studies were available on the lactones, *delta*-decalactone and *delta*-dodecalactone, which are formed from the hydrolysis of their respective glycerol esters glycerol 5-hydroxydecanoic acid and glycerol 5-hydroxydodecanoic acid. Groups of rats were fed a mixture of 30% *delta*-decalactone, 60% *delta*-dodecalactone, and 10% butyric acid at a concentration of 0.01% or 1% *delta*-decalactone or *delta*-dodecalactone in the diet for 49 weeks. These concentrations were calculated to result in average daily intakes of 1.5 or 150 mg/kg bw *delta*-decalactone and 3 or 300 mg/kg bw *delta*-dodecalactone. Histological examination revealed no adverse effects in any group. Haematology, blood chemistry, and urinary analysis showed no significant difference between test and control groups (Wilson, 1961).

2,2,4-Trimethyl-1,3-oxacyclopentane (No. 929)

In rats given 2,2,4-trimethyl-1,3-oxacyclopentane at a dose of 3.8 or 38 mg/kg bw per day for 14 days, there were no signs of toxicity at either dose (deGroot et al., 1974).

*(c) Long-term studies of toxicity and carcinogenicity**Glycerol (No. 909)*

In a study of the tumour promoting potential of glycerol, groups of male and female C3H mice, 6–8 weeks old, were given various carcinogens followed by 0, 0.5, or 1% (v/v) glycerol solution until they were 1 year old. Animals in the control group received either 5% (v/v) glycerol (equivalent to 5000 mg/kg bw per day) or water. The animals were killed, and the incidences of liver and lung tumours were recorded. Among males, the incidence of liver tumours was 23% in those given glycerol and 39% in those given water. The tumour incidence in the lung was 21% with glycerol and 41% with water. Similar results were obtained for female mice. Thus, lower incidences of liver and lung tumours were seen after glycerol treatment. No treatment-related adverse effects were reported (Witschi et al., 1989).

A study in which Sprague-Dawley rats were given glycerol in the diet at a concentration of 0, 5, 10, or 20% (equivalent to 0, 5000, 10 000 or 20 000 mg/kg bw per day) for 50 weeks was evaluated previously by the Committee (Annex 1, reference 41). No significant treatment-related effects were found on growth rate or gross or histological appearance. The NOEL was 20 000 mg/kg bw per day (Atlas Chemical Co., 1969).

A study in which Sprague-Dawley rats were given glycerol in the diet at a concentration of 0, 5, 10, or 20% (equivalent to 0, 2500, 5000, or 10 000 mg/kg bw per day) for 2 years was evaluated previously by the Committee (Annex 1, reference 41). No significant treatment-related effects were found on growth rate or gross or histological appearance. Changes observed in relative kidney weights were not accompanied by histopathological changes. The NOEL was 10 000 mg/kg bw per day (Atlas Chemical Co., 1969).

A study in which Long-Evan rats were given glycerol in the diet at a concentration of 0, 5, 10, or 20% glycerol (equivalent to 0, 2500, 5000, or 10 000 mg/kg bw per day) for 2 years was evaluated previously by the Committee (Annex 1, reference 41). There were no significant treatment-related effects. The NOEL was 10 000 mg/kg bw per day (Hine et al., 1953).

Tributylin (No. 922)

Groups of rats were fed a diet containing tributyrin, butyric acid, or ethyl butyrate to examine the occurrence of gastric lesions. Tributyrin was given at a concentration of 15 or 25% (equivalent to 7500 and 12 500 mg/kg bw per day) for 3–35 weeks. The animals had severely reduced body-weight gain, which represented approximately one-third of that of the control group. The 66 rats receiving tributyrin that were necropsied showed greatly enlarged stomachs with numerous irregular protuberances on the external surface. Microscopic examination revealed hyperplasia, hyperkeratosis, and occasional ulceration. The mucosa of the forestomach was covered in papillomas, resulting in a significant thickening of the forestomach wall (Salmon & Copeland, 1949).

Propylene glycol (No. 925)

A study in which rats were given propylene glycol in the diet at a concentration of 2.45% or 4.9% (equivalent to 900 and 1800 mg/kg bw per day) for 2 years was evaluated previously by the Committee (Annex 1, reference 33). No treatment-related adverse effects were found on growth, and histological examination revealed no treatment-related effects (Morris et al., 1942).

A study in which rats received propylene glycol in the diet at a concentration of 0, 310, 630, 1300, or 2500 mg/kg bw per day for 2 years was evaluated previously by the Committee (Annex 1, reference 33). No treatment-related adverse effects on body-weight gain, haematological, urinary, or clinical chemical end-points, or organ weights were found. The NOEL was 1300 mg/kg bw per day (Gaunt et al., 1972).

A study in which dogs received propylene glycol in the diet at a concentration of 0, 2000, or 5000 mg/kg bw per day for 2 years was evaluated previously by the Committee (Annex 1, reference 33). Increased erythrocyte destruction was found at the higher dose. No significant treatment-related effects on haematological, clinical chemical, or urinary end-points, or on gross or histological appearance were found (Weil et al., 1971).

Lactic acid (No. 930) and pyruvic acid (No. 936)

As pyruvic acid is reduced to lactic acid *in vivo*, data on lactic acid can be used to evaluate the safety of pyruvic acid.

Groups of male and female Fischer 344 rats were fed diets containing the calcium salt of lactic acid at a concentration of 0, 2.5, or 5% for 2 years, calculated to provide a dose of 0, 2500, or 5000 mg/kg bw per day, respectively. No adverse effects were observed, and no evidence was found of a significant dose-related increase in the incidence of tumours in any organ or tissue of treated animals. No specific dose-related changes were observed in any of the haematological and biochemical parameters measured (Maekawa et al., 1991).

Pyruvaldehyde (No. 937)

In a study to examine the tumour promoting potential of pyruvaldehyde in a two-stage model of stomach carcinogenesis, groups of male Wistar rats were given drinking-water containing 0.25% pyruvaldehyde for 32 weeks, alone or after 8 weeks' treatment with a known tumour initiator, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG). Pyruvaldehyde alone caused no increase in the incidence of stomach hyperplasia or tumours. In rats pretreated with MNNG, pyruvaldehyde did not enhance the development of adenocarcinomas in the pylorus of the glandular stomach, but it significantly increased the incidence of hyperplasia (Takahashi et al., 1989).

(d) Genotoxicity

The results of studies of genotoxicity with these substances are shown in Table 4.

Table 4. Results of studies of the genotoxicity of aliphatic acyclic diols, triols and related substances

No.	Flavouring agent	End-point	Test system	Concentration	Results	Reference
<i>In vitro</i>						
909	Glycerol	Reverse mutation	<i>S. typhimurium</i>	11 780 ppm	Negative ^a	Cortruvo et al. (1977)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	Negative ^b	Doolittle et al. (1988)
		Reverse mutation	<i>S. typhimurium</i> TA97, TA102	0–10 000 µg/plate	Negative ^c	Fujita et al. (1994)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100	0.05–1000 µg/plate	Negative ^d	Haresaku et al. (1985)

		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1537, TA1538	10 000 µg/plate	Negative ^b	Haworth et al. (1983)
		Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	50 000 µg/plate ^a	Negative ^b	Ishidate et al. (1984)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	Negative ^b	Lee et al. (1988)
		Reverse mutation	<i>S. typhimurium</i>	NR	Negative	McCann & Ames (1976)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	5-5000 µg/plate	Negative ^b	Shimizu et al. (1985)
		Reverse mutation	<i>S. typhimurium</i> TA100	1000 µmol/plate	Negative	Stolzenberg & Hine (1979)
		Reverse mutation	<i>S. typhimurium</i> TA100	500 µg/ml	Negative ^b	Yamaguchi (1982)
		Reverse mutation	<i>E. coli</i> WP2uvrA	5-5000 µg/plate	Negative ^b	Shimizu et al. (1985)
		Reverse mutation	<i>Saccharomyces cerevisiae</i> D3	12 000 ppm	Negative ^a	Cortruvo et al. (1977)
		Modified reverse mutation	<i>E. coli</i> Sd-4-73	12–31 µg/plate	Negative	Szybalski (1958)
		Gene mutation	Chinese hamster ovary cells K1-BH4, <i>hprt</i> locus	0–1000 µg/ml	Positive ^b	Doolittle et al. (1988)
		Sister chromatid exchange	Chinese hamster ovary cell line	100-1000 µg/ml	Negative ^b	Doolittle et al. (1988)
		Sister chromatid exchange	Chinese hamster ovary cells	1000 µg/ml	Negative ^b	Lee et al. (1988)
		Chromosomal aberration	Chinese hamster ovary cell line	1000 µg/ml	Negative	Doolittle et al. (1988)
		Chromosomal aberration	Chinese hamster lung fibroblasts	1000 µg/ml	Negative	Ishidate et al. (1984)
		Chromosomal aberration	Chinese hamster ovary cells	1000 µg/ml	Negative ^b	Lee et al. (1988)
		Mutation	Chinese hamster ovary cells	1000 µg/ml	Negative ^b	Lee et al. (1988)
		Unscheduled DNA synthesis	Rat hepatocytes	1000 µg/ml	Negative	Doolittle et al. (1988)
			Rat hepatocytes		Negative	

		Unscheduled DNA synthesis		10–100 000 µg/ml		Fautz et al. (1991)
		Unscheduled DNA synthesis	Rat hepatocytes	1000 µg/ml	Negative	Lee et al. (1988)
918	Glycerol mono-stearate	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	50 µg/plate	Negative ^b	Blevins & Taylor (1982)
		Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100	0–400 µg/plate	Positive ^b	Kuroda et al. (1985)
925	Propylene glycol	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1–10 000 µg/plate	Negative	Clark et al. (1979)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	230 µg/plate	Negative ^b	Florin et al. (1980)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	100–10 000 µg/plate	Negative ^b	Haworth et al. (1983)
		Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	10 000 µg/plate	Negative ^b	Ishidate et al. (1984)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100	NR	Negative ^b	Kawachi et al. (1981)
		Reverse mutation	<i>S. typhimurium</i>	NR	Negative	McCann & Ames (1976)
		Reverse mutation	<i>S. typhimurium</i> TA100	1000 µmol/plate	Negative	Stolzenberg & Hine (1979)
		Host-mediated mutation	<i>S. typhimurium</i> TA1530 and G46	0.01–0.25 ml	Negative	Weir (1974)
		Host-mediated mutation	<i>Saccharomyces cerevisiae</i>	0.01–0.25 ml	Positive	Weir (1974)
		Mutation	<i>Bacillus subtilis</i> rec	NR	Negative ^d	Kawachi et al. (1981)
		Chromosomal aberration	Human embryonic lung cells	0.001–0.1 µg/ml	Negative	Weir (1974)
		Chromosomal aberration	Hamster lung fibroblasts	32 000 µg/ml	Positive ^d	Ishidate et al. (1984)
		Chromosomal aberration	Hamster lung fibroblasts	NR	Positive ^d	Kawachi et al. (1981)
		Micronucleus formation	Chinese hamster ovary Don-6 cell line	3800–23 000 µg/ml	Negative	Sasaki et al. (1980)
		Micronucleus formation	Human fibroblastic cell line HE2144	3800–23 000 µg/ml	Negative	Sasaki et al. (1980)
		Sister chromatid	Hamster lung fibroblasts	NR	Negative ^d	Kawachi et al. (1981)

		exchange				
		Sister chromatid exchange	Hamster lung fibroblasts	NR	Negative	Kawachi et al. (1981)
		Sister chromatid exchange	Chinese hamster ovary Don-6 cell line	3800–23 000 µg/ml	Positive	Sasaki et al. (1980)
		Sister chromatid exchange	Human fibroblastic cell line HE2144	7600 µg/ml	Negative	Sasaki et al. (1980)
		Mutation	Mice	2700 mg/kg bw	Negative	Solt & Neale (1980)
		Chromosomal aberration	Mice	0.6–24 mg/kg bw	Negative	Vargova et al. (1980)
		Chromosomal aberration	Rat	30–5000 mg/kg bw	Negative	Weir (1974)
		Micronucleus formation	Mice	0–20 000 mg/kg bw	Negative	Hayashi et al. (1988)
930	Lactic acid	Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA104	0.5–2.0 µl/plate	Negative ^b	Al-Ani & Al-Lami (1988)
		Chromosomal aberration	Chinese hamster ovary K1 cells	900–1400 µg/ml	Positive ^b	Morita et al. (1990)
931	Ethyl lactate	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	NR	Negative ^b	Clary et al. (1998)
935	Butyl butyryl lactate	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	0–3600 µg/plate	Negative ^b	Wild et al. (1983)
		Micronucleus formation	Mice	1500 mg/kg bw	Negative	Wild et al. (1983)
		Sex-linked recessive lethal mutation	<i>Drosophila melanogaster</i>	5400 µg/ml	Negative	Wild et al. (1983)
936	Pyruvic acid	Reverse mutation	<i>S. typhimurium</i> TA100	200 µg/plate	Negative ^b	Yamaguchi (1982)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100	10–10 000 µg/plate	Negative ^b	Bjeldanes & Chew (1979)
937	Pyruvaldehyde	Reverse mutation	<i>S. typhimurium</i> TA98	30 µg/plate	Positive ^b	Yamaguchi (1982)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA104	NR	Positive ^d	Kato et al. (1989)
		Reverse mutation	<i>S. typhimurium</i>	76 µg/plate	Positive	Kim et al. (1987)
937	Pyruvaldehyde	Reverse mutation	<i>S. typhimurium</i> TA100, TA102,	5–500 µg/plate	Positive ^b	Shane et al. (1988)

			TA104			
		Reverse mutation	<i>S. typhimurium</i> TA102, TA104	0–72 µg/plate	Positive ^b	Migliore et al. (1990)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102	0.29-29 µg/plate	Positive ^b	Aeschbacher et al. (1989)
		Reverse mutation	<i>S. typhimurium</i> TA100	1000 µg/plate	Positive ^d	Nagao et al. (1986)
		Reverse mutation	<i>S. typhimurium</i> TA100	220-500 µg/plate	Positive ^d	Dorado et al. (1992)
		Reverse mutation	<i>E. coli</i> WP2uvrA/pKM101	NR	Positive ^d	Kato et al. (1989)
		Chromosomal aberration	Chinese hamster ovary cells	10–50 µg/ml	Positive	Nishi et al. (1989)
		Chromosomal aberration	Human lymphocytes	320 µg/ml	Positive ^b	Migliore et al. (1990)
		Sister chromatid exchange	Chinese hamster ovary cells	7.2 µg/ml	Negative	Tucker et al. (1989)
		Sister chromatid exchange	Chinese hamster ovary cells	14–36 µg/ml	Positive	Tucker et al. (1989)
		Sister chromatid exchange	Chinese hamster ovary cells	7.2–54 µg/ml	Positive	Faggini et al. (1985)
		Sister chromatid exchange	Human lymphocytes	320 µg/ml	Positive ^b	Migliore et al. (1990)
		Micronucleus formation	Human lymphocytes	320 µg/ml	Positive ^b	Migliore et al. (1990)
		DNA strand breaks	Calf thymus DNA	NR	Positive	Rahman et al. (1990)
		Micronucleus formation	Rat	400 mg/kg bw ^e	Negative	Martelli et al. (1989)
		Micronucleus formation	Rat	800 mg/kg bw ^f	Weakly positive	Martelli et al. (1989)
		Sister chromatid exchange	Mice duodenal cells	400 mg/kg bw	Negative	Migliore et al. (1990)
		Sister chromatid exchange	Mice duodenal cells	600 mg/kg bw	Weakly positive	Migliore et al. (1990)
		Unscheduled DNA synthesis	Rat pyloric mucosa cells	50 mg/kg bw	Negative	Furihata et al. (1985)
		Unscheduled DNA synthesis	Rat pyloric mucosa cells	200–600 mg/kg bw	Positive	Furihata et al. (1985)

		Chromosomal aberration	Mice duodenal cells	400–600 mg/kg bw	Negative	Migliore et al. (1990)
938	Ethyl pyruvate	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	32–20 000 µg/plate	Negative ^b	Anderson & Jensen (1984)

a With and without ozonation

b With and without metabolic activation

c With metabolic activation

d Without metabolic activation

e Administered in drinking-water

f Administered by gavage

The only consistently positive results both *in vitro* and *in vivo* were found with pyruvaldehyde (No. 937). This substance caused reverse mutation in bacteria and chromosomal aberrations in Chinese hamster ovary and human cells, sister chromatid exchange in Chinese hamster ovary cells, micronuclei in human lymphocytes and in rat cells, and unscheduled DNA synthesis in rat cells. Pyruvaldehyde is a natural component of some foods and is readily oxidized to the endogenous substance, pyruvate, *in vivo*. The estimated intake from its use as a flavouring agent is well below the estimated intake from natural sources.

(e) Reproductive toxicity

Glycerol (No. 909)

A seven-generation study of reproductive toxicity in rats given glycerol at a concentration of 0 or 30% (equivalent to 15 000 mg/kg bw per day) was evaluated previously by the Committee (Annex 1, reference 41). On average, the pups of treated dams weighed 20% less than those of the control group (Guerrant et al., 1947).

Propylene glycol (No. 925)

In a study to examine the potential of di(2-ethylhexyl) phthalate and its metabolites to cause testicular damage in rats after oral administration, a control group of six male Sprague Dawley rats were given propylene glycol orally at a dose of 2000 mg/kg bw per day for 5 days. On day 6, the animals were killed, and the testis, the ventral lobes of the prostate, and the liver were removed and studied. The testes of animals given propylene glycol were reported to contain occasional degenerated cells most of which were in early meiotic prophase or undergoing meiotic division (Sjoberg et al., 1986).

The effects of 15 chemicals, including propylene glycol, on differential ovarian follicle counts and reproductive performance were compared. Propylene glycol had no effect on reproductive function (Bolon et al., 1997).

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ENDNOTES

¹ When evaluating these flavouring agents, the Committee questioned whether some substances in this group (see footnote to Table 1) were used as flavouring agents and therefore appropriate to be evaluated using this Procedure. Information to address this question will be sought from relevant manufacturers.

² ADI 'not specified' is used to refer to a food substance of very low toxicity, which, on the basis of the available data (chemical, biochemical, toxicological, and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

See Also:

[Toxicological Abbreviations](#)



Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Addictiveness and Attractiveness of Tobacco Additives



SCENIHR adopted this opinion by written procedure on 12 November 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

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ABSTRACT

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked to evaluate the role of tobacco additives in the addictiveness and attractiveness of tobacco products.

The criteria for dependence established in humans indicate that tobacco has a high addictive potential, but it remains difficult to assess the addictiveness of individual additives. In animal studies the addictive potency of the final tobacco product cannot be assessed. The reinforcing potency of drugs is measured after intravenous injections and suggests that the abuse liability of pure nicotine is weaker than the addictive potential of tobacco products in humans. The currently used methods to define addictiveness of nicotine and additives are thus not considered adequate.

In humans, the positive correlation between tobacco consumption and dependence suggests that individuals with high nicotine levels in their blood are more dependent. In animal studies using self-administration, an inverted U-shaped dose-response curve has generally been revealed suggesting that the addictiveness of nicotine is not directly linear with the dose. There is however substantial variation in the response to nicotine in both animals and humans, and genetic factors probably play an important role.

No tobacco additives which are addictive by themselves have so far been identified. However, sugars, polysaccharides and cellulose fibres which are naturally present in tobacco, or sugars added in high quantities to most tobacco products, give rise to numerous aldehydes, such as acetaldehyde, in tobacco smoke. Acetaldehyde given intravenously is self-administered and enhances the addictiveness of nicotine in experimental animals. Additives that facilitate deeper inhalation (e.g. menthol) or inhibit the metabolism of nicotine may enhance the addictiveness of nicotine indirectly.

Substances such as ammonia that increase the pH of the tobacco and the smoke, result in higher amounts of uncharged nicotine. However, it is uncertain if more nicotine is absorbed with higher smoke pH. For smokeless tobacco it seems that an increased pH enhances nicotine absorption in the mouth.

The methods used to quantify the addictive potency of additives have limitations because of technical challenges in experimentally manipulating the presence or absence of an additive in a tobacco product. Such experiments require large technical and financial resources. In addition, there are ethical issues if testing in humans is considered. Due to these limitations, the available methodologies are not considered adequate.

A number of technical characteristics of cigarettes (paper, filter, packing, geometry) influence the content of different substances in the smoke and the size of smoke particles. Many smokers compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. The particle size of the smoke aerosol does not seem to substantially influence the exposure to nicotine. The technical characteristics of cigarettes may thus modulate smoking behaviour but it is uncertain if this leads to a higher risk of addiction.

Attractiveness is defined as the stimulation to use a product. The attractiveness of tobacco products may be increased by a number of additives but is also influenced by external factors such as marketing, price etc. Animal models do not currently exist for the assessment of attractiveness. In humans, the attractiveness of individual tobacco products may be compared in panel studies, surveys, and by experimental measures. Another method is to experimentally adjust tobacco products to exclude or include individual additives and test responses to them. However, this type of research is difficult due to ethical considerations that will usually preclude human testing of tobacco products, particularly among non-users or children.

The use of fruit and candy flavours seems to favour smoking initiation in young people. Menthol also attracts a number of smokers, in particular African Americans. Some additives decrease the harshness and increase the smoothness of the smoke. Certain

additives yield a full and white smoke and other additives reduce the lingering odour of the smoke in order to favour the acceptability of smoking to people around.

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products. However, it remains difficult to distinguish the direct effects of these additives from indirect effects such as the marketing towards specific groups.

Keywords: addictiveness, additives, attractiveness, cigarettes, cigars, nicotine, SCENIHR, smokeless tobacco, smoking, target groups, tobacco, waterpipe

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EXECUTIVE SUMMARY

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked to evaluate the role of tobacco additives in the addictiveness and attractiveness of tobacco products. A summary of the answers are presented below.

1. Criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product

In human studies there are clinical criteria for dependence, laboratory measures of self-administration, as well as preference studies. These criteria indicate that tobacco in humans has a high addictive potential, but they have limitations when assessing the addictiveness of individual additives in the final tobacco product. There is no widely-agreed universal standard for human studies and as a result various possible endpoints exist. In addicted individuals a modified regulation of neural networks exists, and the potential to induce such modifications should be the criteria used to define the addictive potency of a product.

In animal studies the reinforcing potency of a drug is used as a criterion for the addictive potential. Self-administration studies indicate that the abuse liability of pure nicotine is weaker than the addictive potential of tobacco products in humans.

2. Methods currently used for assessing the addictive potency of a substance

Many different methods are used in humans, but there is a lack of consistency between them. Human studies have limitations in design (e.g. the use of conditioned cues, and the need to work with smokers). Furthermore, ethical issues may arise when testing substances in humans.

There is currently no animal model to assess the addictive potency of the final tobacco product; however, pure nicotine has been studied extensively. The experimental animal models are mainly based on self-administration in rodents, usually rats. The evaluation of addictiveness is based on the re-inforcing properties of the drug. However, there is no consensus on the predictive validity for the addictiveness of tobacco products in humans. In animal studies pure nicotine is injected intravenously and shows only a weak addictive potential whereas in humans, tobacco is used differently (e.g. inhalation, oral consumption) and is highly addictive. No method currently used to define the addictive potency of a compound can therefore be considered as adequate.

3. Dose-dependency of development of nicotine addictiveness

In humans, there are little data available on pure nicotine use. However, tobacco consumption (e.g. number of cigarettes smoked per day) is positively correlated with dependence. This suggests that individuals who maintain higher nicotine levels in their blood are more dependent than individuals who maintain low levels.

In animal studies, an inverted U-shaped dose-response curve has generally been revealed suggesting that the addictiveness of nicotine is not directly linear with the dose. As mentioned before, pure nicotine is only weakly addictive in animal studies.

There is substantial variation in the response to nicotine and its addictive potential in both animals and humans, and genetic factors probably play an important role.

4. Additives in tobacco products that are addictive by themselves

No tobacco additives which are addictive by themselves have so far been identified. However, sugars, polysaccharides and cellulose fibres, which are naturally present in tobacco, or sugars added in high quantities to most tobacco products, produce numerous aldehydes, such as acetaldehyde, when burned. Acetaldehyde is self-administered by animals and may thus be considered as potentially addictive.

However, experiments using denicotinised cigarettes show that besides nicotine, a mixture of factors in cigarette smoke probably plays an important role in craving and reinforcement. Although these factors do not have pharmacological effects similar to nicotine, they play a role in smoking behaviour.

5. Additives that enhance the addictiveness of nicotine

Sugars or their derivatives produce numerous substances upon burning. Among them are aldehydes which, as observed with acetaldehyde, enhance the addictiveness of nicotine when injected into experimental animals, probably by inhibiting monoamine oxidases (MAO) in the brain.

Smokers have decreased levels of MAO in the brain. There is, however, no proof that acetaldehyde in the smoke contributes significantly to blood levels of acetaldehyde, and it is likely that aldehydes other than acetaldehyde intervene directly or through the generation of new compounds in the smoke, in the inhibition of MAO.

Additives that facilitate deeper inhalation (e.g. menthol) may enhance the addictiveness of nicotine indirectly. Other substances may enhance the addictiveness of nicotine by inhibiting its metabolism. Substances such as ammonia that increase the pH of the tobacco (and the smoke) result in higher amounts of uncharged nicotine that is more easily absorbed by the cells. However, due to the high buffer capacity of the lining fluid in the lungs it is uncertain if more nicotine is absorbed with higher smoke pH. For smokeless tobacco it has been shown that more nicotine is absorbed in the mouth when the pH of the product is increased.

6. Methods to quantify the potency of additives in enhancing the addictiveness of nicotine

The methods used to quantify the potency of additives in enhancing the addictiveness of nicotine or tobacco products are described above. The limitations of these methods arise from technical challenges in experimentally manipulating the presence or absence of an additive in the tobacco products used in these experiments. Such experiments have probably been carried out by the tobacco industry for some additives, especially sugars and their derivatives, but they require large technical and financial resources. In addition, there are ethical issues if testing in humans is considered. Because of these limitations, the available methodologies are not considered adequate.

7. Technical characteristics that enhance the addictive potential of tobacco products

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and carbon monoxide (CO)) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Many smokers compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. Based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol. The technical characteristics may thus influence smoking behaviour but it is not certain whether this leads to a higher risk of addiction.

8. Criteria for considering an additive or a combination of additives as attractive

The criterion for attractiveness is the stimulation to use the product. Attractiveness of additives refers to factors such as taste, smell and other sensory attributes. In addition, a number of external factors (e.g. ease of use, flexibility of the dosing system, cost etc.) contribute to the attractiveness of the product.

The attractiveness of tobacco products may be increased by a number of additives that create a specific taste/flavour in order to attract certain target groups. An attractive

effect may be obtained by changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant experience of smoking. In order to make smoking more acceptable to other people nearby, some additives reduce lingering odour or side-stream smoke visibility.

9. Methods currently used for assessing attractiveness

Animal models do not currently exist for the assessment of attractiveness.

In humans, the attractiveness of individual tobacco products may be compared with other tobacco products by panel studies and surveys, and by experimental measures. When examining what is known about the additive content of these products, judgements can be made as to the role of individual additives in the overall attractiveness of the product.

Another method is to experimentally adjust tobacco products to include or exclude individual additives and test responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed.

However, this type of research is difficult due to ethical considerations that will usually preclude human testing of different tobacco products, particularly among non-users or children. The methods currently used are thus not adequate.

10. Additives that increase attractiveness of tobacco products

Numerous additives are used in order to increase the attractiveness of tobacco products but it is very difficult to identify the role of individual additives in enhancing attractiveness.

Various sugars constitute a large proportion of additives, and the sweetness of the product is an important characteristic. The use of fruit and candy flavours in high amounts seems to favour smoking initiation by young people. Menthol also attracts a number of smokers (in particular African Americans), possibly due to its action on sensory nerve endings, resulting in a cooling effect.

Some additives decrease the harshness and increase the smoothness of the smoke. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by additives such as propylene glycol and glycyrrhizin, a substance in liquorice.

Certain additives yield a full and white smoke (e.g. magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate, calcium carbonate). Other additives reduce the lingering odour of the smoke in order to favour the acceptability of smoking to people around (e.g. acetylpyrazine, anethole, limonene, vanillin, and benzaldehyde).

In several countries there is a growing trend of using “natural” tobacco products advertised as containing no additives.

11. Association between additives and tobacco consumption – target groups

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products although it is difficult to distinguish the direct effects of these additives from indirect effects such as marketing towards specific groups. In the USA, the consumption of menthol cigarettes is relatively high among African Americans. Cigarettes with certain flavours (e.g. fruit, candy) appear to be developed to target young people.

Additives and design characteristics may modify consumption patterns. However, in spite of the many additives commonly used, tobacco products overtly marketed as containing additives (e.g. menthol cigarettes) command a relatively small market share in EU countries and there is presently a trend in several countries to use products labelled “without additives”.

It is notable that waterpipe smoking is becoming increasingly popular in some EU countries (and elsewhere), potentially due to the flavoured tobaccos used and the mild/cool smoke that may facilitate the inhalation of large volumes into the lungs. Smokeless tobacco products have gained increased interest from the industry because they may be used in places where smoking is prohibited.

1. BACKGROUND

Some 72-92% of adult cigarette smokers meet the criteria for dependence¹. While nicotine is recognised as an addictive substance in the tobacco leaf, the risk of addiction to pure nicotine products is very low compared to cigarettes¹. Currently, it is being discussed in the public health community whether lowering the levels of nicotine in tobacco products would make people less addicted and accordingly reduce the consumption of tobacco products.

Tobacco additives were hardly used before 1970, but today they represent up to 10% of the cigarette weight. By altering the taste and smell of cigarettes the products are made more attractive and the smoke more palatable which leads to an increase of smoking initiation. At present, the role of additives in enhancing the addictiveness of tobacco products is not clear.

In order to make tobacco products more attractive, design features are introduced, e.g. package design and cigarette form. In addition, these features are used to undermine the effect of the maximum limits set by the Tobacco Products Directive 2001/37/EC on tar, nicotine, and carbon monoxide (CO) yields in cigarettes.

Legal background

Article 13 of the Tobacco Products Directive (2001/37/EC)² stipulates that Member States can keep or introduce, in accordance with the Treaty, more stringent rules concerning the manufacture, import, sale, and consumption of tobacco products which they deem necessary in order to protect public health. Member States may prohibit the use of ingredients which have the effect of increasing the addictive properties of tobacco products.

Article 12 of the Tobacco Products Directive invites the Commission to submit a proposal providing a common list of ingredients authorised for tobacco products, taking into account, *inter alia*, their addictiveness.

In its comments to the Green Paper *Towards a Europe free from tobacco smoke: policy options at EU level*³, the European Parliament invited the Commission to propose, by 2008 if possible, an amendment to the Directive including an evaluation and authorisation procedure for tobacco additives and an immediate ban on all additives that are addiction-enhancing⁴. In its 2nd Report on the implementation of the Tobacco Products Directive⁵ the Commission stresses the need for further work on the addictiveness of tobacco additives.

DG SANCO wishes to have a better understanding of the criteria based on which an additive can be considered (classified) as an addictive and/or attractive substance, the role of additives in tobacco products and the role of design features in the attractiveness and addictiveness of a tobacco product.

¹ Henningfield JE, Zeller M. Could science-based regulation make tobacco products less addictive? *Yale J Health Policy Law Ethics* 2002; 3:127-38.

² http://eur-lex.europa.eu/pri/en/oj/dat/2001/l_194/l_19420010718en00260034.pdf

³ http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/gp_smoke_en.pdf

plus report on consultation:

http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/smoke_free_frep_en.pdf

⁴ <http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//NONSGML+REPORT+A6-2007-0336+0+DOC+PDF+V0//EN>

⁵ http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/tobacco_products_en.pdf

2. TERMS OF REFERENCE

In the light of the most recent scientific information, the Scientific Committee is requested to answer the following questions:

1. Which are the criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product?
2. What are the methods currently used for assessing the addictive potency of a substance and are they considered adequate?
3. Is the development of nicotine addictiveness dose-dependent?
4. Which additives are addictive themselves in tobacco products?
5. Which additives enhance the addictiveness of nicotine and how?
6. Which are the methods used to quantify the potency of additives in enhancing the addictiveness of nicotine and are they considered adequate?
7. Which technical characteristics enhance the addictive potential of tobacco products?
8. Which are the criteria based on which an additive or a combination of additives can be considered (classified) attractive?
9. What are the methods currently used for assessing attractiveness and are they considered adequate?
10. Which additives increase attractiveness of tobacco products?
11. What is the association between additives and tobacco consumption (independent of any addictive potential they might have)? Which additives are used to target specific groups?

3. SCIENTIFIC RATIONALE

3.1. Introduction

According to a report from WHO (2008), about 100 million people died in the 20th century from tobacco use. The number of deaths in 2007 due to tobacco related diseases was about 5.4 million and if current smoking patterns continue, more than 8 million deaths are expected to occur each year due to tobacco smoking by the year 2030. In the EU, about a third of the adult population are smokers. The number of deaths from smoking per year is currently about 500,000 in the EU and more than 1.5 million in the whole European region (WHO 2007a).

The vast majority of smokers use cigarettes, while other ways of smoking are less frequent (e.g. cigars, pipes, waterpipes). Apart from smoking tobacco, other tobacco forms (i.e. smokeless tobacco) may also have deleterious public health effects (SCENIHR 2008). In addition, exposure to tobacco smoke in the environment, so-called “passive smoking” or “second-hand smoking” is an important cause of excess mortality and morbidity. Passive smokers have a significantly increased risk for several diseases such as lung cancer (IARC 2004), respiratory diseases (Jaakkola and Jaakkola 2002a, Jaakkola and Jaakkola 2002b) and cardiovascular diseases (Whincup et al. 2004).

Table 1 shows that tobacco has a very high addictive potential in humans (Anthony et al. 1994, O'Brien and Gardner 2005) whereas numerous studies indicate that the risk of addiction to pure nicotine is low.

Table 1 Risk of addiction (adapted from Anthony et al. 1994, O'Brien and Gardner 2005)

	Ever used ^a (%)	Dependence ^b (%)	Risk of addiction ^c (%)
Tobacco	75.6	24.1	31.9
Heroin	1.5	0.4	23.1
Cocaine	16.2	2.7	16.7
Alcohol	91.5	14.1	15.4
Cannabis	46.3	4.2	9.1

^a **Column 1: Ever used – Prevalence of ever use in the total population**

^b **Column 2: Dependence – Prevalence of dependence in the total population**

^c **Column 3: Risk of addiction - Ratio of column 2 (dependence) and column 1 (ever used)**

The addictiveness of nicotine is enforced by substances in tobacco leaves that inhibit the action of monoamine oxidase (MAO) in the body (Berlin and Anthenelli 2001). Apart from naturally occurring substances in tobacco leaves, a number of ingredients in the final product may create or increase dependence. The tobacco industry has admitted the use of 599 different cigarette additives in the United States (US), which are claimed to improve taste and reduce harshness of the smoke (Rabinoff et al. 2007). Current US-style cigarettes contain about 10% of additives by weight; mainly sugars, humectants, cocoa and liquorice. Most other additives are used in small amounts. As discussed later in this opinion, cigars, pipe tobacco and smokeless tobacco generally contain fewer additives than cigarettes. Tobacco used in waterpipes is characterised by a high content of water and various sugars.

Certain flavours (e.g. candy and fruit) have been used largely to make tobacco products more appealing to children (called “young adults” by the tobacco industry). In order to decrease the appeal of cigarettes to children, the US Food and Drug Administration (FDA)⁶ banned the use of a number of flavours as additives in cigarettes in September

⁶ <http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/FlavoredTobacco/default.htm>

2009. Menthol is not one of the banned additives, but is currently being evaluated by the Tobacco Products Scientific Advisory Committee of the FDA. In other parts of the world (e.g. Canada, Australia, New Zealand), legal measures on additives are established or are in preparation. In Europe, some countries, such as Germany, United Kingdom, Austria, Romania and France, use positive and/or negative lists which respectively allow or prohibit the use of specific compounds as tobacco additives, whereas other countries do not have such a regulation.

It is the purpose of the present opinion to examine the criteria for classifying tobacco additives as addictive or attractive, and to evaluate their role for the creation or maintenance of dependence on tobacco products. This would serve as the scientific basis for regulation of the use of additives in order to reduce the toxicity and the addictiveness of the final tobacco product. An important question is whether some additives are addictive by themselves or if they act by increasing the addictiveness of nicotine. The different methods of assessing addictiveness of an additive, alone or in combination with other substances, will be reviewed. In addition to the interactions between additives and constituents of tobacco, the burning of tobacco creates other complex chemical substances that may be toxic or favour addiction. An example of this is aldehydes, such as acetaldehyde, formed by the pyrolysis of various sugars and polysaccharides in the tobacco (see section 3.8.1.4). The technical characteristics of tobacco products, in particular of cigarettes, may also influence their addictive potential. A number of additives favour attractiveness of tobacco products, and may thus promote smoking initiation. In this context special attention will be paid to how additives may be used to target specific groups.

3.2. Methodology

A public call for information⁷ was launched in November 2009, giving all stakeholders the opportunity to submit relevant scientific information concerning tobacco additives. The information asked for concerned: 1) details about the manufacturing process of tobacco products; and 2) methods applicable for assessment of attractiveness. A number of organisations and major tobacco companies responded. The information received has been evaluated carefully and was in many cases useful for writing the opinion. A particular problem in the area of tobacco products is that a number of studies relevant for this opinion have never been published but exist as internal documents of the tobacco industry. Some of the documents contain sensitive information showing health risks associated with smoking. In 1992, 60 documents were destroyed by Imperial Tobacco Canada in order to avoid exposure of the company to liability or embarrassment. Hammond et al. (2009a) have recently reviewed the contents of these documents that were recovered at the British American Tobacco headquarters in the United Kingdom and were released in 1998 through court disclosure in a trial in Minnesota. The author concludes that most of the studies that were carried out by researchers employed by the industry were scientifically valid. They gave evidence that cigarette smoke was carcinogenic and addictive. Since then, a great number of industry documents have become publicly available and can be found in two searchable databases, <http://tobaccodocuments.org> and <http://legacy.library.ucsf.edu>. The collections continue to be updated and currently contain more than 60 million pages in over 11 million documents.

Furthermore, a tobacco documents bibliography is also available which includes papers and publications based on documented research, broadly classified into several groups. Some examples of publications based on research of industry documents appearing under the heading of “Ingredients and Design” illustrate the tobacco industry research and development strategy on issues including: smoker preferences (Chaiton et al. 2005); smoking behaviour and product design (Hammond et al. 2006); targeting consumer groups with specific psychological needs (Cook et al. 2003); research on nicotine (Hurt

⁷ http://ec.europa.eu/health/ph_risk/committees/04_scenihr/scenihr_call_info_08_en.htm

and Robertson 1998); addictiveness (Scharfstein 1999, Slade et al. 1995, Stevenson and Proctor 2008, Vagg and Chapman 2005); manipulation/free base nicotine (Wayne et al. 2006, Wayne and Carpenter 2009); flavoured cigarettes (Lewis and Wackowski 2006); menthol (Kreslake et al. 2008a, Wayne and Connolly 2004); youth targeting (Wayne and Connolly 2002); and particle size (Wayne et al. 2008a). Relevant publications are discussed in subsequent chapters of this opinion.

For the purpose of the present opinion, the health risks of tobacco products and additives have been investigated within different lines of evidence such as epidemiological studies, experimental studies in humans, experimental studies in animals, cell culture studies and *in silico* studies. In order to answer the questions in the Terms of Reference to this opinion, a weighted approach has been used, where data from all the available lines of evidence were integrated as appropriate. A more detailed description of how such weighting is performed is given in an earlier opinion of the SCENIHR (SCENIHR 2009). The primary sources for this opinion were original scientific reports published in peer-reviewed scientific journals. The secondary sources used were the stakeholder information mentioned above and reports and opinions of other Scientific Committees, as well as reports of various governmental bodies. In addition to the reports cited in the text and included in the list of references, various publications were noted but not considered appropriate for the purposes of developing the opinion.

3.3. Definitions

A number of terms related to tobacco products are explained below. For the list of abbreviations, see chapter 7. A full glossary can be found in chapter 9.

3.3.1. Technical characteristics

A wide variety of tobacco products are available worldwide such as cigarettes, cigars, pipe tobaccos, smokeless tobacco products (STP) etc. Each of these types is produced by using different tobaccos and additives and by using different manufacturing practices (Reviewed in IARC Monographs: 1985; 1986; 2004; and 2007).

Cigarette: The most common form of tobacco is the manufactured cigarette. Cigarettes are made from fine-cut tobacco leaves and are wrapped in paper or other non-tobacco material, filter-tipped or untipped, approximately 8 mm in diameter and 70-120 mm in length. Cigarettes are highly engineered, exquisitely designed “nicotine delivery devices”. Design features encompass a wide range of design variables such as tobacco type and blend, chemical processing and additives, and in addition, physical features such as paper, filter and ventilation. It is also important to consider factors such as tobacco weight or density, and cigarette geometry (circumference and length). Cigarette additives have a range of purposes; e.g. to facilitate manufacture, increase shelf life, control burn rates, nicotine delivery, flavour and harshness/irritation etc. The physical design characteristics of the tobacco product interact with its chemical composition to influence its function and effect (WHO 2001). For example, the size of the cuttings of the tobacco in cigarettes and non-combusted and non-heated tobacco, and its level of acidity (measured as pH), interact to influence the release of nicotine from the product (Callicutt et al. 2006, Stevenson and Proctor 2008). Cigarette ventilation designs also modify free nicotine levels in the smoke. Similarly, the physical and chemical characteristics of cigarettes interact to alter the size distribution of the aerosol particles that convey nicotine and other chemicals, and thus influence absorption (WHO 2007b).

Roll your own (RYO) tobacco denotes any tobacco product which, because of its appearance, type, packaging, or labelling, is suitable for use and likely to be offered to, or purchased by, consumers as tobacco for making cigarettes. RYO cigarettes are cheaper substitutes for commercially manufactured brands and have gained popularity worldwide.

A cigar⁸ is a roll of tobacco wrapped in leaf tobacco or any other substance containing tobacco. There are four main types of cigars: little cigars, small cigars ("cigarillos"), regular cigars and premium cigars. Little cigars contain air-cured and fermented tobacco and are wrapped either in reconstituted tobacco or in cigarette paper that contains tobacco and/or tobacco extract. Some little cigars have cellulose acetate filter tips and are shaped like cigarettes. Cigarillos are small, narrow cigars with no cigarette paper or acetate filter. Regular and premium cigars are available in various shapes and sizes and are rolled to a tip at one end.

Pipe tobacco can be a blend of as many as 20-25 different tobaccos, or made of Burley varieties only. Some pipe tobaccos contain midrib tissues, and casings and sauces are frequently added.

A waterpipe is one of the ancient forms of tobacco use. Cut or shredded tobacco is smouldered inside the head, which is covered by a perforated aluminium foil on which the glowing charcoal is placed. The smoke is drawn through a tube inside the waterpipe, filtered through water in a container and reaches the smoker's mouth via a long flexible tube. A great variety of tobaccos, or mixture of tobaccos with additives, is used in such pipes.

Smokeless tobacco is consumed without burning the product, and can be used orally or nasally. It comes in two main forms: snuff (finely ground or cut tobacco leaves that can be dry or moist, loose or portion packed in sachets, and administered to the mouth, or the dry products to the nose or mouth); and chewing tobacco (loose leaf, in pouches of tobacco leaves, "plug" or "twist" form). According to the Tobacco Products Directive (2001/37/EC) chewing tobacco is not included in the definition of "tobacco for oral use", the sale of which is banned in all EU countries except Sweden. Swedish-type moist snuff (snus) consists of finely ground dry tobacco (Kentucky and Virginia tobacco), mixed with aromatic substances, salts (sodium chloride), water, humidifying agents and chemical buffering agents (sodium carbonate). The large variety of smokeless tobacco products available worldwide has been described in detail elsewhere (SCENIHR 2008).

Electronic cigarettes, or e-cigarettes, are battery-powered devices that vaporise nicotine, flavouring, and other chemicals into an inhalable vapour (Pauly et al. 2007). Chemical analyses have detected tobacco-associated chemicals that may be harmful to humans, including known human carcinogens (Kuehn 2009). E-cigarettes have been marketed recently for a range of uses, including, as a cessation aid and as an alternative to cigarettes in smoke-free zones. The different brands vary greatly in content of nicotine and other chemicals, but the health risks or efficacy as cessation aids have not yet been sufficiently documented (Bullen et al. 2010).

3.3.2. Contents, ingredients, and additives

According to the terminology used in the WHO Framework Convention and the recommendation by the Scientific Advisory Committee in 2003, the term "contents" is used synonymously with the term "ingredients". Consequently, it means all product components, the materials used to manufacture those components, residual substances from agricultural practices, storage and processing, substances that can migrate from packaging into the product, as well as what may be termed "additives" and "processing aids" in some countries and regions (WHO 2007b).

⁸ According to the Council Directive 2010/12/EU of 16 February 2010, the following shall be deemed to be cigars or cigarillos if they can be and, given their properties and normal consumer expectations, are exclusively intended to be smoked as they are: (a) rolls of tobacco with an outer wrapper of natural tobacco; (b) rolls of tobacco with a threshed blend filler and with an outer wrapper of the normal colour of a cigar, of reconstituted tobacco, covering the product in full, including, where appropriate, the filter but not, in the case of tipped cigars, the tip, where the unit weight, not including filter or mouthpiece, is not less than 2,3 g and not more than 10 g, and the circumference over at least one third of the length is not less than 34 mm.

Based on the 2nd Report on the Application of the Tobacco Products Directive (EC 2007b), the current definition of “ingredients” in Article 2 (5) covers any substance or constituent used in the manufacture or preparation of a tobacco product and still present in the finished product even if in an altered form, including paper, filter, inks and adhesives. It does not cover the tobacco leaf itself or other natural or unprocessed tobacco plant parts.

For the purpose of this report, we consider that the WHO definition is the most useful, as some of the added ingredients (e.g. different forms of sugar) are already present in the tobacco leaves. Tobacco leaves may also in some cases contain various toxic substances such as cadmium or radioactive isotopes. The possible presence of residual substances from agricultural practices will not be addressed in this report.

In order to avoid misunderstandings, the present report uses the term additives for added ingredients or substances. Additives are defined as any substance that is added, except water, during the course of manufacture of a tobacco product, including preservatives, humectants, flavours, and processing aids.

Natural or clean cigarettes are being marketed as having no chemicals or additives and the filters are made from natural cellulose. However, smoke from these cigarettes still contains all the carcinogens and toxins that come from the tobacco itself (Malson et al. 2002, McDaniel and Malone 2007).

Herbal cigarettes, although they may not contain tobacco, yield tar and carbon monoxide when smoked, and are thus also dangerous to health (Chen et al. 2007a, Gan et al. 2009).

3.3.3. Addiction and addictiveness

Addiction is the commonly used term referring to what is technically known as “dependence” and is widely employed to connote severe substance dependence, as has been demonstrated to occur in tobacco users. Dependence has been defined by the WHO Expert Committee on Drug Dependence (WHO 2003) and The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines (WHO 1992).

Addictiveness refers to the pharmacological potential of a substance to cause addiction. Abuse liability of a drug is the likelihood that its use will result in addiction (dependence) and it can be assessed in laboratories by methods referred to as abuse liability testing (Schuster and Henningfield 2003, Wayne and Henningfield 2008b, WHO 2003).

The terms “dependence-causing” and “dependence potential” have been used as synonyms for “addictive” and “addictiveness”, respectively. In addition to the neurobiological characteristics of the substance itself, dependence potential is related to the dose, speed of absorption, metabolism, and to physical and chemical features of the formulation (WHO 2007b).

3.3.4. Attractiveness

According to the WHO, the terms “attractiveness” or “consumer appeal” refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use (WHO 2007b). Physical product characteristics are often integrated with marketing (WHO 2007b). For example, a flavour such as “menthol”, “mint”, or “cherry”, which is intended to appeal to a target population, may be incorporated into the product name or descriptors and marketed to reach out to that population (WHO 2007b). Attractiveness is also related to nicotine dosing characteristics, which is why smokeless tobacco product companies may include products ranging from lower dosing and slower onsetting “starter” products to higher dose maintenance products (FDA 1995, FDA 1996).

Although the risk of dependence on any substance is partially related to the attractiveness and/or ease of use of the delivery system, these features are not typically evaluated in dependence-potential testing but rather are generally described as factors affecting “consumer appeal” or “attractiveness”. Addictiveness and attractiveness go hand in hand as the real world liability for abuse of and addiction to a tobacco product is to a large extent also related to the attractiveness of the tobacco product.

Attractiveness is powerfully determined by imagery and cultural associations that are cultivated by the tobacco industry and effects may therefore be indirect. Attractiveness is also influenced by product sensory characteristics using flavours, and product characteristics (as well as marketing) that are intended to reduce concerns or undesirable features (e.g. reduce concerns about cancer with “light” branding, and reduce noxious throat burn with various chemicals and “smoke smoothers”) (Wayne and Henningfield 2008b).

3.4. Tobacco - manufacturing process

The manufacturing process for cigarettes has been described in several publications (Davis and Nielsen 2006, Hoffmann and Hoffmann 1997, IARC 2004, Wigand 2006). However, while the exact composition of each brand remains a trade secret, according to the Tobacco Products Directive (2001/37/EC) tobacco industries have to report the full list of additives in tobacco products, including the exact amount, to the competent authorities in the Member States.

Both the make-up of cigarettes and the composition of cigarette smoke have gradually changed in the last 50-60 years, including the use of a larger range of additives. The sales-weighted average “tar” and nicotine yields have declined. These changes have been primarily achieved by the introduction of filter tips, with and without perforation, selection of tobacco types and varieties, utilization of highly porous cigarette paper, and incorporation into the tobacco blend of reconstituted tobacco, opened and cut ribs, and “expanded tobacco” together with the use of a large number of additives/ingredients. At least four of the physical parameters of cigarettes have a decisive influence on smoke yields. These are the length of a cigarette, its circumference, the cut of the tobacco, and the packing density (Hoffmann and Hoffmann 1997). Agronomic factors such as production practices and soil characteristics, and environmental conditions such as rainfall, reportedly influence the accumulation of metals, including cadmium, beryllium, chromium, nickel and arsenic in the leaf.

Commercial tobacco products are predominantly produced from *Nicotiana tabacum*, while *Nicotiana rustica* is used on a limited commercial scale. Within the species *N. tabacum* one distinguishes four types: bright (Virginia), Burley, Maryland, and Turkish tobaccos. Bright tobacco is flue-cured by drying with artificial heat; Burley and Maryland tobaccos are air-cured; Turkish tobaccos are sun-cured. The properties of tobacco are based primarily on curing methods, locality of growth, position on the stalk from which the leaves have originated and factors such as colour quality and ripeness at harvest. Curing is the process for drying freshly harvested tobacco with partially or fully controlled temperature and moisture schedules. Freshly cured leaf is then threshed to separate stem from lamina, sometimes blended with other tobacco lamina and then re-dried to a uniform moisture level then packed into bales or hogsheads.

Virginia tobacco leaves contain a higher carbohydrate (e.g. sugars) level and lower nitrogen level than Burley leaves. The natural drying of the Burley leaves at relatively low temperatures allows plant respiration which continues to consume sugars during the process, leaving negligible sucrose and reducing sugars in the cured leaf. Burley leaves contain higher levels of nitrogen than Virginia leaves. The smoke of Virginia or flue-cured leaves is more aromatic and less alkaline than that of Burley tobacco, with a slight acidic taste resulting from the high levels of natural sugars. Burley tobacco produces a more alkaline smoke than flue-cured tobacco (Weeks 1999) and therefore imparts a bitter aroma and taste to cigarettes. Oriental leaves tend to have a low nitrogen content and

moderate levels of carbohydrates, but fewer proteins, than the other varieties (Philip Morris 2010, Wolfe 1962).

A comprehensive integrated pest management programme is used to avoid insect infestation, e.g. chemical fumigation. The tobacco then undergoes aging and fermentation, usually for 1-3 years.

For the manufacture of cigarettes, specific tobacco blends utilizing desired tobacco types are prepared. Blending is the selection and thorough mixing of the tobacco-based components plus any associated casings, humectants and flavouring required for a particular product or brand. The tobacco based components may include the leaf lamina, cut and rolled stem, reconstituted sheet and expanded tobacco.

The tobaccos stored in bales are broken up, cut into specific dimensions, and combined with other blend components such as casing and top dressing, and adjustment of the moisture content. American blend cigarettes contain the four types of tobacco mentioned above plus reconstituted or homogenised sheet tobacco. This is made from tobacco dust, fines and particles, and leaf ribs and stems (IARC 2004). Reconstituted tobacco or homogenised sheet tobacco is a paper-like sheet approaching the thickness of tobacco laminae. It is made from tobacco dust, fines, and particles, and from ribs and stems; various additives may be incorporated. In the past, most of these “tobacco by-products” were wasted. The introduction of reconstituted tobacco or RECON is the primary means by which ammonia chemistry and other chemicals are introduced into a cigarette. Expansion is a process which increases the shred filling power, e.g. puffed tobacco. Puffed, expanded, and freeze-dried tobaccos are modified preparations of cigarette tobacco and have up to twice the filling power, thus requiring less tobacco per cigarette. The principle applied here is to expand the tobacco cell walls by quick evaporation of water and other agents that readily volatilize.

Blending is carried out to achieve specific pH, taste, burning characteristics, and nicotine content. The pH strongly influences the concentration of free (i.e. non-protonated) nicotine in tobacco smoke, whereas the nitrate content influences the carcinogenic potential of smoke (IARC 2004).

Table 2 presents the classification of tobacco types based on curing methods and function.

Table 2 Classification of tobacco types based mainly on curing methods (IARC 2004, US Department of Agriculture 2001)

Tobacco type	Characteristics/ alternate names	Main use
Flue-cured	Leaves are yellow, blond, bright therefore also called Bright or Virginia	Cigarettes and also roll your own (RYO) cigarettes and pipe tobacco
Fire-cured	Light to dark brown cured over open fires (Kentucky)	RYO, chewing tobacco, cigars and smoking tobacco
Light air-cured	Burley (cured without supplementary heat) Maryland Perique	Mainly in cigarettes (also RYO, pipe tobacco and cigars) Cigarettes Pipe tobacco
Dark air-cured	Light to medium brown	Chewing tobacco and snuff, snus, dark cigarettes
Sun-cured	Oriental tobacco varieties Latakia	Turkish cigarettes (also RYO and pipe tobacco) Some pipe tobaccos
Cigar filler, Cigar binder, Cigar wrapper	Tobacco types for use as cigar fillers, binders and wrappers	Used for cigars

Two principal types of commercial cigarettes have traditionally been sold throughout the world: (i) American Blend cigarettes, which are made from a blend of Virginia, Burley and Oriental tobaccos; and (ii) Virginia cigarettes, which contain exclusively Virginia tobacco.

Casing refers to the sauce composed of a variety of ingredients such as humectants, sugars, cocoa, liquorice and fruit extracts (Hoffmann and Hoffmann 1997). The basic material of casing for reducing harshness is sugar. A commercial solution of tannin also sweetens and softens the smoke of tobacco. The best known example of an additive that changes markedly and even masks the taste of tobacco is the use of cloves. Addition of menthol is another example, but in this case the tobacco taste is still discernible. Burley leaf has the ability to absorb up to 25% of its weight of added material (Akehurst 1981).

Casings are usually applied to tobacco strips or leaf early in the primary processing scheme to tone down or mute the strength or harshness of tobacco smoke, improve processibility of tobacco and add deep flavour notes to the smoke. Casings are traditionally added to US blended styles of product that contain significant proportions of Burley type tobacco blends. These casings are added to the Burley tobacco line through the means of the casing cylinder or Cased Leaf Dryer.

Ammonia technology has been used with US blended styles of products containing cased Burley tobacco. Ammonium salts could be added at the Cased Leaf Dryer (CLD) stage or with the manufactured reconstituted tobaccos.

There are no fixed rules as to where humectants, flavours and flavourings are added to the processed tobacco but generally the more volatile ingredients are added as late as possible during tobacco processing to prevent losses. Those tobacco blends that contain flavours and flavourings are usually held in a bin to allow for equilibration across the blend before it is passed to the making machine as the final blend. Top flavourings are generally applied to the total tobacco blend as one of the last steps in processing. They

are usually carried in an alcohol base. They are used to improve quality of smoke, impart a pleasant pack aroma and side-stream aroma. Menthol may be added at any of the following stages; spraying onto the final blend, through addition to the filter via a thread, or by application to the cigarette paper or the foil used to wrap the cigarettes. Due to the high level of volatility of menthol, different manufacturers have over the years developed a variety of methods for producing mentholated products that are as consistent as possible in terms of their finished product menthol levels (BAT 2010).

In cigarettes, flavours may be added to tobacco, cigarette paper, or the filter, in a plastic pellet placed in the filter or the foil wrapper, in an attempt to enhance the tobacco flavour, mask unpleasant odour, and deliver a pleasant cigarette-pack aroma. Internal industry documents reveal additional flavour technologies such as flavour microencapsulation in the paper, carbon beads, and polymer-based flavour fibres inserted into the filter, flavoured tipping etc. (WHO 2007b).

As described above, the physical elements of the cigarette such as packing density, particle size distribution, rag cut per inch, colour appearance, resistance to draw, the appropriate paper, filter, tobacco type and the final tobacco blend, are carefully controlled (Wigand 2006). The final product is manufactured using high speed automated machines.

Over the years the tobacco industry has developed genetically modified (GM) tobacco plants with an aim, among others, to manipulate nicotine levels (Dunsby and Bero 2004). Reductions of nicotine levels have been in the range of 80-98%.

Philip Morris sought to use anti-sense biotechnology to disrupt enzymes involved in nicotine biosynthesis (US Patent 5684241). In 2003, Vector Tobacco began marketing a new cigarette that is produced from GM tobacco containing trace amounts of nicotine. The GM plant was produced by disrupting expression of the gene for quinolinate phosphoribosyl transferase, which encodes one of the rate-limiting enzymes in the nicotine biosynthetic pathway (Bonetta 2001). Vector Tobacco market Quest Cigarettes, which exist in three forms, ranging in nicotine content from 0.6 mg per cigarette to 0.05 mg per cigarette. They are marketed as a smoking cessation or reduction aid, with the manufacturer claiming that graded reduction of nicotine exposure through the gradual use of increasingly lower nicotine content cigarettes will lead to the eventual extinction of nicotine dependence and conditioned associations with related cues (Bonetta 2001).

Large scale field-trials have also been conducted despite consumer opposition and fear of tobacco growers that GM crops would be turned down by several countries.

3.4.1. Conclusions on manufacturing

Cigarettes, which are the predominant tobacco product, are highly engineered nicotine delivery devices that are mass produced by the major industries by integrated automation.

The properties of tobacco products depend on locality of growth, position of leaves on the stalk, ripeness and curing method. The different curing methods (drying procedures) determine the sugar content and colour of the tobacco leaves. During the manufacturing process of cigarettes, a number of substances are added at different stages for various reasons, such as providing consistency of the product, creating a unique brand, and promoting attractiveness.

3.5. Technical characteristics of cigarettes

Parts of cigarettes, like the paper and filter have technical features which affect the constitution of main-stream and side-stream smoke.

3.5.1. Introduction

Considering the natural origin of tobacco leaves, their content will, both qualitatively and quantitatively, depend on the season, local weather conditions and geographical origin. Consumers do not like to smoke a product that changes over time, i.e. smoking a constant product is preferred. In order to produce a constant product, i.e. to mask the batch to batch variation in taste, tobacco companies use a large variety of additives in the manufacture of tobacco products. In addition, the tobacco companies strongly prefer to maintain the same TNCO values (tar, nicotine and carbon monoxide) of their products. To achieve consistency in TNCO values, tobacco producers change, amongst others, the ventilation of the products. The ventilation through the filter can be increased by punching more (or wider) ventilation holes. The ventilation of a cigarette can also be changed by using commercially available cigarette paper wraps with another grade of porosity.

Relevant technical characteristics of cigarettes are the following:

- Ventilation of the paper (paper porosity);
- Ventilation holes in the filter;
- Ventilation holes in the paper wrap;
- Packing of tobacco (dense or loose); and
- Geometry (length, diameter).

Ventilation

Large efforts have been made by the tobacco industry to investigate the effect of ventilation on the size distribution of the smoke aerosol. Depending on the size, the smoke particles enter and deposit at different levels of the airways (upper or lower airways). The purpose of this research was either to enhance the absorption of nicotine, to decrease the toxic potential of the product or to manipulate the taste of the smoke.

The main effect of ventilation is the dilution of the tobacco smoke. As such, the concentration of smoke components is reduced which not only leads to a lower dose of nicotine, but also to a lower concentration of other (toxic) components. It appears, however, that smokers compensate for the lower dose of nicotine per puff (due to increased ventilation) by increasing their puff volume, puff frequency, and deeper inhalation of the smoke (Jarvis et al. 2001, Scherer 1999). Many other smokers consciously or unconsciously block a part of the ventilation holes with their fingers so that more concentrated smoke is inhaled.

Another feature of ventilation is that it may affect the particle size and particle size distribution of the smoke aerosol, i.e. increasing the ventilation is supposed to decrease the mean particle size of the aerosol. It is difficult to assess whether an increase in ventilation indeed reduces the particle size as only few studies are reported in publicly available literature.

3.5.2. Technical limitations

It is difficult to determine the size of the particles and their distribution in cigarette smoke, mainly because the half-life of the particles is very short (0.1-1 sec). Rapid ageing of the aerosol results in larger particles as they have time to coalesce, i.e. a secondary aerosol containing larger particles at the expense of smaller particles is rapidly formed (Harris and Kay 1959). Therefore, only sophisticated on-line sampling and detection allows a proper measurement of the particle distribution of the smoke aerosol. Obviously, these techniques require large financial resources and highly qualified technical personnel.

A number of variables other than ventilation may affect the particle size; moisture of the cigarette (relative humidity), puff volume, puff number (e.g. first or last puff), butt

length, length of the cigarette, electrostatic charges, etc. Different unities are used in the studies to express the size of the particles (mean diameter, count median aerodynamic diameter, mass median aerodynamic diameter) which hampers quantitative comparison of the data. The aerosol is produced during burning, i.e. directly behind the burning cone at the tip of the cigarette the superheated vapour condenses and forms an aerosol; the longer the aerosol stays in the cigarette, the larger the size of the particles.

Due to the number of different particle sizing methods, instrumentation and sampling and detection techniques applied, as well as differences in the cigarettes and smoking conditions, variable results are found and the results of different investigations are difficult to compare. Important limiting factors for many techniques are low time of resolution and the ageing of the smoke. Over time various methods have been developed to improve the accuracy of the measurements.

3.5.3. Smoke particles

Particle size may be relevant for the absorption of nicotine into the bloodstream.

Cigarette smoke particle size has generally been reported with mass median diameter (MMD) in the size range of 0.3-0.5 μm and count median diameter (CMD) in the range of 0.2-0.4 μm (Bernstein 2004, Wayne et al. 2008a). Particles larger than 1 μm are mostly trapped within the cigarette, whereas ultra-fine particles (less than 0.1 μm - nano-particle range) probably will adhere to the surface of the paper, tobacco and filter, or coagulate into larger particles (Stratton et al. 2001), see section 3.5.4. Differences in particle size found in many studies were quite small and some internal tobacco documents concluded that the measurable influence of conventional design changes was insignificant (Philip Morris 1991, Wayne et al. 2008a). Of the four variables applied by Philip Morris to change the size of the particles (filler, filter, paper and ventilation) only ventilation had any significant effect (Cox et al. 1992). In addition, butt length and puff volume affect the size of the particles. There is a clear trend of decreased size of the particles at shorter butt lengths; the average size at 20 mm was 0.29 μm and at 55 mm it was 0.34 μm . Cox et al. (1992), taking all the variables mentioned above into account, reported deviations of about 10 to 30%. Surface mean diameter increased from 0.32 to 0.42 μm when the ventilation was increased from 0 to 60%. Based on their results, Cox et al. (1992) suggested that aerosol coagulation in the cigarette rod is the main mechanism for change in particle size.

Bernstein (2004) reviewed the available data of the tobacco smoke particulates which go back to 1950s. The main findings include:

- No difference in particle size between plain (non-filter) and filter cigarettes.
- Particle size depends on puff number (e.g. first vs. last puff).
- Relative humidity of the tobacco does not affect or only marginally affects particle size.
- Aged tobacco smoke contains larger particles than fresh smoke.

Considering all the studies reviewed by Bernstein, the size of the smoke particles range roughly from 0.17 to 0.60 μm , either expressed as CMD or MMD.

A study by McCusker et al. (1983) compares mass median aerodynamic diameter of ultra-low-tar, low-tar and medium-tar rated cigarettes (with and without filter). Particle size was less than 0.6 μm and not affected by the cigarette filters. Among the 10 brands tested ventilation ranged from 22 to 94%. The mass median aerodynamic diameter ranged from 0.36 μm to 0.56 μm , but did not correlate with ventilation efficiency. The number of particles was, however, reduced by 20–90% by applying the commercial filters and the particles were present in the higher puff numbers. Interestingly, blocking of the ventilation holes on the filters of ultra-low-tar cigarettes increased the particle concentration. This is explained by the longer residence time (longer transit time from cone to filter) of the newly formed particles in the cigarette rod.

As mentioned in section 3.5.2, only sophisticated on-line sampling and detection allows a proper assessment of the particle size and distribution. Moreover, the relevance of ultra-fine particles for nicotine absorption has only been taken seriously for the last two decades; therefore, most of the older studies did not focus on the presence of ultra-fine particles.

Recently, using on-line measurement of the particle size (range measured 5–1000 nm), Adam et al. (2009) reported that non-ventilated cigarettes smoked under an intense regime, which includes blocking the ventilation holes resulted in a count median diameter of 0.18 μm , whereas 70% ventilated cigarettes smoked under a milder standard smoking regime led to a diameter of 0.28 μm . The particle size of mainstream smoke of Virginia cigarettes, smoked under a standard smoking regime, was 0.22 μm and 0.25 μm at 0 and 70% ventilation, respectively. For the intense smoking regime the respective particle sizes were 0.18 and 0.22 μm . Interestingly, when the ventilation was increased from 0 to 70% the total number of particles decreased dramatically from 2.3×10^{12} to 0.3×10^{12} , and the total mass of particles dropped from 17.2 to 2.3 mg (standard smoking regime). In another recent paper by Gowadia et al. (2009) the particle size (mass median aerodynamic diameter) was found to be approximately constant (0.9–1.0 μm) for three different puffing regimes. The smoke was collected in a conditioning chamber and the particle size distribution was determined by UV spectrometry.

The particle size of waterpipe smoke was shown to be somewhat smaller than that of cigarette smoke. Monn et al. (2007) reported waterpipe smoke particle median diameter of 40 nm in a full smoking set containing charcoal, tobacco and water; the smoke of the heated tobacco alone ranged from 10 nm to 200 nm while the burning of charcoal was mostly responsible for the particles smaller than 50 nm. Fromme and colleagues found two phases of particle emission during a waterpipe session; when the charcoal was lit, the particle diameter was around 100 nm and during the smoking session it decreased to 17 nm (Fromme et al. 2009). Daher et al. (2010) found similar particle sizes to the Monn study in side-stream smoke from waterpipes, which were significantly smaller than particle sizes in side-stream smoke from cigarettes with a median diameter of 139 nm and a large number of particles smaller than 100 nm.

3.5.4. Deposition of particles

Although the size of the particle is an important factor for the deposition in the lung, the relationship between particle size and deposition in the lung is complex and factors other than size alone, such as respiration rate, depth of inhalation and flow rate, affect lung deposition (Sarangapani and Wexler 2000).

In figure 1 the relative deposition of particles (dependent on the aerodynamic diameter) in humans is depicted. Particles larger than 1 μm will mainly deposit in the extra-thoracic region. Smaller particles will deposit in different regions, but the general statement that smaller particles deposit deeper in the lung is not entirely true. Very small particles (a few nm) will mainly deposit in the extra-thoracic region. Peak alveolar deposition is around 30-20 nm and becomes less important at sizes less than 8-9 nm (ICRP 1994, Oberdörster et al. 2005). The question whether the ultra-fine particle size is relevant for mainstream tobacco smoke is unanswered. From a theoretical point of view removal of ultra-fine particles is to be expected due to adherence to the surface of the paper or to the tobacco and filter, or due to coagulation into larger particles (Stratton et al. 2001) (see section 3.5.3), however this needs to be confirmed experimentally.

Other points of concern in the inhalation of ultra-fine particles are the translocation of these particles: (1) from the lumen of the lung to the circulation; and (2) from the olfactory nerve endings in the nose to the brain. These two events have been described for several solid nanoparticles in the lungs of animals and humans (Kreyling et al. 2002, Nemmar et al. 2002), and in the noses of rodents (Oberdörster et al. 2002). These phenomena have not been shown for tobacco smoke derived particles which are not solid nanoparticles (although combustion derived particles have been studied in the lung);

therefore, only theoretical/hypothetical considerations can be made (which fall outside the scope of this opinion).

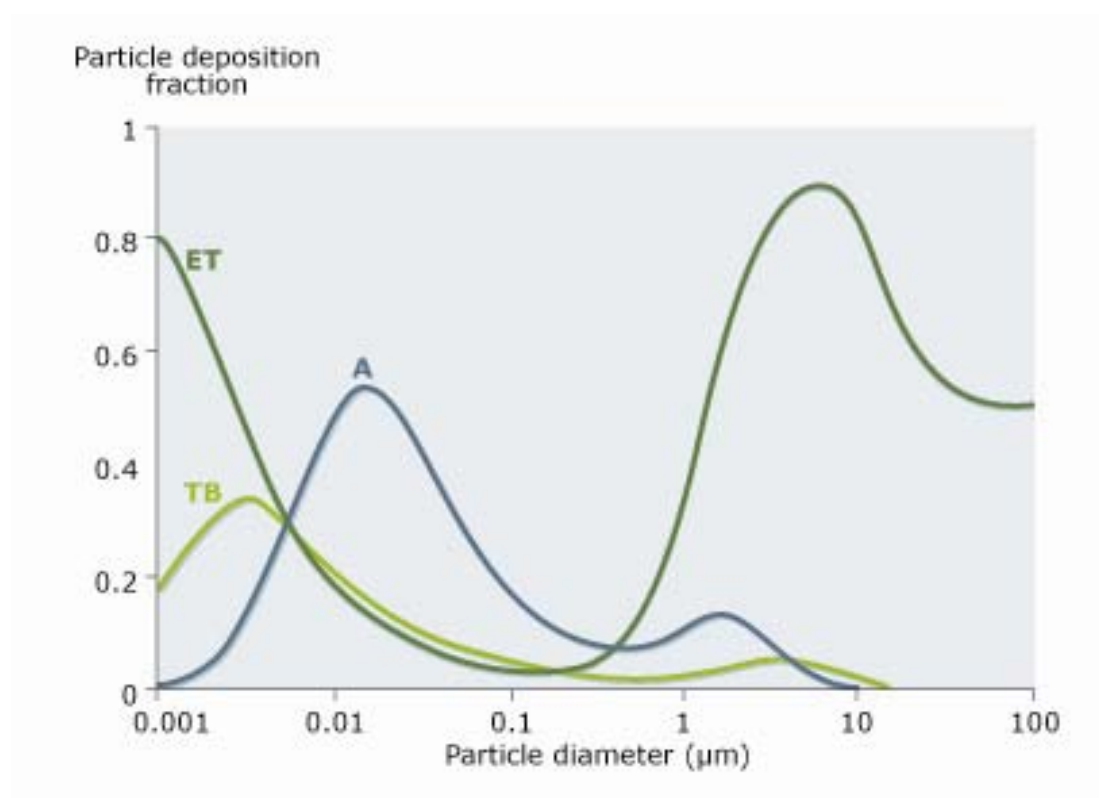


Figure 1 Predicted deposition of inhaled particles during nose breathing. Fractional deposition in: extrathoracic (ET); trachea-bronchial (TB); and alveolar (A) regions (adapted from ICRP 1994).

3.5.5. Light cigarettes as an example of cigarettes with high ventilation

The best known application of changing ventilation is the development of light cigarettes. Light cigarettes have been marketed as products with a lower health risk as they should deliver less tar and other toxic compounds in the smoke inhaled. As will be described in detail in section 3.10.1 many smokers of light cigarettes inhale the smoke deeper and increase the number of puffs, so the health risks are probably not lower than for smokers of regular cigarettes (Frost et al. 1995). Animal studies have shown that self-administration of a low dose of nicotine at a high frequency gives a more reinforcing effect as compared to self-administration of a higher dose at a low frequency (in this comparison total dose self-administered is the same) (Harris et al. 2008, Harris et al. 2009, O'Dell et al. 2007).

3.5.6. Conclusions on technical characteristics

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and CO) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Smokers usually compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. Data obtained in animal studies suggest that cigarettes with high ventilation (often described as “light” or “low tar”) may favour addiction to nicotine in the smokers of these products, because of an increased smoking frequency.

The particle size of smoke aerosol of commercial cigarettes is around 0.4 to 2 μm . A large fraction of ultrafine particles ($<0.1 \mu\text{m}$) probably adheres to the surface of the paper or the filter, or coagulates into larger particles, and will thus not be present in the smoke as such. The small smoke particles (submicron meter range) will enter the lower airways and alveoli, while larger particles (micron meter range) will be deposited increasingly in the upper airways.

Considering the manufacturing of cigarettes, the change of the technical characteristics of cigarettes may affect the mean particle size and, therefore, the distribution of the smoke aerosol. However, based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol.

3.6. Nicotine

3.6.1. Pharmacological effects (incl. metabolism of nicotine)

3.6.1.1. Brief historical overview

Nicotine is the principal component alkaloid of tobacco, occurring throughout the plant (*Nicotiana tabacum*), especially in the leaves. The plant and the compound are named after Jean Nicot, a French ambassador to Portugal, who sent tobacco seeds to Paris in 1550. Crude nicotine was known by 1571, and the compound was obtained in purified form in 1828; the correct molecular formula was established in 1843, and the first laboratory synthesis was reported in 1904. It is one of the few liquid alkaloids; colourless and extremely toxic. Nicotine is commercially obtained from tobacco scraps; it has been used as an insecticide and as a veterinary vermifuge.

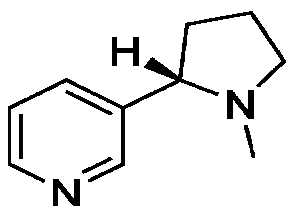


Figure 2 Structure of nicotine (CAS number 54-11-5)

3.6.1.2. General pharmacodynamic (physiological) effects

Nicotine administration induces a series of multifaceted effects which show great interindividual variability, i.e. the effects vary greatly from person to person. This is reflected in a non-linear and complex dose-response relationship ensuing from a summation of stimulatory and inhibitory actions in the central and peripheral nervous systems.

Low doses of nicotine, including those in the range of inhaled cigarette smoke (1-2 mg), produce stimulation of ganglionic neurotransmission (vegetative ganglia). This generates a complex response which results from a mix of sympathetic and parasympathetic actions. Thus, tachycardia and rise of blood pressure are to a large extent the consequence of sympathetic ganglia activation that induces an increased adrenaline release in the adrenal medulla (via splanchnic nerve stimulation). At the same time, the nicotine action on the carotid and aortic chemoreceptors and on the brain regulating centres modifies the cardiovascular effects determining the great variability observed in the final response. Therefore, the direct nicotine effects on heart rate and blood pressure are rapidly counterbalanced by the peripheral and central cardiovascular compensatory reflexes. Similarly, nicotine-induced activation of parasympathetic ganglia and cholinergic terminals causes an increase of the gastrointestinal peristalsis. In susceptible subjects, first doses may cause nausea, vomiting and related effects of hypercholinergic

activation. Nicotine also increases blood glucose levels and the activity of exocrine glands. In the brain, nicotine is clearly a stimulant at low doses. It produces a pattern of alertness in the electroencephalogram (EEG), mediates fast synaptic transmission, and positively modulates a range of cognitive functions. As a result, it improves attention, learning, arousal, motor skill, facilitates memory functions and decreases irritability and anxiety, among other central nervous system (CNS) functions (Balfour and Fagerström 1996, Benowitz 2008, Fattinger et al. 1997, Grybko et al. 2010).

An important pharmacological characteristic of nicotine is the rapid development of tolerance to its unwanted effects. Although there is a great individual variability, in many cases tolerance to the peripheral effects appears a few days after the first exposure (Benowitz 2008).

3.6.1.3. Toxicity effects

At high doses, after the initial stimulation, nicotine rapidly produces a ganglionic blockade due to the inhibition of transmission, which is a consequence of a persistent depolarisation of all autonomic ganglia. This depression of all autonomic ganglia results in bradycardia, hypotension, impairment of adrenaline release, etc. Similarly, a biphasic nicotine-induced action is also observed in the adrenal medulla (a discharge of catecholamines is evoked by small doses whilst their release is blocked by larger doses). It should be noted that most peripheral effects are influenced by compensatory reflexes. In the CNS large doses induce a generalised mental depression, tremors, nausea, and convulsions. The acute lethal dose of nicotine in an adult human is estimated to be about 60 mg (Benowitz 2008, García-Estrada and Fischman 1977, Solarino et al. 2010). This dose (less than 1 mg/kg) is derived from old reported cases of intoxication when nicotine was widely used as an insecticide (Grusz-Harday 1967, Lockhart 1939). In rats the LD₅₀ is ~50 mg/kg and in mice ~3 mg/kg (Okamoto et al. 1994). Acute nicotine poisoning has occurred in children who accidentally ingest tobacco or are occupationally exposed to wet tobacco leaves. Children have played a role, and they continue to do so in many places, in agricultural production of tobacco, where absorption of nicotine from the plant is likely to happen. This nicotine-induced acute condition is known as green tobacco sickness. Clinical features are similar to those observed in adults (Gehlbach et al. 1974, McKnight and Spiller 2005).

Ingestion of tobacco products is a major reason for infant and child toxic exposures reported to poison control centres. The large majority (90%) of such accidental poisonings in the population involve children up to 6 years of age (Connolly et al. 2010). However, ingestion of cigarettes and cigarette butts by children aged ≤ 6 years resulted in minor toxic effects (CDC 1997).

Malizia et al. (1983) described four children who ingested two cigarettes each and developed salivation, vomiting, diarrhoea, tachypnoea, tachycardia, and hypotension within 30 minutes, and depressed respiration and cardiac arrhythmias within 40 minutes. Convulsions occurred within 60 minutes of ingestion. All recovered after gastric lavage with activated charcoal, intermittent positive pressure ventilation, and 5 mg diazepam intravenously for convulsions.

A prospective review of 51 cases of tobacco ingestion and five cases of nicotine resin chewing gum exposure was conducted to evaluate the incidence and degree of toxicity caused by these products in children. A dose-response relationship was observed for cigarette exposures. Nine of 10 children ingesting more than one cigarette or three cigarette butts developed signs or symptoms (Smolinske et al. 1988).

3.6.1.4. The nicotinic acetylcholine receptor

Nicotine acts on a class of cholinergic receptors which are ligand-gated ion channels (nicotine acetylcholine receptors: nAChR). These kinds of receptors are structurally similar to the ones operated by GABA, glycine, glutamate, 5-HT₃, etc. Nicotine binding to the nAChR opens the channel and increases its ionic permeability for monovalent cations

(Na⁺, K⁺) and divalent cations (Ca²⁺, Mg²⁺), although with difficulty for the latter and depending on the subtype of nAChR. Neuronal nAChR embrace a conjunct of at least 20 homologous subtypes that mediate fast synaptic transmission throughout the central and peripheral nervous systems (Xiu et al. 2009).

Neuronal nAChR are pentamers of homomeric or heteromeric combinations of α (α_2 to α_{10}) and β (β_2 to β_4) subunits, which possess different pharmacological and biophysical properties and locations in the brain (Gotti et al. 2006).

The nAChRs in the CNS are localised both in postsynaptic and presynaptic neural membranes. Studies in recent years have shown that the primary site of nicotine action is presynaptic, and that nAChRs facilitate the release of neurotransmitters when localised in non-cholinergic terminals. In fact, nAChRs are present in the terminals of most of the neurotransmitter systems (GABAergic, glycinergic, glutamatergic, dopaminergic, serotonergic, etc.). Likewise, nAChRs have been identified, in different densities, in most of the brain areas.

Nine individual subunits of nAChRs in the human brain have been identified and cloned, and they combine in various conformations to form individual receptor subunits. The structure of individual receptors and the subtype composition are not completely understood. Only a finite number of naturally occurring functional nAChR constructs have been identified (Luetje 2004).

The pentameric structure of the neuronal nAChR and the considerable molecular diversity of its subunits offer the possibility of a large number of nAChRs with different physiological properties. The stoichiometry of most nAChRs in the brain is still uncertain (Kuryatov et al. 2000).

For example, the neuronal nAChR subunits on presynaptic terminals of dopamine neurons projecting to the striatum have been fully defined (Luetje 2004), as has the complete subunit composition of four major presynaptic nAChR subtypes in the striatum (Salminen et al. 2004).

It should also be noted that chronic exposure to nicotine induces a marked increase in the density of nAChRs in most neurotransmitter systems and brain areas (Walsh et al. 2008).

3.6.1.5. Nicotine pharmacokinetics and metabolism

Nicotine as a weak base (pK_a = 8.0) is rapidly absorbed across biological membranes where the pH is at physiological (7.4) or slightly alkaline levels. This is the case for nicotine in cigarette smoke when it reaches the lung alveoli (Pankow et al. 2003). The average nicotine content of a cigarette (6-10 mg) delivers about 1 mg of nicotine (0.5-2 mg) systemically through the smoker's lungs (Henningfield et al. 1993). The pulmonary bioavailability (the amount absorbed from smoke) of inhaled nicotine is 80-90%. After inhalation it reaches high levels in the brain within 10-20 seconds, thus being equivalent to, or even faster than, an intravenous administration (Gourlay and Benowitz 1997, Hukkanen et al. 2005). In both cases the hepatic first-pass effect (metabolism) is avoided allowing higher levels of unmetabolised nicotine to be delivered to the brain. In addition, nicotine easily crosses the blood-brain barrier.

In contrast, the buccal and gastric bioavailability of nicotine is low (20-40%) due to the acidic environment at which nicotine is protonated and therefore poorly absorbed through local membranes. Better absorption is obtained in the intestinal mucosa because of its alkaline pH. The liver first-pass metabolism contributes to the impairment of the bioavailability to a great extent. The time of nicotine blood maximal concentration for oral administrations is about 60-90 min. Nicotine bioavailability through the skin is high (75-100%).

Nicotine is widely distributed in the body (liver, kidney, lungs, etc.; with adipose tissue showing the lowest affinity). Brain tissue exhibits a high affinity for nicotine. It has been

reported that nAChR binding capacity for nicotine is increased in smokers compared to non smokers (Breese et al. 1997, Perry et al. 1999). This reflects the higher density of nAChRs in the brain of smokers (nicotine-induced up-regulation of nAChRs). However, the quantity of nicotine delivered from the tobacco product which reaches the brain is higher in non dependent smokers than in heavy smokers (Rose et al. 2010a).

The blood half-life ($t_{1/2}$) of nicotine after cigarette smoking or intravenous administration is about 2 hours ($t_{1/2}$ = 100-150 min). The disposition of nicotine shows a multiexponential elimination (Hukkanen et al. 2005). However cotinine, the main metabolite of nicotine, has a $t_{1/2}$ $\approx$ 19 hours. It was found recently that every puff of a cigarette induces a peak of nicotine in the arterial blood (Berridge et al. 2010) with a $t_{1/2}$ of 45 seconds, but that these peaks do not occur in the brain (Rose et al. 2010a). This finding rules out that the lack of efficacy of nicotine replacement therapy (NRT) (e.g. gums or patches) is due to a continuous delivery of nicotine. In the liver nicotine is mostly metabolised in the endoplasmic reticulum by the cytochrome P450 (CYP) system, mainly by CYP2A6 and CYP2B6. The major metabolite produced by CYP through nicotine oxidation is cotinine, which is further converted to cotinine glucuronide and other metabolites. It should be noted that CYP oxidative metabolism of nicotine to cotinine and its glucuronide conjugation are inhibited by menthol, a commonly used cigarette additive. The pathway of nicotine to cotinine represents around 70-80% of nicotine biotransformation in humans and, therefore, is commonly used as a quantitative biomarker of nicotine exposure as well as of CYP2A6 metabolic activity, which exhibits an important variation in function in humans (Benowitz 2008, Dempsey et al. 2004, Hukkanen et al. 2005, Hukkanen et al. 2010). Many other minor metabolites of nicotine are produced by CYP, glucuronidation, demethylation and other enzymatic pathways. These metabolites have no nicotinic activity, with the exception of nornicotine which is produced by N-demethylation of nicotine in humans and other mammals (besides being a major tobacco leaf alkaloid). Although nornicotine is a minor metabolite, it has been shown that after repeated nicotine administration it accumulates in the brain at pharmacologically relevant concentrations acting as agonist on nAChRs but with about 10-fold lower potency (Dwoskin et al. 2001, Hukkanen et al. 2005).

Renal excretion is the major route of elimination of nicotine and its metabolites (>90% of a dose). Unchanged nicotine accounts for about 10%, and nicotine glucuronide and nicotine N'-oxide for about 5% each, of the total nicotine-derived amount present in urine. Trans-3'-hydroxycotinine (35-40%) and its glucuronide (~10%) are the principal nicotine metabolites determined in urine, both after a single dose and in smokers; unchanged cotinine (10-15%), cotinine glucuronide (~15%) and cotinine N'-oxide (~4%) represent the rest of the cotinine metabolic pathway excreted. Small amounts of a large array of nicotine metabolites produced in the minor biotransformation pathways are also detected in urine. Nevertheless, the pattern of nicotine metabolites and their amounts are highly variable in humans due to the important polymorphism of CYPs and the other enzymatic pathways involved in the metabolic disposition of xenobiotics (Benowitz et al. 2006, Benowitz 2008, Hukkanen et al. 2005). It has been suggested that this genetic variation in xenobiotic metabolism, especially that of CYP2A6, has a role in smoking behaviour and nicotine dependence (Malaiyandi et al. 2005).

3.6.1.6. Conclusions on nicotine pharmacology

The main effect of nicotine (besides its action on the cholinergic system) is the presynaptic release in the brain of neurotransmitters such as acetylcholine, noradrenaline, dopamine, serotonin, glutamate, GABA and opioid peptides. This allows the possibility that many compounds may modify the action of nicotine on the presynaptic nicotine receptors, and consequently modify the activity of nicotine in the brain. There is substantial interindividual variability in the action and metabolism of nicotine and many aspects of its pharmacology are still not fully understood.

Nicotine metabolism may be modified by compounds inducing or inhibiting the activity of the cytochrome P450 system and other metabolic pathways, thus determining

pharmacokinetic changes. While the half-life of nicotine in the arterial blood is short, nicotine levels in the brain remain at high levels for much longer.

3.6.2. Addictive properties of nicotine

Nicotine exposure produces adaptive changes in the central nervous system (CNS) leading to an addictive process characterised by compulsive tobacco use, loss of control over tobacco consumption despite the harmful effects, the appearance of withdrawal symptoms upon the cessation of tobacco smoking, and relapse after periods of abstinence (McLellan et al. 2000). As in other addictive processes, the initiation of nicotine addiction has been related to its capacity to induce rewarding/reinforcing effects. However, the negative consequences of nicotine abstinence have a crucial motivational significance for maintenance and relapse of this addictive behaviour (Koob and Le Moal 2008). The terms “reward” and “reinforcement” are often misused and confused. Reward describes stimuli that have appetitive (desirable) consequences and/or produce a hypothetical pleasurable internal state (hedonia). Reinforcement refers to the ability of a stimulus to promote behavioural responses in order to obtain (positive reinforcement) or to avoid (negative reinforcement) such a stimulus. A drug like nicotine that produces rewarding effects will also promote behavioural responses to obtain the drug, i.e. positive reinforcing effects. On the other hand, the effects induced by a drug can be associated with some particular neutral stimuli. After learning the association, this neutral stimulus becomes a conditioned stimulus associated with the drug that can also promote behavioural responses by itself. Several animal models of drug reward/reinforcement are based on these conditioning processes.

The neurobiology of nicotine addiction is a complex phenomenon in which various transmitter systems are involved (Berrendero et al. 2010). The experimental animal models that have been used to investigate nicotine addiction are mainly models of nicotine reward/reinforcement and have been useful to define the neurobiological substrate involved in this behavioural response that is crucial for the nicotine addictive process. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed more recently (Belin et al. 2008, Deroche-Gamonet et al. 2004, Vanderschuren and Everitt 2004). These models of addiction are extremely complex and have been validated only for cocaine addiction. Due to their complexity, these models have still not been used to investigate the neurobiology of drug addiction. Therefore, all the valuable information currently available about drug addiction, including nicotine addiction, is based on the results obtained in experimental models that evaluate drug rewarding/reinforcing effects (see section 3.9 for details about significance of the models).

3.6.2.1. Nicotinic acetylcholine receptors subunits and nicotine rewarding/reinforcing effects

The mesocorticolimbic system plays a crucial role in the rewarding/reinforcing properties of nicotine (Koob and Le Moal 2008). An important component of this system is the dopamine (DA) projection from the ventral tegmental area (VTA) to the frontal cortex and limbic structures, such as the nucleus accumbens (NAc). Nicotine administration increases DA activity in the NAc and other limbic structures (Di Chiara and Imperato 1988) by direct stimulation of nicotinic acetylcholine receptors subunits (nAChRs) within the VTA (Nisell et al. 1994). $\alpha_4\beta_2$ containing nAChRs located on DA cell bodies contribute decisively to the final activation of VTA DA neurons (Mansvelder and McGehee 2003). Indeed, the administration of selective $\alpha_4\beta_2$ antagonists block nicotine-self-administration in rodents (Grottick et al. 2000). In agreement, mice with the β_2 subunit knocked out do not self-administer nicotine (Picciotto et al. 1998). The specific location of nAChRs containing the β_2 subunit in the VTA plays a crucial role in the mediation of nicotine reinforcement as demonstrated by genetic studies in mice (Maskos et al. 2005). In addition, α_4 knockout mice fail to show nicotine-dependent enhancement of DA release in the NAc (Marubio et al. 2003), whereas a single nucleotide mutation rendering α_4

containing nAChRs hypersensitive to nicotine (Tapper et al. 2004) demonstrates that this subunit is sufficient to induce nicotine reward (Tapper et al. 2004). The precise role of the α_7 homomeric nAChRs in nicotine reinforcing effects remains unclear since conflicting results have been obtained in mutant mice lacking this subunit and in rodents injected with selective α_7 nAChR antagonists (Markou and Paterson 2001, Walters et al. 2006). On the other hand, repeated exposure to nicotine leads to up-regulation and desensitisation of nAChRs (Quick and Lester 2002), which are involved in the development of nicotine tolerance and the appearance of a withdrawal syndrome following smoking cessation. The brain regions underlying nicotine physical dependence have not yet been fully clarified, although an involvement of nAChRs located in the medial habenula and the interpeduncular nucleus has been recently reported (Salas et al. 2009).

Recent genome-wide association studies in humans have revealed a clear linkage between genetic variations in the nAChRs and the risk for nicotine dependence (Bierut 2009). Thus, the region on chromosome 15 that includes the family of α_5 - α_3 - β_4 nAChR genes has been associated with the development of nicotine dependence (Berrettini et al. 2008, Thorgeirsson et al. 2008) and lung cancer (Amos et al. 2008, Hung et al. 2008, Thorgeirsson et al. 2008). These studies differ on whether the connection between the genetic variant at chromosome 15 and lung cancer is direct (Amos et al. 2008, Hung et al. 2008) or mediated through a modification of smoking behaviour (Thorgeirsson et al. 2008).

3.6.2.2. Involvement of glutamatergic receptors in nicotine rewarding/reinforcing effects

Nicotine stimulates nAChRs on glutamatergic terminals that release glutamate in several brain regions including the VTA (Fu et al. 2000). Glutamate receptors located on postsynaptic DA neurons are critically involved in nicotine reinforcing effects (Liechti and Markou 2008). Thus, nicotine-induced DA release in the NAc is blocked by the administration of NMDA and AMPA ionotropic receptor antagonists (Kosowski et al. 2004). In addition, the blockade of NMDA receptor decreases intravenous nicotine self-administration in rats (Kenny et al. 2009). Several studies have also involved postsynaptic mGlu5 and presynaptic mGlu2/3 metabotropic receptors in nicotine reinforcing effects. Thus, mGlu5 receptor antagonists decrease nicotine self-administration (Paterson et al. 2003) and the incentive motivation for nicotine in rodents (Paterson and Markou 2005). The administration of a mGlu2/3 agonist also decreases nicotine self-administration in rats (Liechti et al. 2007). This last result is in accordance with previous studies showing that presynaptic mGlu2/3 receptors modulate glutamate release in a negative manner (Schoepp et al. 2003). The administration of mGlu5 receptor antagonists (Bespalov et al. 2005) or mGlu2/3 receptor agonists (Liechti et al. 2007) also decreases cue-induced reinstatement of nicotine-seeking in rats. Cholinergic and glutamatergic inputs from the pedunculopontine tegmental nucleus (PPTg) to the VTA seem to play a crucial role in nicotine reinforcement since complete lesion of the PPTg reduces nicotine self-administration (Lança et al. 2000, Picciotto and Corrigall 2002). On the other hand, the negative affective changes of nicotine withdrawal are related to a hyperactivity of corticotropin-releasing-factor neurons in the central nucleus of the amygdala (Brujinzeel et al. 2007, Panagis et al. 2000) and a decrease of DA activity in the NAc (Hildebrand et al. 1999) that seems to be modulated by the glutamatergic system. Thus, mGlu2/3 receptor antagonists, which increase extracellular glutamate in the NAc, attenuate reward deficits associated with nicotine withdrawal in rodents and could also alleviate the depression-like symptoms related to nicotine abstinence in humans (Kenny et al. 2003, Liechti and Markou 2008).

3.6.2.3. Involvement of GABA receptors in nicotine rewarding/reinforcing effects

DA neurons in the VTA are under the inhibitory control of GABAergic inputs that also participate in nicotine rewarding/reinforcing effects. Hence, the administration of the GABA-B receptor agonists such as baclofen, as well as several GABA-B receptor positive allosteric modulators, decrease nicotine self-administration in rats (Paterson et al. 2004, Paterson et al. 2008). Baclofen also inhibits nicotine-induced conditioned place preference in rats (Le Foll et al. 2008). Although GABA neurons are also activated by nicotine, $\alpha_4\beta_2$ nAChRs located on GABA cells tend to desensitise rapidly during repeated nicotine exposure (Mansvelder et al. 2002). Desensitisation of these receptors following repeated nicotine exposure contributes to the final activation of mesolimbic DA neurons induced by the chronic administration of this drug of abuse. Recent studies have reported that the GABA system also participates in nicotine relapse. Thus, the administration of GABA-B receptor agonists decreases cue-induced reinstatement of nicotine-seeking behaviour in rodents (Fattore et al. 2009, Paterson and Markou 2005). In agreement, baclofen also prevents the reinstatement of nicotine conditioned place-preference triggered by nicotine priming in rats (Fattore et al. 2009).

3.6.2.4. Endogenous opioid system in nicotine rewarding/reinforcing effects

Nicotine administration has been reported to enhance the release of endogenous opioids in the CNS. Thus, an increased concentration of β -endorphin has been found in the hypothalamus after acute nicotine administration in rodents (Marty et al. 1985). In addition, chronic nicotine administration has been found to increase mRNA expression of prodynorphin and μ -opioid receptors (Wewers et al. 1999) in the striatum (Isola et al. 2008). An enhancement of proenkephalin expression has also been observed in the striatum of mice following acute or chronic nicotine administration (Dhatt et al. 1995).

Nicotine induces opposite responses on anxiety-like behaviour related to the development of nicotine addiction that are modulated by the endogenous opioid system. Thus, nicotine anxiolytic-like effects were blocked by a μ -opioid antagonist, and its anxiogenic-like effects were enhanced by a δ -opioid antagonist (Balerio et al. 2005). In addition, a reduction of nicotine anxiogenic-like effects was reported in knockout mice lacking β -endorphin (Trigo et al. 2009). The opioid system also plays an important role in nicotine rewarding effects. The efficacy of naltrexone on smoking cessation in humans supports the involvement of opioid receptors in nicotine reward (Rukstalis et al. 2005). In rodents, nicotine-induced elevations of extracellular DA levels in the NAc were modulated by the activation of μ -opioid receptors localised in the VTA (Tanda and Di Chiara 1998). In agreement, nicotine rewarding properties were blocked in knockout mice lacking μ -opioid receptors (Berrendero et al. 2002) or the proenkephalin gene (Berrendero et al. 2005), revealing an involvement of endogenous enkephalins through the activation of μ -opioid receptors. In addition, proenkephalin knockout mice showed a reduction of nicotine-enhanced DA extracellular levels in the NAc (Berrendero et al. 2005). Mice lacking β -endorphin also showed a reduction of nicotine rewarding effects (Trigo et al. 2009). κ -Opioid receptors and their endogenous ligands modulate nicotine reward in the opposite way to enkephalins and β -endorphins. Hence, knockout mice deficient in the prodynorphin gene showed an enhanced sensitivity to nicotine self-administration, probably due to the modulation of its aversive effects (Galeote et al. 2009).

The opioid system is also involved in the development of nicotine tolerance. Thus, chronic nicotine exposure produces cross-tolerance with morphine (Biala and Weglinska 2006, Zarrindast et al. 1999), and increases the functional activity of μ -opioid receptors in the spinal cord (Galeote et al. 2006). In addition, μ -opioid receptor knockout mice developed faster nicotine tolerance than wild-type mice, suggesting that increased activation of μ -opioid receptors could be an adaptive mechanism to counteract the establishment of nicotine tolerance (Galeote et al. 2006). The involvement of the opioid system in nicotine withdrawal has also been demonstrated. In humans, the opioid antagonist, naloxone

induces somatic signs of withdrawal in heavy chronic smokers (Krishnan-Sarin et al. 1999). In rodents, opioid antagonists precipitate somatic manifestations of withdrawal in nicotine-dependent animals (Balerio et al. 2004). In addition, somatic manifestations of nicotine withdrawal were reduced in mice lacking μ -opioid receptors (Berrendero et al. 2002) or the proenkephalin gene (Berrendero et al. 2005). Different studies also indicate that the opioid system participates in the negative emotional states associated with nicotine withdrawal. Thus, naloxone induced aversive effects in nicotine-dependent rodents, which reflects the motivational manifestations of nicotine withdrawal (Balerio et al. 2004, Watkins et al. 2000).

3.6.2.5. Involvement of cannabinoid receptors in nicotine rewarding/reinforcing effects

Several studies demonstrate that the endocannabinoid system plays an important role in the rewarding/reinforcing effects of nicotine (Maldonado et al. 2006). Indeed, the selective CB1 receptor antagonist rimonabant reduces nicotine self-administration in rats (Cohen et al. 2002) and nicotine-induced conditioned place preference in rats and mice (Le Foll and Goldberg 2004, Merritt et al. 2008). In addition, rimonabant pre-treatment blocks nicotine-enhanced DA extracellular levels in the NAc (Cheer et al. 2007, Cohen et al. 2002) and in the bed nucleus of the stria terminalis (Cheer et al. 2007). Nicotine conditioned place preference was also absent in knockout mice lacking CB1 receptors (Castañé et al. 2002, Merritt et al. 2008). The endocannabinoid system has also been involved in the relapse to nicotine-seeking behaviour (De Vries and Schoffelmeer 2005b). Thus, rimonabant attenuates the reinstatement of nicotine seeking-behaviour induced by nicotine-associated cues (Cohen et al. 2005, De Vries et al. 2005a), and reinstatement of nicotine-induced conditioned place-preference provoked by nicotine priming (Biala et al. 2009). The cannabinoid antagonist AM251 also reduced the reinstatement produced by the combination of nicotine-associated cues and a nicotine priming dose (Shoaib 2008). Based on the behavioural and biochemical results obtained in rodents, several clinical trials were developed to evaluate the efficacy of rimonabant for smoking cessation (STRATUS, studies with rimonabant and tobacco use) (Cahill and Ussher 2007). Rimonabant was effective in obtaining a significant smoking cessation in two clinical trials (STRATUS-NORTH AMERICA and STRATUS-WORLD WIDE), although this effect was not significant in the STRATUS-EUROPE trial. The different clinical trials performed with rimonabant have reported several gastrointestinal and psychiatric side effects including nausea, anxiety and depression. Due to these psychiatric side effects, the European Medicines Agency (EMA) recommended the suspension of the marketing authorisation for rimonabant on 23 October 2008.

3.6.2.6. Other neurotransmitters involved in nicotine rewarding/reinforcing effects

The serotonergic (5-HT) system, mainly through the activation of the 5-HT_{2c} receptor subtype, seems to be involved in nicotine reward/reinforcing by exerting an inhibitory influence on DA activity in the VTA (Di Matteo et al. 1999). Thus, 5-HT_{2c} agonists reduce nicotine-self-administration (Grottick et al. 2001), although responding for food was also attenuated by these antagonists. In contrast, no modification on nicotine-induced conditioned place preference was observed by a 5-HT_{2c} agonist in a recent report (Hayes et al. 2009). On the other hand, tobacco smoke contains monoamine oxidase (MAO) inhibitors which are thought to enhance the reinforcing effects of nicotine. Behavioural studies have confirmed this statement since nicotine self-administration was facilitated in rats pre-treated with MAO inhibitors (Villégier et al. 2006a, Villégier et al. 2007). Recently, the hypothalamic neuropeptides hypocretins acting in the insula have also been involved in nicotine reward (Hollander et al. 2008).

3.6.2.7. Conclusions on addictive properties of nicotine

Animal models of nicotine reward/reinforcement have enabled the neurobiological substrate involved in this behavioural response that is crucial for nicotine addictive processes. Similar animal models have been widely used to define the neurobiological substrate of the addictive properties of all drugs of abuse. Results obtained in these models suggest that the neurobiology of nicotine addiction is complex involving various transmitter systems in the CNS. Multiple neurotransmitter pathways are activated by nicotine, including dopaminergic, GABAergic and opioidergic pathways. The complexity of the mechanisms of addiction is further underlined by the involvement of the endocannabinoid system, and the serotonergic system also seems to be involved. Dose-dependency appears to have been shown in animal studies. In general, an inverted U-shaped dose-response has been revealed, which suggests that, such as for other drugs of abuse, the addictiveness of nicotine is not directly linear with the dose. The experimental animal models used for evaluating addiction are described in section 3.9.

3.6.3. Conclusions on nicotine

The action of nicotine on the CNS is multifaceted and the mechanisms of addiction are still poorly understood. There are substantial inter-individual differences in the action of nicotine and in its metabolism, which are in part genetically determined. A number of different compounds may in principle interfere with the binding of nicotine with its receptors, while others may interfere with the metabolism of nicotine via the cytochrome P450 system or other pathways. Addiction to nicotine is difficult to measure directly and is usually assessed experimentally with reference to reinforcement assessed in self-administration paradigms.

3.7. Possibilities to make tobacco more addictive or attractive

3.7.1. Introduction

Tobacco products are manipulated by tobacco companies by the addition of chemical compounds, most of which are flavours. Obviously, the flavours are added to the natural tobacco to give the product a better taste thereby increasing the attractiveness of these products. This includes the addition of humectants which keep the humidity of the tobacco product at a desired level; dry tobacco generates an unpleasant harsh smoke.

“Light” cigarettes were introduced on the market in the 1970s. Typical for light cigarettes is their high grade of ventilation. Due to the delivery of less tar, the impact and taste of the “diluted” smoke is also decreased. It is therefore probable that the light cigarettes were “enriched” by adding more substances, and in higher amounts, to compensate for reduced taste and impact. For details see sections 3.5.5 and the different sections reviewing specific tobacco additives such as section 3.8.

An important reason for using additives is to give the product a specific and standardised taste. A specific taste is important for the company to be competitive on the consumer market in view of the large variety of brands available. A unique product binds the customer/consumer to this specific product. The specific taste of a certain product must be preserved (standardised) to compensate for the yearly variation of the natural tobacco, because consumers do not like to smoke a product that changes from year to year. To circumvent this, some 40 or more substances per product are added to the majority of the brands in order to mask the variation.

3.7.2. Additives with direct or indirect addictive potency

In the following two sections, various approaches to increase the addictive and attractive potency of tobacco products have been briefly described. Details of these additives and further information about their effectiveness can be found in later sections (see section 3.8.1).

The addictive potency of tobacco products may in theory be increased by:

1. Direct enhancement of the nicotine content;
2. Addition of substances which increase the bioavailability of nicotine;
3. Addition of substances which facilitate the inhalation of tobacco smoke;
4. Addition of substances which generate compounds in the mainstream smoke which increase the addictiveness of nicotine;
5. Changing the physical properties of tobacco smoke, e.g. particle size.

The five approaches are briefly described below.

1. Direct enhancement of the nicotine content

No examples of increasing the content of nicotine in tobacco are known. Moreover, in cigarettes sold (or produced) in the EU nicotine yield has to remain below a maximal level of 1 mg per cigarette. Some Member States also have upper limits for roll your own (RYO) tobacco. Genetic techniques or classical selection of variants are available to produce tobacco with relatively high nicotine content. From public sources it cannot be deduced or concluded that such approaches are indeed used by tobacco growers or tobacco companies.

2. Addition of substances which increase the bioavailability of nicotine

- a) Increase the bioavailability of nicotine by adding alkalising ingredients which increase the pH of tobacco (such as ammonium compounds). At higher pH (pH >8.0) more nicotine is in its free uncharged form, which would therefore more easily pass the (lung) membrane i.e. higher absorption leading to higher blood and brain nicotine levels. For details see section 3.8.3.2 on ammonia and other compounds affecting smoke pH.
- b) Increase the bioavailability of nicotine by adding ingredients which serve as a carrier for nicotine.
- c) Increase the effect of nicotine by inhibiting its metabolism.

3. Addition of substances which facilitate the inhalation of tobacco smoke

- a) Certain ingredients have local anaesthetic effects. As a result coughing due to inhalation of irritating smoke is dampened and the smoker can inhale the smoke deeper (and more frequently). Examples are etheric oils, such as menthol and thymol. For details see later sections e.g. section 3.8.1.
- b) Compounds which have bronchodilating properties (opening/broadening the airways) would enable the smoker to inhale deeper (a larger volume of) tobacco smoke implying an increase in the bioavailability of nicotine. It has been proposed that theobromine, generated from cocoa, caffeine and glycyrrhizine, serves such a function.

4. Addition of substances which generate compounds in the mainstream smoke which increase the addictiveness of nicotine

- a) It has been suggested that certain natural components in tobacco promote the addictiveness of nicotine. Examples are components like sugars, which when pyrolysed generate aldehydes. The combination of acetaldehyde and nicotine appears to be more addictive than nicotine alone. The addition of sugars may thus increase the addictive nature of tobacco products. In tobacco smoke or *in vivo*, tryptophan may react with aldehydes to form beta-carbolines, like harman and norharman. Both beta-carbolines are inhibitors of monoamine oxidases (MAO). Monoamine oxidases are enzymes that degrade neurotransmitters involved in addiction such as dopamine, serotonin and noradrenaline. As such, tryptophan as an ingredient may potentiate nicotine addiction.

- b) Acetaldehyde and other aldehydes can react *in vivo* with biogenic amines to yield carbolines or isoquinolines, which have affinity for the opiate receptor. These ligands are, however, formed in very low amounts.

5. Changing the physical properties of tobacco smoke, e.g. particle size

It is possible to change the physical properties of tobacco smoke, for example the particle size of the tobacco smoke aerosol. Considering the entry of particles to deeper lung levels, there is probably an optimum in size. Cigarette paper and/or filters can be modified in a technological way to attain an optimal particle size (see section 3.5).

The size and its distribution of smoke particles can be changed to obtain an optimum so that particles enter deeper levels of the lungs. As a result, a more efficient absorption of nicotine from the particles and higher blood nicotine levels can be attained. Examples of such applications are the use of cigarette paper with a higher porosity and filters with higher ventilation (see section 3.5).

3.7.3. Additives with attractive properties

A large number of tobacco additives are flavours, which are mostly aromatic compounds or generate aromatic compounds found in the smoke. Flavours are mainly applied for two reasons: firstly, to enhance the attractiveness of a product (appeal to consumers); and secondly, to produce a unique product, typical in “taste” and markedly different from competitor products. The aim here is to get and maintain a certain and stable market share. Note that each of the many flavours is added to tobacco in minute amounts (nano to microgram range per unit). As reported by the tobacco industry to several national competent authorities and as described on tobacco industry websites, cigarettes contain up to 40 (sometimes even more) different additives.

Sugars are natural components of tobacco, but they are also added to tobacco products during manufacturing. The heating of sugars in the tobacco product initiates a caramelisation, generating secondary products which have an attractive smell and taste.

Other additives which may increase the attractiveness of tobacco products, e.g. menthol, are mentioned later (see section 3.8).

A number of additives have an effect on colour, smell, visibility, taste, and harshness of the smoke.

Note that some additives may fall into several of the above mentioned groups.

3.7.4. Conclusions on addictive and attractive additives

Section 3.7 has provided a preview of the additives used in tobacco which may have addictive or attractive properties. Conclusions about their efficacy are found at the end of the individual sections, which describe their effects in full detail. The addictiveness of tobacco products can theoretically be increased by additives in a number of ways including generation of new compounds upon combustion, enhancing the bioavailability of nicotine, promoting smoke inhalation and influencing particle size. Attractiveness can similarly be improved in a number of ways, such as by adding flavours. Importantly, some additives may at the same time have addictive and attractive properties, or may influence addictiveness indirectly, for example by promoting smoke inhalation.

3.8. Classification of additives

According to the EU Tobacco Products Directive (2001/37/EC) tobacco companies are obliged to provide information about the ingredients added to tobacco products, and their function, to the local authorities. In Germany, this information is published on the website of the Federal Ministry of Nutrition, Agriculture, and Consumer Protection⁹.

⁹ http://service.ble.de/tabakerzeugnisse/index2.php?site_key=153&site_key=153

Consumers can search for brands and ingredients. The reports from 2008 showed the amount of each ingredient listed. However, only the amounts of major ingredients such as sucrose, propylene glycol or cocoa are disclosed to the public. Furthermore, only 22 of the 50 most-used ingredients have been specified by name. In the reports for the general public the tobacco industry does not reveal the nature of all flavourings, colours, or adhesives used. Quantitatively, sugars and humectants (e.g. glycerol, propylene glycol) are the dominant additives in cigarettes. Furthermore, compounds which influence the taste of the cigarette are used in many brands; relevant substances are cocoa (incl. cocoa powder, cocoa extracts, shells of cocoa bean etc.) and liquorice (incl. liquorice extract). Other ingredients are part of the cigarette paper, the filter, or are used as glue. Even if the tobacco companies are secretive about the exact amount of flavours used in each brand, some information is available on the websites of the tobacco companies (e.g. BAT¹⁰). Most of the tobacco companies disclose only the highest amount of ingredients used in their brands (i.e. Quantity Not Exceeded (QNE)). Therefore, it is not possible to draw conclusions about the average amount added or about the percentage of brands that contain a particular ingredient. As an example the information on the Philip Morris website¹¹ for German cigarettes has been evaluated. In the compilation the maximum use levels are given, i.e. Philip Morris only discloses the highest amount used in its brands. Most of the flavours are added in very small amounts. On the other hand, menthol and lactic acid are flavours used in milligram amounts per cigarette (see table 3). For the calculation it was assumed that each cigarette contains about 700 mg of tobacco.

¹⁰ <http://www.bat-ingredients.com/>

¹¹ <http://www.pmintl-technical-product-information.com/aspx/IngredientsInformation.aspx>

Table 3 **Ingredients added to the tobacco based on a table presented by Philip Morris International (PMI) on cigarettes manufactured for sale in Germany¹¹**

Ingredient	Maximal use level(w/w%)	Maximal use level(mg/cigarette (700 mg))
sucrose	4.2	29.4
propylene glycol	3.9	27.3
glycerol	2.2	15.4
invert sugar	2.1	14.7
l-menthol	1.1	7.7
d-sorbitol	1.1	7.7
liquorice extract	0.9	6.3
lactic acid	0.7	4.9
guar gum	0.6	4.2
benzoic acid	0.3	2.1
benzoic acid sodium salt	0.3	2.1
carob bean and/or extract	0.2	1.4
cocoa and cocoa products	0.2	1.4
acetic acid	0.01	0.07
lovage extract	0.01	0.07
peppermint oil	0.01	0.07
vanillin	0.01	0.07
benzoin, resinoid	0.005	0.035
phenylcarbinol	0.005	0.035
coffee extract	0.005	0.035
ethyl acetate	0.005	0.035
ethyl hexanoate	0.005	0.035
ethyl vanillin	0.005	0.035
fenugreek extract	0.005	0.035
maltol	0.005	0.035
methyl-cyclopentenolone	0.005	0.035
3-methyl-butylaldehyde	0.005	0.035
orange oil, sweet	0.005	0.035
piperonal	0.005	0.035
spearmint oil	0.005	0.035
veratraldehyde	0.005	0.035
bergamot oil	0.001	0.007
ethyl heptanoate	0.001	0.007
ethyl maltol	0.001	0.007
isoamyl acetate	0.001	0.007
isoamyl formate	0.001	0.007
orris root extract	0.001	0.007
2,3,5,6-tetramethylpyrazine	0.001	0.007
valerian root extract	0.001	0.007

3.8.1. Addictiveness

3.8.1.1. Introduction

Only few scientific articles have addressed the possibility that individual additives may cause addiction. It is probable that many additives have not been examined/analysed or the results (either positive or negative) have simply not been described in publicly available literature.

The available documentation on additives in respect to a direct addictive effect is reviewed in section 3.8.1.2. Examples of additives causing addictiveness indirectly are provided in section 3.8.1.3. Finally, an assessment of how different forms of sugar may have an indirect addictive effect due to combustion products such as aldehydes is presented in section 3.8.1.4.

3.8.1.2. Additives with addictive properties (direct effect)

In the peer-reviewed scientific articles assessed there is no documentation for certain individual additives to cause addiction directly.

The following compounds, used as tobacco additives, may have an effect on the central nervous system: acetophenone, isoamyl alcohol, valerian oil, theobromine, and valerenic acid (Lington and Bevan 1994, Moreno 1978, Oliva et al. 2004, Ortiz et al. 1999, Reynolds 1983a, Reynolds 1983b, Simons et al. 1985, Yuan et al. 2004). However, the fact that these additives may have an effect on the central nervous system (CNS) does not imply that they are addictive. Moreover, they are present in the products in very low amounts.

Although several articles point out that some of the above mentioned additives may create dependence, it is probably more likely that they are acting by attractiveness, as they induce a more pleasant experience of smoking and therefore reduce the barrier in relation to smoking initiation.

3.8.1.3. Additives enhancing addictiveness indirectly

Additives which increase the absorption of nicotine or potentiate in whatever way the effect of nicotine on the nervous system implicitly increase the addictiveness of tobacco products.

Examples of additives

Ammonium salts

It has been proposed that the free nicotine content of smoke increases with increasing pH, which would lead to a higher uptake of nicotine in the bloodstream. A higher pH also increases the nicotine/tar ratio (Wayne and Carpenter 2009) as well as the harshness of the smoke (Hurt and Robertson 1998). The increased harshness will be disguised by using different additives that remove the smoker's sensation of harshness. Ammonium salts are used as additives to increase the pH of tobacco. See Section 3.8.3.2 for a full description of ammonia technology.

Menthol

Because of its local anaesthetic properties, menthol allows a deeper inhalation of the irritating tobacco smoke. As such, more smoke could be inhaled and deeper puffs could be attained, resulting in a higher nicotine dose. See section 3.8.3.1 for detailed description of the action of menthol.

Theobromine

Theobromine is found in cocoa beans; therefore this substance is present in cocoa and chocolate, both of which are used as additives in tobacco. Theobromine is a bronchodilator and has been used in the treatment of asthma (Simons et al. 1985). It has been proposed that the bronchodilating effect of the substance may contribute to the absorption of nicotine in connection with smoking (Bates et al. 1999, Fowles 2001). In a document from the New Zealand Ministry of Health (Fowles 2001) it is reported that up to 3% of the weight of cigarettes is cocoa extract and another 0.2% is chocolate. There is typically 0.2% theobromine in cocoa (Rambali et al. 2002). In most of the types of cigarettes containing cocoa and chocolate, which were reported to the Danish competent authorities¹² in 2006, the contents of cocoa and chocolate are 0.3-0.5% and 0.2%, respectively. Based on the information available on the PMI and BAT websites the percentage of cocoa used in cigarettes ranges from 0.2% to 0.66%. Taking this information into account, the content of theobromine per cigarette will be too low to have a bronchodilating effect on the lungs and thereby increase the absorption of nicotine.

Eucalyptol

Like theobromine, eucalyptol has an effect on the lungs as a bronchodilator (Hasani et al. 2003, Juergens et al. 2003). For eucalyptol it is also clear that the contents per cigarette are not large enough to exert this effect. However, even though the doses of theobromine and eucalyptol are so low in cigarettes that they probably do not have a bronchodilating effect, it cannot be excluded that there are other additives with a similar effect.

Lactones

The addictive effect of nicotine may be increased if the metabolism rate of nicotine is reduced. Reduction of the metabolic rate of nicotine, e.g. by inhibition of the metabolic enzymes involved in nicotine degradation, implicates a higher bioavailability of nicotine (nicotine is present in the body for a longer time or at a higher blood level). The additives gamma-heptalactone, gamma-valerolactone, gamma-decalactone, delta-decalactone, gamma-dodecalactone, delta-undecalactone and gamma-hexalactone are mild to weak inhibitors of CYP2A6, an enzyme within the P450 enzyme system, involved in the metabolism of nicotine (Juvonen et al. 2000). However, with IC₅₀-values in the range 560-12,000 µM it seems unlikely that these compounds will inhibit nicotine metabolism at the amounts used in cigarettes.

3.8.1.4. Additives enhancing addictiveness indirectly by combustion of sugar

Sugar is already present naturally in considerable amounts in the tobacco leaf (up to 20%) and the quantities remaining in the final product depend on the curing methods. Sugar in different forms is also one of the most common additives in tobacco (see table 3 in section 3.8). When the sugars, including complex polysaccharides like cellulose (Seeman et al. 2002) in the tobacco product are combusted, various aldehydes are generated such as formaldehyde, acetaldehyde, propanal, 2-butenal, 2-methylpropenal, butanal, methylbutanal, furfural, benzaldehyde, methylfurfural, methoxybenzaldehyde (Adam et al. 2006, Baker et al. 2004b).

Acetaldehyde is claimed to increase the addictiveness of nicotine in a synergistic way (Belluzzi et al. 2005, Charles et al. 1983, Philip Morris 1992). The mechanism of action may be that acetaldehyde forms secondary condensation products which inhibit monoamine oxidase (MAO). The inhibition of MAO by aldehydes has already been demonstrated many years ago (Townee 1964, Williams et al. 1992) and part of the inhibition is probably irreversible (Sowa et al. 2004, Wood et al. 2006).

¹² <http://www.sst.dk/Sundhed%20og%20forebyggelse/Tobak/Indberetning/Indberetninger.aspx>

However, even during heavy smoking, only minor amounts of the acetaldehyde in the smoke is absorbed into the blood stream (McLaughlin et al. 1990), suggesting no direct addictive effect of sugars through acetaldehyde when used as a tobacco additive. Moreover, alcohol consumption leads, in contrast to smoking, to a significant increase in the acetaldehyde blood level by its metabolism. Acetaldehyde is very reactive and forms adducts with proteins and DNA. Chen et al. (2007b) found only a small contribution of chronic smoking to the formation of acetaldehyde DNA adducts, whereas alcohol consumption had a much higher effect, suggesting again that in chronic smokers lower amounts of acetaldehyde enter the circulation than in alcohol consumers. Acetaldehyde is rapidly oxidised in the body by dehydrogenases which, however, are much less efficient for oxidation of more complex aldehydes that are formed in the smoke by combustion of sugars. The decrease in the level of monoamine oxidases which has been repeatedly found in brains of smokers may thus be due to inhibition by aldehydes other than acetaldehyde which are present in the smoke.

Finally, the addition of sugars to tobacco increases the content of acids in the smoke, resulting in a lowering of the pH value of the tobacco smoke. This may be one of the reasons why ammonia compounds are added to neutralise these acids.

Examples of sugar additives

The sugars added to tobacco are mainly inverted sugar (fructose and glucose), and sucrose (Philip Morris 2002, Seeman et al. 2003), and are often added in the form of syrups (Covington & Burling 1992, Reynolds 1985). The main part of sugar substances in tobacco is non-volatile and only a small part is transferred unmodified into the mainstream smoke. The sugar substances are not hazardous to health by oral consumption, but are transformed to a number of toxic compounds under pyrolysis. These mainly include formaldehyde, acetaldehyde, acetone, acrolein, furans (Burton 1976) and different complex aldehydes. The pyrolysis products have a hazardous effect on health; formaldehyde is classified as a carcinogen to humans (IARC 2006, IARC 2009), whereas acetaldehyde and acrolein are highly irritating to the respiratory tract.

Mono- and disaccharides (natural sugars like glucose, fructose, sucrose)

Mono- and disaccharides are derived from a number of sources including brown sugar, honey, corn syrup, molasses, sugar cane, fig juice and prune juice. Sugars are flavourings that constitute the largest part of additives in cigarettes (Bates et al. 1999). According to table 3 in section 3.8 the levels of sugars applied to the cigarette tobacco blends constitute more than 10% of the total amount of additives. They are added to the tobacco in order to contribute to the taste and flavour (Philip Morris 2002, Reynolds 1985, Reynolds 1994. This reduces irritation and makes the taste milder (Covington & Burling 1986, Covington & Burling 1987a, Seeman et al. 2002).

Inverted sugars are responsible for a large part of the contents of formaldehyde in smoke and also contribute to the formation of furfural, furan, levoglucosan, and acetaldehyde (Baker et al. 2004b, Baker et al. 2004d, Philip Morris 2002).

Polysaccharides (e.g. cellulose, pectin, starch)

Apart from the sugar substances mentioned, cellulose fibres are a natural part of the tobacco, and are also added as a binding agent (Baker et al. 2004b, Baker 2006, Fox 1993). Pyrolysis of cellulose fibres results in the formation of volatile aldehydes and levoglucosan (Seeman et al. 2002). The amount of pyrolysis products varies depending on the sugar contents and the temperature within the cigarette. It is difficult to estimate the relative contribution of pyrolysis products of simple sugars in relation to polysaccharides (Covington & Burling 1986). The pyrolysis products of polysaccharides and simple sugars are similar, but their yields differ (Fox 1993, Rodgman 2002, Sanders et al. 2003, Seeman et al. 2002). It is estimated that more formaldehyde and less acetaldehyde and acetone are generated from the pyrolysis of simple sugars compared to polysaccharides (Burton 1976).

Addictive potential of acetaldehyde

Animal studies have shown that acetaldehyde can maintain self-administration behaviour equal to, or probably more effectively than, nicotine (Charles et al. 1983, Philip Morris 1992). Belluzzi et al. (2005) found that acetaldehyde has reinforcing properties (Belluzzi et al. 2005).

A number of studies have elaborated on the interaction between nicotine and acetaldehyde (Belluzzi et al. 2005, Cao et al. 2007, Charles et al. 1983, Philip Morris 1992). The combination of nicotine and acetaldehyde increases the degree of self-administration in young rats (Belluzzi et al. 2005). It is possible that norepinephrine contributes to the age-dependent difference in acetaldehyde uptake in rats (Sershen et al. 2009). A study by Cao et al. (2007) shows that acetaldehyde potentiates hyperlocomotive effects of nicotine in young as well as adult rats, but that these effects are more pronounced in adult rats. No effect of acetaldehyde on the nicotine level in the brain was observed (Cao et al. 2007). In the Philip Morris publications, the interaction between nicotine and acetaldehyde is examined with the purpose of increasing the reinforcing effect of tobacco (Charles et al. 1983, Philip Morris 1992). The synergistic interaction between nicotine and acetaldehyde is substantiated by experiments where the combination of nicotine and acetaldehyde results in a rewarding effect that exceeds the additive effects of each substance in rats (Philip Morris 1992). It is likely that the combination of nicotine plus acetaldehyde is more reinforcing than nicotine alone, as a long-lasting instrumental conditioned response in young rats was observed (maintains lever pressing at a higher rate than nicotine alone) (Charles et al. 1983, Philip Morris 1992). However, the effect of acetaldehyde seems not to be mediated by opioid receptors in the CNS and the substance does not cause physiological addictiveness (Charles et al. 1983). Cao et al. (2007) discussed whether acetaldehyde may pass the blood-brain barrier and directly affect the CNS. It is proposed that acetaldehyde has to be present in high concentrations ($>100\text{ }\mu\text{M}$) in the blood to overcome aldehyde dehydrogenase in the blood-brain barrier (Tabakoff et al. 1976). It should be noted that the experiments in animals used intravenous infusion of acetaldehyde, and as mentioned before, it is uncertain whether the acetaldehyde in smoke contributes significantly to the blood level of this substance (Chen et al. 2007b, McLaughlin et al. 1990). However, acetaldehyde is definitely not the only aldehyde produced by burning of sugars. Because the chemical aldehyde group has a potent inhibiting effect on monoamine oxidase activity (Townee 1964, Williams et al. 1992, Wood et al. 2006), it is suggested that those aldehydes which are more complex than acetaldehyde are responsible for monoamine oxidase inhibition and increased addictiveness of tobacco.

Proposed mechanisms of action

The reinforcing effect of acetaldehyde may be due to the reaction between acetaldehyde and catecholamines, which results in the formation of tetraquinolines (beta-carboline and tetrahydroquinoline) (DeNoble 1994, Philip Morris 1992, Rahwan 1975). Tetraquinoline derivatives may act as false neurotransmitters and therefore promote addictiveness of the product (DeNoble 1994, Rahwan 1975).

Others argue that acetaldehyde has an addictive effect because of the formation of the condensation products harman and norharman, which inhibit the enzyme monoamine oxidase (MAO). Inhibition of MAO results in a slower metabolism of the biogenic amines, like dopamine, noradrenaline and serotonin in the brain, so that the brain levels are increased by MAO-inhibition. However, it is only proven that harman could have significance for tobacco addiction by virtue of its inhibitory effect on MAO-A (Guillem et al. 2006). Indeed, harman is formed in the smoke (0.1 to 5.8 microgram per cigarette). At this level, harman, following its absorption, may be responsible for 3 to 11% of the inhibition of MAO-A (note that drinking a cup of coffee delivers 1 to 8 microgram orally). Nevertheless, whatever the active product, one smoked cigarette decreases MAO in the monkey heart by 25% (Valette et al. 2005). Smokers have decreased MAO-A and MAO-B activities in brain (Fowler et al. 1996), which recover following smoking cessation. However, harman and norharman are not irreversible inhibitors of monoamine oxidases

and it has been shown that only an irreversible blockade of MAO-A and MAO-B increases the reinforcing effects of nicotine (Guillem et al. 2005; Villégier et al. 2006a). This suggests that aldehydes, which are probably irreversible inhibitors of monoamine oxidases (Sowa et al. 2004, Wood et al. 2006), could be the inhibitors of monoamine oxidases responsible for the increased reinforcing effects of nicotine in tobacco and tobacco smoke. Acetaldehyde is rapidly inactivated in the body. Therefore, more complex aldehydes are possible candidates for the observed monoamine oxidase inhibition. In summary, the mechanistic explanation for the role of sugars in smoking addiction is still unclear.

3.8.1.5. Denicotinised cigarettes

Nicotine plasma levels are associated with cigarette smoking behaviour and nicotine is considered the main factor driving cigarette addiction. In apparent contradiction to this observation, nicotine replacement therapy, as a smoking cessation treatment, does not show the expected effectiveness. Therefore, it has been assumed that non-nicotine components are important in smoking reinforcement. The exact nature of these factors (chemical composition) is largely unknown, but constituents which provide reinforcing sensory stimulation and/or minimize excessive irritation from inhaled nicotine are considered to play an important role in non-nicotine effects in cigarette smoke (Rose 2006).

In this chapter several studies with denicotinised cigarettes are briefly described to highlight the importance of the non-nicotine components in tobacco.

Denicotinised cigarettes have the appearance, draw and taste of standard cigarettes but contain (and deliver) virtually no nicotine (<0.06 mg). However, they deliver tar and carbon monoxide (CO) in a comparable way to traditional cigarettes (Pickworth et al. 1999).

In short term (for a few hours; maximum up to 24 hours) experiments, smoking volunteers were placed under tobacco (nicotine) abstinence and were allowed to smoke denicotinised or conventional cigarettes.

- In 1999, Pickworth et al. reported that the denicotinised cigarettes did not increase heart rate or activate the EEG, but subjects reported that both conventional and denicotinised cigarettes reduced (subjective) measures of tobacco craving and withdrawal (Pickworth et al. 1999).
- In a study by Eid et al. (2005) a stimulating effect on heart rate of denicotinised cigarettes was reported. Smoking of either denicotinised or conventional cigarettes caused a significant reduction in the craving score. The authors could not find a correlation between the nicotine yield and behavioural effects.
- Perkins et al. (2010) simulated different stressful situations (negative affects) during smoking abstinence and studied how relief was perceived after smoking. The authors did not find an association between the relief of several negative affects and smoking (also not from denicotinised cigarettes) but the relief was not dependent on nicotine intake, therefore, challenging the assumption that nicotine in smoking alleviates negative affects.
- Brody et al. (2009) found that, compared to conventional cigarettes, smoking denicotinised cigarettes (0.05 mg nicotine) resulted in a decrease in occupancy of the brain nicotine acetylcholine receptor (nAChR), as predicted on the basis of nicotine concentration. They did not observe occupancy of the nAChR with other factors, suggesting that only nicotine in cigarette smoke is capable of binding this receptor (Brody et al. 2009).

These acute studies show that denicotinised cigarettes, compared to conventional cigarettes, do not exert the same pharmacological effects, but cravings and symptoms of withdrawal can be diminished and this phenomenon is, in many cases, independent of

the delivered nicotine. Some components of tobacco smoke, other than nicotine, may be biologically active; thus it has been suggested that non-nicotine components of tobacco smoke decrease brain levels of monoamine oxidase A and B which possibly change sensitivity to the actions of nicotine and/or exert independent behavioural effects (Eid et al. 2005).

Recently, Rose et al. (2010b) found that denicotinised smoke was self-administered more than any other alternative (i.v. nicotine self-administration or sham puffs) in established smokers, even after a few days of nicotine abstinence. This preference for denicotinised smoke compared to i.v. nicotine was inversely correlated with subjective ratings of “comfort” (normally) associated with nicotine; therefore non-nicotine aspects of cigarette smoking have potent reinforcing effects in established smokers. These authors, therefore suggested that in contrast to current smoking cessation pharmacotherapies, which address only the nicotine component of nicotine (tobacco) addiction, future cessation strategies should also be designed to target non-nicotine factors such as added flavour constituents (e.g. menthol).

In conclusion, besides nicotine, a mixture of other factors in cigarette smoke probably plays an important role in craving and reinforcement. Although these unknown factors do not have pharmacological effects similar to nicotine and are probably not addictive, they definitely play a role in smoking behaviour.

3.8.1.6. Conclusions on how additives can increase the addictiveness of tobacco products

Certain tobacco additives may affect the central nervous system in smokers directly, but their concentration in tobacco products is probably too low to have a physiological effect. However, an indirect addictive effect of certain substances cannot be excluded.

Some additives increase the pH of the smoke, thereby increasing the quantity of nicotine delivered to the smoker.

Sugars generate aldehydes such as acetaldehyde during combustion. When given intravenously to animals, acetaldehyde potentiates the addictive effect of nicotine. The mechanism of action of the reinforcing effect of acetaldehyde in animals is not clear, although an inhibition of MAO is the most likely reason. Inhibition of MAO has also been observed in human smokers. However, acetaldehyde, generated from the sugars during combustion, is presumably not absorbed into the blood stream, and this suggests that other aldehydes, produced by sugar combustion are responsible for the inhibition of monoamine oxidases and the increased addictiveness of tobacco products.

Natural tobacco already contains considerable amounts of sugars, especially Virginia tobacco. In addition, polysaccharides and cellulose fibres in the tobacco leaves generate acetaldehyde and other aldehydes upon combustion. In this respect addition of sugars to tobacco may lead to a significant increase in the addictiveness of the product.

3.8.2. Attractiveness

3.8.2.1. Introduction

A number of additives increase the attractiveness of tobacco products. This may be attained by creating a better experience of the product (e.g. appearance of the product, white and full smoke) or by making it easier to start smoking (e.g. by means of a cool, sweet and mild smoke, as well as causing less irritation in the lungs).

For many additives, attractiveness depends on multiple functions which may be difficult to distinguish clearly. One of the reasons to use additives is to attract the smoker to a specific product and to promote/encourage (young) people to start using the product. Other reasons for using additives are to produce a unique product, typical in taste and

markedly different from competitor products, and to maintain the stability of the taste of the product.

3.8.2.2. Better experience of the product

Preservation of humidity of the tobacco product

Humectants are added to tobacco products to retain the water, i.e. to prevent them from drying out, and consequently increase the shelf life of the products.

Examples of additives

Examples of additives include: glycerol, propylene glycol and sorbitol.

Appearance, smell and irritation of tobacco smoke

In order to make the smoke more attractive to the smoker, but also to other people in the proximity of the smoker, it is important that the smoke is appealing and not annoying. This may be attained with additives which make the smoke whiter and more attractive to people seeing the smoke. The smell of the smoke may be also changed so that it is also more attractive and less irritating (Connolly et al. 2000, Ling and Glantz 2005).

Connolly et al. (2000) examined tobacco industry patents covering the function of environmental tobacco smoke masking. These strategies include reducing smoke odour, and reducing side-stream smoke visibility and emissions.

Methods to neutralize or reduce lingering smoke odour include addition of acetylpyrazine, anethole and limonene to modify the side-stream odour. These compounds have rather low odour thresholds, and are subsequently easily picked up, while they elicit no trigeminal nerve response. Aroma precursors, e.g. polyanethole provided a noticeable fresher, cleaner and less irritating cigarette side-stream aroma, while others (e.g. cinnamic aldehyde, pinanediol acetal) produce slightly sweet, spicy, clean, fresh, and less cigarette-like aroma. Also, more "classic" additives (e.g. vanillin, benzaldehyde, bergamot oil, cinnamon/cinnamon extract, coffee extract and nutmeg oil) modify side-stream odour.

Reduced visibility of side-stream smoke is accomplished by the addition of magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate and calcium carbonate to the wrapper (cigarette paper). This has an effect on particle size; particles become smaller and therefore do not easily scatter light and become less visible.

Reducing side-stream emissions is based on encapsulating the smoke in an impermeable cone using different types of additives such as potassium succinate, potassium citrate and magnesium carbonate.

By combining the use of additives and the look of the tobacco product, greater acceptance of the smoke may be created. Less resistance may be encountered from persons that do not smoke, and at the same time greater pleasure for the smoker may be created. The same agents may also be used to target the individual product at certain target groups (Carpenter et al. 2005a, Connolly 2004).

Taste and experience of the smoke

Cis-3-hexenol is added to increase the organoleptic characteristics of tobacco and it has a characteristic smell of newly mown grass (Alford and Johnson 1970). *Cis*-3-hexenol adds a green, foliaceous taste and a smell of chlorophyll to the tobacco smoke (Leffingwell et al. 1972). Apart from adding a taste and flavour of fresh tobacco to the tobacco smoke, the substance has another important characteristic: *cis*-3-hexenol reduces irritation (Alford and Johnson 1969).

The American tobacco company Brown & Williamson has tested the effect on the characteristics of the smoke when adding *cis*-3-hexenol to cigarettes (Alford and Johnson 1969, Alford and Johnson 1970). Cigarettes with added *cis*-3-hexenol in concentrations of 0.05, 0.10 and 0.15 mg per cigarette were tested against control cigarettes without added *cis*-3-hexenol by having an expert panel smoke the various cigarettes. All cigarettes with *cis*-3-hexenol were preferred to the control cigarettes (Alford and Johnson 1969, Alford and Johnson 1970). The effect of *cis*-3-hexenol was *"A dramatic increase in smoke freshness and acceptability. Irritation is also markedly reduced."*

Harshness

According to the tobacco industry definition, harshness is a chemically induced physical effect associated with a roughness, rawness experience generally localised in the mouth and to a lesser degree in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. Harshness can also cause a drying, rasping, coarse, astringent sensation usually associated with the smoke flavour of Virginia or air-cured type tobaccos.

Harshness is classically measured in four degrees: (i) Free – an absence of harshness; (ii) Touching – a slight awareness of a sensation; (iii) Scratchy – some discomfort, a stinging effect; and (iv) Harsh – rough, raw, raspy, coarse, astringent, painful inhalation.

Reducing the harshness of the smoke makes it possible to inhale deeper and increase the number of puffs, as more physical barriers will be reduced (Wayne and Henningfield 2008b).

The ratio between nicotine and tar is an important parameter in relation to the smoker's experience of the cigarette. If the concentration of nicotine in relation to tar is too high, the harshness of the smoke will be much higher (Hurt and Robertson 1998). Nicotine is irritating in high doses compared to other substances in the smoke (Baker 1990).

The irritating effect of nicotine on the lungs and the bad experience at too large amounts of nicotine in relation to the amount of tar may be remedied by additives that may drown or reduce the harshness of the smoke. This may also be achieved by adding nicotine salts that do not cause the same irritation, but are still delivering nicotine or keeping the nicotine effect by means of a quicker absorption by ensuring larger amounts of free nicotine (Bates et al. 1999, Keithly et al. 2005).

Smoothness

Tar provides a strong flavour and mouth sensation, masking the harsher, bitter taste of nicotine which may be unpalatable to new smokers and uncomfortable to established smokers. Certain highly flavoured additives may also have the same properties to "smoothen" or reduce the harsh irritation of nicotine in tobacco smoke.

A central feature of tobacco marketing strategy has been to promote the perception that some cigarettes are less hazardous than others, so that smokers worried about their health are encouraged to switch brands rather than quit. Products bearing the word "smooth" or using lighter coloured branding mislead people into thinking that these products are less harmful to their health. Adults and children are significantly more likely to rate packs with the terms "light", "smooth", "silver" and "gold" as lower tar, lower health risk and either easier to quit (adults) or their choice of pack if trying smoking (children). For example, more than 50% of adults and youth reported that brands labelled as "smooth" were less harmful than the "regular" variety. The colour of packs was also associated with perceptions of risk and brand appeal. For example, compared to Marlboro packs with a red logo, cigarettes in packs with a gold logo were rated as lower health risk by 53% and easier to quit by 31% of adult smokers.

Plain packs significantly reduced false beliefs about health risk and ease of quitting and were rated by the children as less attractive and appealing (Hammond et al. 2009a).

Examples:

Propylene glycol

The addition of propylene glycol (1,2-dihydroxypropane) to tobacco results in a milder smoke (Danker 1958). It was found that propylene glycol reduces the delivery of nicotine, while the formation of tar is increased (Shepperd and Bevan 1994b). In another study, also by the Brown & Williamson Tobacco Company, a reduction of nose irritation was observed and a reduced delivery of nicotine was confirmed (Shepperd 1994a). It was suggested that the sensation of reduced effect and irritation in cigarettes with added propylene glycol is caused by reduced liberation of nicotine, since the tar/nicotine ratio is of importance to the sharpness of the smoke (Danker 1958, Shepperd and Bevan 1994b).

Levulinic acid and levulinate

Based on the information submitted by the tobacco industry to the competent authorities of the EU Member States, these two substances have in many cases not been included in the reports, but have been used and mentioned several times in the internal documents of the tobacco industry.

These organic salts would also be able to reduce the harshness of nicotine, as the salts do not cause the harshness that otherwise characterises high levels of nicotine (Bates et al. 1999). In a study of the published literature up until 2004, Keithly has also shown that the primary purpose of levulinic acid as an additive in tobacco is to make the smoke sweeter and softer and at the same time increase the nicotine absorption and the effect of nicotine in the brain. Keithly also describes the use of nicotine levulinate and levulinic acid to cause less harshness (Keithly et al. 2005).

3.8.2.3. Easier to start smoking

Tobacco products may also be designed in such a way that they are easier to start smoking with. This may be attained by making it easier to inhale the smoke in the lungs and by creating a sweeter, milder or “colder” smoke. By reducing and changing the harshness of the smoke, special target groups may be reached (Carpenter et al. 2005a, Carpenter et al. 2005b, Cummings et al. 2002, Klein et al. 2008, Wayne and Connolly 2002).

In a number of countries, sweet and tasteful tobacco products are the most preferred tobacco products among children and adolescents as well as experimenting smokers (Ashare et al. 2007, Giovino et al. 2005, Klein et al. 2008).

How to make inhalation of smoke less aversive

Liquorice

Glycyrrhizin is the active substance of liquorice i.e. the root extract of *Glycyrrhiza glabra* and has a sweet taste (Hodge and Shelar 1979). Apart from glycyrrhizin, liquorice also contains sugar substances, cellulose fibres and essential oils (Covington & Burling 1987b).

The taste and flavour of tobacco with liquorice/liquorice root added are described as sweet, woody and round (Leffingwell et al. 1972), but adding liquorice/liquorice root also has the objective of camouflaging the unpleasant taste of tobacco (Covington & Burling 1987b).

The use of adding liquorice/liquorice root to tobacco has the following advantages (Vora 1983); it reduces the harshness of tobacco smoke, the dryness in the mouth and throat, and it provides a pleasant sweet undertone to the smoke.

Menthol

The additive menthol is relevant for how a smoker experiences the smoke in the lungs and the concentration of menthol may be an important issue for the group that the cigarette brand is targeted at. This is described further in section 3.8.3.1, which broadly outlines the potency of menthol to inhale smoke more easily and deeply.

Cooler and milder smoke

Certain substances make the smoke milder and cooler, e.g. menthol (see section 3.8.3.1), liquorice and propylene glycol. However, many more additives probably have these effects on the smoker's lungs, but they have not yet been evaluated, or have not been described in the literature.

Sweeter taste

The presence of sugars in cigarettes is associated with a more favourable taste. The experience of the smoke is less negative and the irritability is somewhat masked.

The tobacco producers have used additives that create sweetness and taste in the smoke to make it easier for new smokers to start smoking, since these tobacco products do not have the same harshness and bad experience at the first inhalations (Cummings et al. 2002, Wayne and Connolly 2002).

3.8.2.4. Conclusions on how certain additives can increase the attractiveness of tobacco products

The attractiveness of tobacco products may be increased by a number of additives. An attractive effect may be obtained in a number of ways, such as changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant experience of smoking. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by certain additives such as propylene glycol or levulinates. Various sugars constitute a large proportion of additives, and the sweetness of the smoke is an important characteristic.

Many different additives are used to create a specific taste/flavour in order to attract certain target groups. In order to make the smoke less aversive and permit deeper inhalation, additives such as liquorice and menthol are used. Finally, in order to make smoking more acceptable to people around, some additives have the function of reducing lingering odour or side-stream smoke visibility.

3.8.3. Most prominent additives in tobacco products

3.8.3.1. Menthol

Menthol is an important tobacco additive and it is the only additive explicitly declared to the consumer. For more than 40 years, scientific discussions have covered the health effects of the addition of menthol to tobacco. Menthol is a monocyclic terpene alcohol. It is a naturally occurring compound of plant origin which gives plants of the *Mentha* species the typical minty smell and flavour (Eccles 1994). Mentholated cigarettes have a major share of the market in the USA. However, in most European countries, the market shares for mentholated cigarettes range between 1 and 5% (Giovino et al. 2004). The menthol content has been investigated in the USA in 48 commercially available mentholated cigarette sub brands. Menthol content per g tobacco was reported to range between 2.88 and 5.75 mg menthol (Celebucki et al. 2005). In Germany, the menthol content was analyzed in non-mentholated cigarettes as well as in raw tobacco. Menthol content in raw tobacco and home grown tobacco was in the range 0.02-0.18 µg menthol/g tobacco. Menthol content per g tobacco in non-mentholated cigarettes ranged between 0.019 and 13.3 µg menthol (Merckel et al. 2006). These data clearly prove three points: firstly, menthol occurs naturally in very small amounts in tobacco;

secondly, some brands contain no added menthol at all and in some brands, microgram amounts of menthol have been added; and finally, mentholated brands contain milligram amounts of menthol per g tobacco.

The tobacco industry advertises menthol as a substance which alleviates harshness and enhances taste and smoothness, but menthol may also facilitate nicotine delivery and increase the sensory impact of cigarettes.

Menthol can be applied to cigarettes in a number of ways; it can be applied directly to the tobacco or introduced into the cigarette filter, or it can be applied to the cigarette packaging (see section 3.4.).

The fate of menthol in the cigarette has only been investigated by the tobacco industry. Philip Morris showed with ^{14}C -labelled menthol that 29% of the activity went into the mainstream smoke, and 98.9% was as unchanged menthol (Jenkins et al. 1970). The transfer of menthol from tobacco into smoke was investigated by another company in 11 cigarette brands; the values ranged from 19 to 31% (Brozinski et al. 1972).

A report by Schmeltz and Schlotzhauer raised concerns about the pyrolysis of menthol. The authors pyrolysed menthol under nitrogen at 860°C and analysed the pyrolysate by paper-chromatography and thin-layer chromatography. They found approximately 400 µg benzo[a]pyrene per g menthol (Schmeltz and Schlotzhauer 1968). However, under normal smoking conditions, the menthol evaporates before being burned. The question was investigated again later on by Baker and Bishop who heated menthol at 30°C per second from 300 to 900°C under a flow of 9% oxygen in nitrogen. The products were analysed by gas chromatography and mass spectrometry. The authors found that 99% of the menthol was unchanged in the gas phase; additional products were menthon (0.9%) and menthen (0.1%) (Baker and Bishop 2004a).

Some companies have investigated the influence of tobacco additives on the composition of smoke constituents. For example, Philip Morris studied experimental cigarettes with many additives. They prepared two sets of cigarettes containing, among other additives, 18,000 ppm menthol, yielding 13 mg menthol per cigarette (Carmines 2002). The cigarettes were machine-smoked and compared to control cigarettes without ingredients added. The benzo[a]pyrene content in the smoke of menthol cigarettes was significantly higher compared to the smoke of the control cigarettes. The smoke of the control cigarettes contained 5.1 ng benzo[a]pyrene per cigarette in comparison to 5.63 and 5.51 ng benzo[a]pyrene per cigarette in menthol cigarettes (Rustemaier et al. 2002).

Recent reviews on health effects of menthol in cigarettes published by the tobacco industry have maintained its claim that menthol does not pose any adverse health effects when used as an additive in cigarettes (Heck 2010 Werley et al. 2007).

The hypothesis that smoking mentholated cigarettes increases lung cancer risk compared with smoking non-mentholated cigarettes was tested in several epidemiological studies. Sidney and colleagues found a 1.45-fold increase of the relative risk for men smoking mentholated cigarettes for 20 years and more (Sidney et al. 1995), whereas three other studies (Brooks et al. 2003, Carpenter et al. 1999, Stellman et al. 2003) did not find a difference between menthol smokers and non-menthol smokers.

Menthol has a cooling effect on the skin or mucosal surfaces. The perceived temperature effect is not caused by evaporation of menthol. Furthermore it is not due to vasodilatation, but is due to a specific action on sensory nerve endings (Eccles 1994). Menthol activates a transient receptor potential channel (TRPM8). This channel is expressed in small-diameter primary sensory neurons (Clapham et al. 2005). The use of menthol causes a subjective sensation of improved airflow without any change in nasal airway resistance, breathing pattern or ventilation (Eccles 1994, Nishino et al. 1997). Furthermore, menthol has a local anaesthetic activity (Galeotti et al. 2001).

It is important to take into account that this cooling and anaesthetic effect may mask early symptoms of tobacco induced respiratory disease (Garten and Falkner 2003). In a follow-up paper, it was postulated, that there is a greater opportunity for exposure and

transfer of the contents of the lungs to the pulmonary circulation. For the smoker of mentholated cigarettes this could result in a greater exposure to nicotine and the particulate matter of the smoked cigarette (Garten and Falkner 2004). Additionally, it was postulated that menthol increases the absorption with other chemicals through permeability and increased salivation. This would mean that menthol facilitates the absorption of other substances from the smoke (Ahijevych and Garrett 2004, Eccles 1994). Two recent biomarker studies addressed the question if the use of mentholated cigarettes would lead to higher exposure to toxic compounds from smoke (Heck 2009, Muscat et al. 2009). Muscat and colleagues investigated a group of 525 smokers and stratified them for sex and race. In the United States, African American smokers preferred mentholated cigarettes (90% of men and 82% of women); whereas European Americans smoked predominantly non-mentholated cigarettes (percentage of menthol cigarettes smoked was 25% and 31%, respectively). European Americans smoked significantly more cigarettes per day than African Americans. There were no significant differences in the mean concentrations of all cigarette smoke metabolites (plasma cotinine, urinary cotinine, plasma thiocyanate and urinary 4-N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL)) between menthol and non-menthol cigarette smokers in African Americans and European Americans, after adjustment for sex and other factors (Muscat et al. 2009). However, the ratio of NNAL-glucuronide to NNAL, a possible indicator of lung cancer risk, was significantly lower in menthol versus non-menthol cigarette smokers. The NNAL-Gluc/NNAL ratio was 34% lower in European Americans ($P < 0.01$) and 22% lower in African Americans (Muscat et al. 2009). In subsequent human liver microsome studies, menthol inhibited the rate of NNAL-O-glucuronidation and NNAL-N-glucuronidation. These results suggest that menthol may modify the detoxification of the potent lung carcinogen NNAL (Muscat et al. 2009).

A similar study has been performed and published by the tobacco industry (Heck 2009). They investigated 112 smokers (28 African Americans and 84 European Americans; 54 menthol cigarette smokers and 58 non-menthol cigarette smokers). Smokers continued smoking *ad libitum* throughout the one week study interval. The participants were provided with a commercially available menthol cigarette brand and several non-mentholated brands of similar smoke yield. Menthol content in smoke was determined as 0.34 mg/cigarette. Content of 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) was determined as 63 ng/cigarette in the mentholated brand and with a range from 45 to 80 ng NNK/cigarette in five non-mentholated brands (Heck 2009). Neither total urinary NNAL nor urinary nicotine equivalents exhibited statistically significant differences between the menthol and non-menthol cigarette smokers (Heck 2009).

Recently, a study was published from the tobacco industry on 3341 cigarette smokers (Wang et al. 2010). The participants smoked either mentholated or non-mentholated cigarettes with different tar yields. European Americans in the menthol group smoked more cigarettes than European Americans in the non-menthol group. No differences were found between menthol and non-menthol groups with respect to nicotine equivalents per day, carboxyhaemoglobin (COHb) and serum cotinine. The authors concluded that menthol did not influence the metabolism of nicotine. However, the study was not designed to answer this specific question.

The possible influence of menthol on the metabolism of nicotine was investigated in a cross-over study in 14 healthy smokers (Benowitz et al. 2004). Subjects were randomly assigned to smoke mentholated or non-mentholated cigarettes for one week, then to cross over to the other type of cigarettes for another week. The blood levels of deuterium-labelled nicotine and cotinine were measured after intravenous infusion of these compounds. It was demonstrated that, when smoking similar numbers of mentholated and non-mentholated cigarettes of similar machine-determined yield and nicotine content, the systemic intake of nicotine and carbon monoxide during non-menthol cigarette smoking is on average not affected by mentholation. Furthermore, it was shown that mentholated cigarette smoking inhibits the metabolism of nicotine. Inhibition of nicotine metabolism by menthol most likely involves inhibition of both oxidative metabolism to cotinine, and glucuronide conjugation (Benowitz et al. 2004). *In*

vitro studies using human liver microsomes showed that menthol inhibits nicotine metabolism (MacDougall et al. 2003). However, mentholated cigarette smoking did not substantially affect cotinine metabolism. Finally, the systemic intake of menthol was determined as 12.5 mg menthol from 20 cigarettes. Thus, on average 20% of menthol contained in each cigarette is absorbed systemically by the smoker (Benowitz et al. 2004).

Studies on the influence of menthol on puff numbers and puff volume gave conflicting results. Puff numbers have been investigated in seven studies, three showing a reduced number of puffs in smokers of mentholated cigarettes (Jarvik et al. 1994, McCarthy et al. 1995, Nil and Bättig 1989). Four other studies did not show any influence of mentholation on the number of puffs (Ahijevych et al. 1996, Caskey et al. 1993, Miller et al. 1994, Pickworth et al. 2002). Puff volume was investigated in six studies, three of them showing a decrease in puff volume when smoking mentholated cigarettes (Jarvik et al. 1994, McCarthy et al. 1995, Nil and Bättig 1989). Two studies did not find any effect of mentholation on puff volume (Ahijevych et al. 1996, Miller et al. 1994) and one study even showed an increase in puff volume (Ahijevych and Parsley 1999).

The results of studies on the CO exhalation in smokers of mentholated and non-mentholated cigarettes are contradictory. In a study with experimental cigarettes smokers inhaled defined volumes of cigarette smoke. The experimental cigarettes had been injected with 0 mg, 4 mg or 8 mg of menthol. The CO content in exhaled air increased from 5.6 ppm to 6.1 ppm and reached 8.1 ppm CO after use of 8 mg menthol cigarettes (Miller et al. 1994). Clark and colleagues did find a non-significant difference of 40.3 ppm CO (mentholated cigarettes) against 35.8 ppm CO (non-mentholated cigarettes) (Clark et al. 1996). In a study in women, smokers of non-mentholated cigarettes showed a higher CO exhalation (10.6 ppm) than smokers of mentholated cigarettes (6.5 ppm) (Ahijevych et al. 1996). In a cross-over study, Benowitz and colleagues did not find any significant difference in the blood carboxyhaemoglobin content in smokers of mentholated and non-mentholated cigarettes (Benowitz et al. 2004). Six other studies also did not show significant differences between CO uptake or CO exhalation in smokers of mentholated or non-mentholated cigarettes (Caskey et al. 1993, Heck 2009, Jarvik et al. 1994, McCarthy et al. 1995, Nil and Bättig 1989, Pickworth et al. 2002).

Menthol may increase the degree of dependence, or promote maintenance of smoking behaviour. Several findings suggest that menthol is involved in tobacco addiction. Some investigators have found that menthol cigarette use increases cotinine levels, and a significant correlation between cotinine and nicotine dependence has been reported, as well as a reduction in time to first cigarette of the day (Pomerleau et al. 1990).

Greater smoking urgency among menthol compared to non-menthol adolescent cessation-treatment seekers has been reported (Collins and Moolchan 2006).

Evaluating the tobacco industry documents, it was shown that cigarettes with low contents of menthol appeal to young smokers, new smokers, and smokers that do not like the harshness of the smoke. This can be due to the fact that lower contents of menthol in the smoke cover the harshness of the smoke, whereas a large dose of menthol causes harshness. On the other hand, cigarettes with a higher concentration of menthol appeal to smokers who are used to the harshness of the smoke (Kreslake et al. 2008b).

3.8.3.2. Ammonia and other additives affecting smoke pH

Armitage et al. (2004) described a study in which 10 volunteers smoked either control cigarettes, cigarettes with diammonium hydrogen phosphate (DAP) or cigarettes with urea added. The venous blood levels of nicotine were independent of the amount of DAP or urea added to the tobacco. Preliminary data of a human study performed by a governmental research group at the RIVM (van Amsterdam et al., submitted for publication), comparing two commercial brands (one with low and one with high

ammonia content) with respect to nicotine absorption, showed no difference in venous blood nicotine levels (no difference in total absorption and peak plasma of nicotine) when smoking the two brands. These findings are in agreement with data of Labstat test laboratory (Rickert 1997) clearly showing that the amount of ammonia in tobacco does not lead to higher yields of nicotine and ammonia in mainstream smoke or a higher smoke pH of 10 commercial products. Furthermore, in a review of Seeman (2007), it is concluded that the fraction of free base nicotine trapped in aged smoke particulate matter has not been shown to be a useful predictor of the amount or total rate of nicotine absorption by smokers.

The bioavailability of nicotine is dependent upon the pH as only uncharged nicotine is volatile and can be absorbed readily across cell membranes. The different ways of manipulating cigarettes so that more free nicotine is delivered have recently been reviewed (Wayne and Carpenter 2009). At lower pH the nicotine molecule will be positively charged and an equilibrium between the three forms of nicotine is created in relation to the pH (see figure 3).

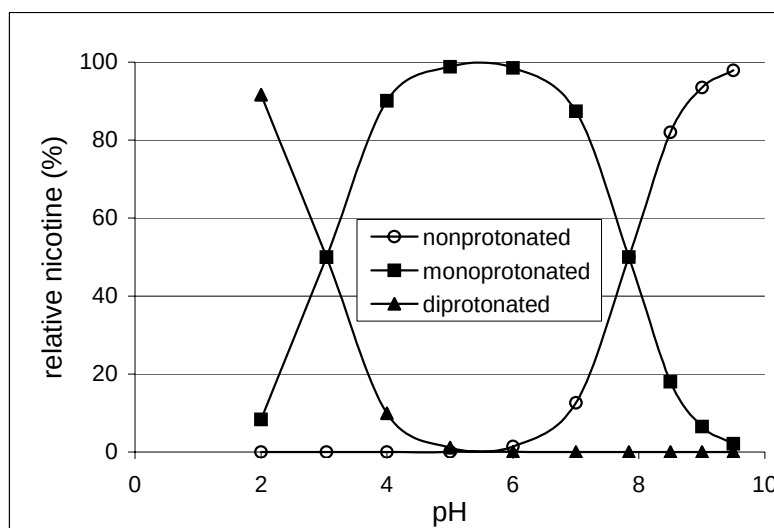


Figure 3: Chemical form of nicotine (charged or free base) and their percentages as function of pH, ranging from 2 to 9.5 (adapted from Hoffmann and Hoffmann (1997)).

Initially, cigarette smoke is lightly acidic and the nicotine is therefore poorly absorbed. However, the pH value is higher in the lungs (7.4) and some of the nicotine is found in uncharged form. Internal documents from the tobacco industry show that manufacturers started to use ammonia to increase smoke pH levels in the early 1970s (Willems et al. 2006). Particular focus has been on ammonia and related compounds, but any compound that contributes to increasing the pH value will have a potential effect in increasing the impact of nicotine and the rate at which inhaled nicotine is absorbed into the bloodstream.

While it has been shown that the absorption of nicotine in smokeless tobacco by the oral mucosa is dependent on the pH of the product (Fant et al. 1999), it is uncertain if the pH in cigarette smoke has a significant impact on the nicotine absorption in the lungs. This is due to the high local buffering capacity of the lung lining fluid which will cause free nicotine to be charged (protonated) again in the deeper airways (Willems et al. 2006). The high buffering capacity of mucus has been shown experimentally in human volunteers (Holma and Hegg 1989).

It is widely accepted that smoke from different pyrolysed tobacco delivery devices (e.g. cigarettes, cigars, waterpipes, etc.) is inhaled differently. For example, cigarette and

waterpipe smoke tends to be inhaled into the lungs, while cigar smoke is typically only inhaled into the mouth (except among former cigarette smokers who have switched to cigar smoking, in which case they often smoke cigars like cigarettes). It has been argued that this may be due to the characteristics of both the delivery device (for example, waterpipes cool the tobacco smoke, thereby allowing easier, deeper inhalation) and the tobacco itself. Waterpipe smoking is associated with greater smoke exposure (a larger volume of smoke is inhaled) than cigarette smoking (Maziak et al. 2009).

This difference in inhalation may be due in part to the more acidic pH of cigarette smoke. The smoke of most cigars has an alkaline pH; as a result, nicotine contained in the smoke can be readily absorbed across the oral mucosa without inhalation into the lung. The more acidic pH cigarette smoke produces a protonated form of nicotine which is much less readily absorbed by the oral mucosa, and the larger absorptive surface of the lung is required for the smoker to receive the desired dose of nicotine. According to the National Cancer Institute (NCI), cigarette smokers must inhale to ingest substantial quantities of nicotine (the active agent in smoke), whereas cigar smokers can ingest substantial quantities of nicotine without inhaling (NCI 1998). The difference may, however, also be explained by the fact that cigar smoke is more concentrated and contains much more nicotine than cigarette smoke.

While there has been considerable research into the effects of product characteristics on cigarette smoking behaviour (such as ventilation holes in “light” cigarettes resulting in compensatory smoking whereby smoke is inhaled more deeply to extract the required dose of nicotine), there is relatively little research into the effects of other delivery devices such as waterpipes. This is despite the rapid growth in the popularity of waterpipe smoking in European countries in recent years.

3.8.3.3. Conclusions on most prominent additives

Menthol is one of the most prominent additives in tobacco. If it is added in milligram amounts to cigarettes it dominates the taste of the smoke and the application is usually mentioned in the brand name. Menthol has a cooling effect on mucosal surfaces and a local anaesthetic activity. The use of menthol causes a subjective sensation of improved airflow without any change in nasal airway resistance, breathing pattern or ventilation. It has been proposed, that the cooling and local anaesthetic effects could lead to deeper inhalation of the smoke and higher exposure to other smoke constituents, but current data are inconclusive. However, menthol has been shown to inhibit the metabolism of nicotine. Furthermore, the taste of menthol could be an important reason for some smokers to consume mentholated cigarettes.

It has been proposed that the addition of ammonia compounds increases the absorption of nicotine in the lungs by raising the pH in smoke, but this seems unlikely because of the high buffering capacity of the lung lining fluid.

3.8.4. Additives in tobacco products other than cigarettes

3.8.4.1. Cigars

Very few additives are used in the classical manufacture of cigars; recently marketed cigarillos being an exception. In general, cigar brands contain only glue as an additive; several compounds are used as glue (e.g. ethyl-2-hydroxy ethyl cellulose, sodium carboxy methyl cellulose, gummi arabicum, methyl hydroxy ethyl cellulose). Several brands contain humectants such as propylene glycol or glycerol. Citric acid is added to influence the burning properties of the cigars. Some companies sum up their flavouring ingredients as “flavouring”, whilst others mention all compounds, including the amounts used.

As written earlier, in Germany, the information about ingredients of cigars can be found on the website of the Federal Ministry of Nutrition, Agriculture and Consumer Protection⁹. Consumers can search for brands and ingredients.

Data from 2008, published on this website, showed that many of the flavourings were added in tiny amounts of 1 ppm. However, other flavourings such as 2-methylbutyric acid were added at a level of 60 ppm and ethyl vanillin was added at levels up to <0.5%. Some cigar manufacturers disclosed probably most, if not all of the additives, for example 211 additives are listed for the brand “7B Bonajuto” starting with 34 mg dextrose down to 8 µg clary sage oil.

3.8.4.2. Pipe tobacco

Pipe tobaccos contain humectants (e.g. glycerol and propylene glycol), preservatives (e.g. sodium benzoate, potassium sorbate), sweetening agents (e.g. dextrose, fructose, invert syrup, honey) and many flavours (e.g. cocoa, prune flavour, apple treacle concentrate, tamarind extract).

The ingredients reported in 2009 in Germany can also be found at the website of the Federal Ministry of Nutrition, Agriculture and Consumer Protection⁹.

3.8.4.3. Water pipes

The use of waterpipes has increased in the eastern Mediterranean region since the 1990s with the introduction of maassel, a sweetened and flavoured tobacco (Maziak et al. 2004a). During recent years, the smoking of waterpipes has become a habit among teenagers in Germany and other European countries, and in the USA (BZgA 2008, Jackson and Aveyard 2008a, Primack et al. 2008). The mild, sweet and flavoured tobacco appeals to many waterpipe smokers, especially young smokers. No information is available about the flavours used in waterpipe tobacco. The nicotine content in flavoured waterpipe tobacco ranged from 1.8 to 6.3 mg nicotine/g tobacco; the average was 3.35 mg nicotine/g tobacco. In contrast, the traditional waterpipe tobacco without flavour contained 30 to 41 mg nicotine/g tobacco (Hadidi and Mohammed 2004).

There are major differences in the consumption of waterpipes compared to other tobacco products. In contrast to cigarettes and cigars, the tobacco in waterpipes is not burned. The waterpipe tobacco is placed in the tobacco head, which is covered by a perforated aluminium foil on which the glowing charcoal is placed. In a study in Lebanon, Shihadeh measured the temperature during a waterpipe session. Within 15 minutes the foil reached a temperature of 400 to 450°C, whereas the waterpipe tobacco reached temperatures ranging from 60°C (after 10 minutes) to 120°C (after 50 minutes) (Shihadeh 2003). To prevent the tobacco from burning, high amounts of humectants are added to waterpipe tobacco. Besides glycerol and propylene glycol, the companies use honey and molasses. The resulting smoke is very mild and it is easy to inhale, even for inexperienced smokers. Since the smoke has almost no harshness the smoker can inhale huge volumes. Some waterpipe smokers refuse to smoke cigarettes. Waterpipe smokers inhale between 0.3 and 1.0 l per puff (Eissenberg and Shihadeh 2009, Monn et al. 2007, Shihadeh 2003, Shihadeh et al. 2004) compared to approximately 0.050 l per puff in cigarette smokers (Kozlowski and O'Connor 2002).

In Germany, the addition of humectants to waterpipe tobacco is restricted to an upper limit of 5%. The Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung (BfR)) argued that it is possible that higher concentrations of glycerine in the waterpipe tobacco could lead to higher contents of acrolein in waterpipe smoke. Acrolein is present in waterpipe smoke as shown in a recent investigation from Lebanon. The authors not only found acrolein, but also high amounts of acetaldehyde. In one waterpipe session, 2520 µg acetaldehyde was measured in the smoke (Al Rashidi et al. 2008).

In 2008, few companies reported added ingredients in waterpipe tobacco to the German authorities. However, the values reported were interesting: in some brands the total tobacco content was small, for example the label “Al-Waha” contained per kg: 200 g tobacco, 710 g fructose, 50 g glycerine and 40 g flavouring. Another label (“Sindbad”) contained per kg: 398 g invert syrup, 210 g water, 42 g propylene glycol, 28.6 g flavouring, 6 g ethyl alcohol and 1.92 g potassium sorbate, leaving 313 g of tobacco. The tobacco content of waterpipe tobacco is thus not very high (20 to 31%).

Studies from Syria show that waterpipe use can be addictive. The frequency of waterpipe use was strongly correlated with the participants’ subjective judgement of how hooked they are on waterpipes (Maziak et al. 2004b).

3.8.4.4. Smokeless tobacco

There are many different types of smokeless tobacco in use around the world, some being more toxic than others.

In Europe, smokeless tobacco is widely used in Sweden (24% of men, 3% of women), in particular in the form of moist snuff called “snus”. Snus is sold in loose weight in boxes or in small “tea-bag”-like sachets. The sale of snus is banned in all other EU countries. As described in the SCENIHR opinion on smokeless tobacco products (SCENIHR 2008), the frequency of smokers in Sweden is lower than in other European countries and the morbidity due to tobacco related diseases is also lower.

On the other hand, according to a recent study in the US, the promotion of smokeless tobacco as a safer alternative to cigarettes is unlikely to result in a substantial health benefit at a population level (Mejia et al 2010). The dual use of smokeless tobacco and cigarettes may in part explain the findings. The tobacco industry has marketed and promoted snus as a way to cope with smokefree environments, at the same time advocating the endorsement of snus as a harm reduction product by public health authorities. This aspect is outside the scope of the current opinion, but as indicated in the SCENIHR report from 2008, all smokeless tobacco products (STP), including snus, contain carcinogenic compounds and cause addiction and dependence (SCENIHR 2008). Moreover, the studies comparing the efficiency of smokeless tobacco with established therapies for smoking cessation are inconclusive.

Due to immigration, many different smokeless tobacco products have found their way into EU countries, and their use is typically clustered in local communities. A similar clustering of use may be seen with now increasingly rare traditional European products such as nasal snuff.

The Swedish “snus” is, according to the manufacturers (Swedish Match¹³, Fiedler & Lundgreen¹⁴), a standardised product using mainly air cured tobacco. Sodium carbonate is added in order to raise the pH to around 8, thus facilitating the uptake of uncharged nicotine in the mouth (Fant et al. 1999). A number of artificial or natural flavours are added according to the brand; the flavours all comply with food regulations. Two sorts of humectants are used, glycerol and propylene glycol. Snus is pasteurised and the fermentation that takes part in other tobacco products is thus inhibited, leading to a lower content of tobacco specific nitrosamines. More than 250 additives are found in different snus brands, most of them are flavours which are used in small amounts. Table 4 shows the 50 substances that are added in greatest amount.

Gutkha is another smokeless tobacco product that is popular among Indian communities in the UK. This is a chewing tobacco that in addition to tobacco contains areca nut, catechu, lime, saffron, saccharine, mint and various flavourings. A table describing the

¹³ www.swedishmatch.com/

¹⁴ www.flsnus.se

many different smokeless products that are rarer in Europe is found in the SCENIHR report from 2008 (SCENIHR 2008).

Table 4 The 50 additives present in greatest amount in different snus brands¹⁵

Ingredient	Maximum percentage added to different snus brands
Sodium chloride	6.7
Ethanol	5.1
Propylene glycol	4.2
Coffee extract	3.7
Plant fibre	3.7
Glycerol	3.6
Sodium carbonate	2.9
Benzyl alcohol	2.1
Anethole (trans-)	1.5
Peppermint oil	1.5
Maltodextrin	1.4
Calcium carbonate	1.2
Licorice and liquorice extract	1.1
Gum Arabic	0.9
Lemon oil	0.7
Ammonium chloride	0.6
Vanillin	0.6
Lime oil	0.4
Ginger extract	0.3
Linalyl acetate	0.3
Menthol	0.3
Ethyl butyrate	0.2
Eucalyptus oil	0.2
Hydroxyphenyl-2-butanone (4-(para-))	0.2
Potassium sorbate	0.2
Sugar, invert	0.2
Acesulfame K	0.1
Acetic acid	0.1
Benzaldehyde	0.1
Buchu leaf oil	0.1
Butyric acid	0.1
Citronellol	0.1
Clary sage oil	0.1
Damascenone	0.1

¹⁵ Data extracted from www.swedishmatch.se

Ingredient	Maximum percentage added to different snus brands
Damascone (beta-)	0.1
Diacetyl	0.1
Dimethyl-1,2-cyclopentadione (3,4-)	0.1
Ethyl 2-methylbutyrate	0.1
Ethyl acetate	0.1
Geraniol	0.1
Geranium rose oil	0.1
Hexen-1-ol (cis-3-)	0.1
Hexen-1-yl acetate (cis-3-)	0.1
Hexenyl butyrate	0.1
Hexenyl formate (cis-3-)	0.1
Hexyl alcohol	0.1
Hydroxy-2,5-dimethyl-3(2H)-furanone (4-)	0.1
Ionone (alpha-)	0.1
Ionone (beta-)	0.1
Jasmone	0.1
Lactic acid	0.1

3.8.4.5. Conclusions on tobacco products other than cigarettes

Compared with the widespread use of cigarettes, other tobacco products are consumed much less commonly. There is a great variety of additives which either have a specific function as humectants, glues, acidity regulators etc., or determine the specific flavour of the product or brand. Most flavours are added in small amounts. There is a lack of data to clarify that any of the additives are by themselves contributing to addictive potential, either directly or indirectly. The additives and the design characteristics of tobacco products are likely to attract specific groups of consumers and perhaps facilitate initiation of tobacco use. The aspects of target groups will be addressed in later sections (3.10.2).

3.8.5. Overall conclusions concerning additives which can increase the addictiveness and/or attractiveness of tobacco products

For most tobacco additives, information about possible effects on addictiveness and attractiveness does not exist. A number of studies have been conducted by the tobacco industry, and there are indications that some additives have effects in relation to addictiveness and attractiveness.

The pyrolysis of sugar substances to acetaldehyde and more complex aldehydes may increase nicotine addictiveness, but the data are not yet conclusive, although an important role of inhibition of monoamine oxidases by tobacco smoke has been repeatedly demonstrated. Additives that increase the pH, and thereby the formation of free nicotine, may contribute to addictiveness, but the efficacy of these compounds has not been shown. In view of the buffer capacity of the body fluids involved (saliva, lung lining fluid), the presence of such an effect is doubtful.

A large number of additives are used to increase attractiveness. Among these, various sugars constitute an important part. Menthol is widely used in certain brands in considerable amounts while most other additives are used in small amounts, and the mixture of additives is characteristic for each brand. This is an important aspect for maintaining consistency of the tobacco products and in targeting special groups.

3.9. Experimental animal models

Several animal models are available to study particular responses that are related to nicotine addiction. Thus, predictive models are available in animals to evaluate the development of nicotine tolerance and physical dependence as well as the rewarding/reinforcing effects produced by nicotine. The animal methods currently used to evaluate nicotine addictiveness are mainly based on the evaluation of its rewarding/reinforcing properties. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently. However, these new models can only be applied for some particular drugs and are not yet available for nicotine addiction.

3.9.1. Experimental models to evaluate the development of nicotine tolerance and physical dependence

Long-term consumption of nicotine produces adaptive changes in the central nervous system leading to the development of tolerance and physical dependence that can be easily evaluated in animal models. Thus, chronic nicotine administration produces tolerance to most of its pharmacological effects (Benowitz 2008). Tolerance to several nicotine responses such as hypolocomotion, convulsive effects, hypothermia or antinociception has been widely described in animal models, whereas an absence of tolerance to the effects on cognitive processes has been currently reported in these studies (Benowitz 2008, Collins et al. 1988, Damaj and Martin 1996, Marks et al. 1986, Miner and Collins 1988).

In humans, cessation of tobacco intake precipitates both somatic and affective symptoms of withdrawal which may include severe craving for nicotine, irritability, anxiety, loss of concentration, restlessness, decreased heart rate, depressed mood, impatience, insomnia, increased appetite and weight gain (Hughes and Hatsukami 1986, Hughes 2007). In rodents, nicotine withdrawal is also characterised by the manifestation of both somatic signs and affective changes similar to those observed in humans. The somatic signs include teeth chattering, palpebral ptosis, tremors, wet dog shakes, and changes in locomotor activity and other behavioural manifestations (Malin et al. 1992). Although the development of nicotine tolerance and physical dependence is concurrent to the development of addiction, they are not aetiologically related to nicotine addiction (Volkow and Li 2005). However, the affective manifestations of nicotine withdrawal seem to play an important role in the maintenance of the nicotine addictive process. These manifestations can be evaluated in rodents by measuring several emotional symptoms such as increased anxiety, aversive effects and reward deficits (Jackson et al. 2008b, Johnson et al. 2008). The aversive manifestations of withdrawal are mainly evaluated in rodents by using the place conditioning paradigm, whereas the associated reward deficits are currently evaluated using intracranial self-stimulation techniques. Both behavioural paradigms have also been extensively used to evaluate nicotine rewarding effects and will be described in the next section.

3.9.2. Experimental models to evaluate nicotine rewarding effects

Drug consumption is promoted and maintained by the rewarding properties of the drug. However, it is important to underline that drug consumption is a requirement for the development of addiction, although addiction is not a necessary consequence of drug intake.

3.9.2.1. Self-administration paradigms

Self-administration methods are widely used to directly evaluate the reinforcing properties of a drug. The procedures are considered by most researchers to be valid and reliable models of drug consumption in humans, and to have a high predictive value. It is assumed that the neurobiological mechanisms involved in drug self-administration in animals are similar to those underlying drug-intake in humans (Sanchis-Segura and

Spanagel 2006). Self-administration methods can be classified considering the route of administration and the behavioural paradigm. From a behavioural perspective, these methods can be classified as operant and non-operant procedures. Non-operant paradigms are centred on the amount of drug consumed whereas the operant procedures require a conditioned response in order to obtain the drug, and the analysis of this response provides valuable information about different behavioural aspects of drug consumption. Non-operant paradigms in animals are mainly restricted to oral self-administration and they are very useful for alcohol research considering the similarities with the route of alcohol consumption by humans. The use of the appropriate route of self-administration for each drug of abuse provides an additional source of validity to these animal models, and these non-operant paradigms are therefore not useful in evaluating nicotine rewarding effects.

The use of operant models is based on the learning contingency defined as “positive reinforcement”. In these models, the drug constitutes a positive reinforcer that is delivered contingently to the completion of the schedule requirements (Sanchis-Segura and Spanagel 2006). The operant chambers are equipped with one or more manipulanda, transmitting the operant response and devices to deliver the drug (reinforcer). Usually, there is an active manipulandum that is linked to the delivery of the drug and an inactive one, which results in the delivery of the drug vehicle or lacks any programmed consequence. The programmes of reinforcement commonly used are the fixed ratio and the progressive ratio schedule and the animal species currently used for nicotine self-administration is the rat. It is suggested that fixed ratio schedules measure the pleasurable or hedonic effects of a drug (McGregor and Roberts 1995, Mendrek et al. 1998), whereas progressive ratio schedules are more related to motivation and provide a better measure of incentive salience or craving (Arnold and Roberts 1997). Under a fixed ratio schedule, the drug is delivered every time that a pre-selected number of responses are completed. For nicotine self-administration, the number of responses required to obtain the drug is generally kept low, and the most used is the fixed ratio 1 (a nicotine delivery after each response in the active manipulandum), although fixed ratio 3 and 5 schedules of reinforcement have also been used (for instance, Shram et al. 2008). Multiple studies have demonstrated that rats easily maintain an operant behaviour to self-administer nicotine under these fixed ratio experimental conditions (Maldonado and Berrendero 2009). In contrast with other drugs of abuse, when dose-response curves have been constructed for nicotine self-administration in rats, they have been relatively flat or inverted U-shaped, which may be because of the aversive effects and toxicity associated with high doses of nicotine (Corrigall and Coen 1989, Shoaib et al. 1997). In a large number of studies the dose of 0.03 mg/kg (free base) per infusion showed very robust self-administration behaviour in rats (Corrigall and Coen 1989, Donny et al. 1999, Shoaib et al. 1997).

Under the progressive ratio schedule, the response requirement to deliver the drug escalates according to an arithmetic progression. The common index of performance evaluated in this schedule is the break point defined as the highest number of responses that the animal accomplished to obtain a single delivery of drug. In rats, several studies have also revealed that nicotine can maintain self-administration on a progressive ratio schedule of reinforcement. The break point achieved for nicotine self-administration has been compared by the authors with other drugs of abuse. They found that it was lower than the final ratio obtained for cocaine under an identical schedule of reinforcement, higher than that reported for heroin under similar progressive ratio schedule, and slightly lower than heroin when a slowly accelerating schedule was used (Donny et al. 1999). However, comparison across studies and drugs is difficult due to procedural differences in training parameters, sequence of progressive reinforcement or degree of drug dependence (Stafford et al. 1998). Increasing doses of nicotine usually resulted in a more linear increase in the performance in the progressive ratio schedule than in the fixed ratio schedule (Donny et al. 1999). The maximum break points usually reached by the adult rats when using the progressive ratio schedule are around 50 responses to obtain a single nicotine injection (Shram et al. 2008). Interestingly, higher break point

values were obtained in adolescent rats (around 95) than in adult rats (Shram et al. 2008).

Operant nicotine self-administration has been difficult to establish in mice. A recent study has reported the validation of a new reliable operant model of nicotine self-administration, extinction and relapse in mice. This model was developed in C57BL/6 mice which are particularly sensitive to the behavioural effects of nicotine (Martín-García et al. 2009). Mice were successfully trained to self-administer a dose of nicotine similar to that previously used in rats (0.03 mg/kg, free base) under a fixed ratio 1 schedule of reinforcement. An inverted U-shaped dose-response function was also obtained using mice to self-administer different doses of nicotine (Galeote et al. 2009). Similar to other drugs of abuse, the break point achieved for nicotine self-administration in mice was lower than in rats. Indeed, the maximum break point (27 responses to obtain a single nicotine injection) was reached by the mice when using the dose of nicotine of 0.042 mg/kg (free base) (Galeote et al. 2009).

3.9.2.2. Conditioned preference paradigms

In the conditioned preference paradigms, the subjective effects of the drug are repeatedly paired to a previously neutral stimulus. Through this repeated conditioning process, this stimulus acquires the ability to act as a conditioned stimulus, and the animal will prefer or avoid this conditioned stimulus depending on the rewarding or aversive effects produced by the drug. The most commonly used paradigms apply a spatial environmental stimulus as conditioned stimulus and the animal will show a conditioned place preference or a conditioned place aversion for the environment associated with the effects of the drug or its withdrawal. Although a conditioned approach/avoidance towards specific stimuli can also occur in humans as a result of drug consumption (Bardo and Bevins 2000), the place conditioning paradigms are not primarily intended to model any particular feature of human behaviour. These paradigms mainly represent an indirect assessment of the rewarding or aversive effects of a drug or its withdrawal, by measuring the response of the animal towards the conditioned stimulus. Drugs of abuse display a differential ability to produce conditioned place preference. Opioids and psychostimulants easily produce robust place preference over a wide range of experimental conditions, whereas other drugs such as ethanol, cannabinoids or nicotine produce more inconsistent results (Sanchis-Segura and Spanagel 2006). Thus, nicotine has been shown to induce in rodents conditioned place preference across a wide range of doses in some experiments, although inverted U-shaped dose-response curves have been often reported, and the magnitude of the effect is generally small and affected by environmental stimuli or previous handling history (Castañé et al. 2006, Forget et al. 2005, Grabus et al. 2006, Le Foll and Goldberg 2004). Nicotine also produced aversive effects when used at high doses in some, but not all, studies (Grabus et al. 2006, Le Foll and Goldberg 2004). These results suggest that the rewarding effects of nicotine may be weaker than other drugs of abuse in this particular experimental paradigm (LeFoll and Goldberg 2004). Interestingly, sex differences were clearly revealed in mice exposed to nicotine in the conditioned place preference paradigm. Thus, female mice responded more to the conditioned rewarding effects of nicotine compared with males (Isiegas et al. 2009).

3.9.2.3. Intracranial self-stimulation paradigms

Intracranial electric self-stimulation procedures were essential in the discovery of the brain reward circuits (Olds and Milner 1954) and are now widely used to study the effects of drugs of abuse in the activity of the reward circuits (Sanchis-Segura and Spanagel 2006). In this paradigm, animals are trained to maintain an operant behaviour in order to obtain an electric pulse through an electrode that has been previously implanted in a reward-related brain site, most frequently the lateral hypothalamic area. The threshold of the minimal current needed to promote intracranial electric self-stimulation is estimated. A drug that stimulates the reward circuit will decrease this threshold, which would be

related to its rewarding properties, whereas a drug having aversive effects will enhance the minimal current required to maintain the self-stimulation (Markou and Koob 1993). Nicotine as well as other drugs of abuse such as psychostimulants, opioids or ethanol, reduces the threshold to promote intracranial electric self-stimulation in some reward brain areas (Huston-Lyons and Kornetsy 1992, Kornetsky and Bain 1992, Wise 1996). Therefore, this behavioural paradigm clearly demonstrates the capability of nicotine to activate the brain reward circuits.

3.9.3. Experimental models to evaluate nicotine addiction

The behavioural models available to evaluate drug rewarding effects have been very useful in clarifying the neurobiological basis of drug taking. However, addiction is not just the taking of drugs, but represents a relapsing disorder characterised by compulsive drug use maintained despite adverse consequences for the user (APA 1994). Behavioural models that resemble the main diagnosis criteria for addiction are difficult to validate in animals. Recently, two independent research groups have validated behavioural models of compulsive drug seeking in rodents that resemble addictive behaviour in humans (Belin et al. 2008, Deroche-Gamonet et al. 2004, Vanderschuren and Everitt 2004). In these models the authors have evaluated the difficulties in stopping drug use by measuring the persistence of drug seeking during a period of signalled non-availability. The extremely high motivation of the addicts to take the drug has been evaluated by using a progressive ratio schedule where the number of operant responses to obtain a single drug injection was increased progressively within the same session. The maximal amount of work that the animal performs before cessation of responding (referred to as the break point) is considered a reliable index of the motivation for the drug. These new animal models of addiction report a break point over 500 to obtain a single cocaine injection in “addict rats” (Deroche-Gamonet et al. 2004). In these new animal models of addiction, the continued use of the drug despite its harmful consequences has been resembled by the persistence of the animal’s responding for the drug when drug delivery was associated with a punishment.

However, these models validated for cocaine consumption are still not available for other drugs, such as nicotine. Indeed, nicotine self-administration has not been reported to be maintained when drug delivery was associated with a punishment. In addition, only moderate break point values were obtained when a progressive schedule of reinforcement was used for nicotine self-administration. Thus, the maximum break points usually reached to obtain nicotine, i.e. around 50 responses in adult rats (see for instance, Shram et al. 2008) and around 95 in adolescent rats (Shram et al. 2008), are far away from the break point values (over 500) reached to obtain cocaine by the “addicted rats” (Deroche-Gamonet et al. 2004).

In contrast, recent advances using animal models of relapse have shown that nicotine seeking after extinction of the operant behaviour can be triggered in rats and mice by nicotine-associated (conditioned) cues (Caggiula et al. 2002, Liu et al. 2007, Martín-García et al. 2009), stressors (Bilkei-Gorzo et al. 2008, Buczek et al. 1999) (e.g. mild footshocks) and re-exposure to the previously experienced drug (Chiamulera et al. 1996, Dravolina et al. 2007, Shaham et al. 1997), which are the same events that trigger nicotine craving and relapse in humans. Nicotine-paired cues have a critical role in sustaining nicotine self-administration after prolonged periods of abstinence and in maintaining smoking behaviour in humans. Indeed, a critical role of the environmental stimuli previously associated with drug consumption has been attributed when explaining the high rate of nicotine relapse (Caggiula et al. 2001, Caggiula et al. 2002, Liu et al. 2007). In agreement, the exposure to the associated cues was the most effective stimulus reinstating nicotine-seeking in mice, whereas stress exposure reinstated nicotine-seeking behaviour in half of the mice, and a priming injection of nicotine only reinstates seeking behaviour in a low percentage of mice (Martín-García et al. 2009). The neurobiological mechanisms involved in the processes underlying relapse to nicotine

seeking are poorly understood. Further studies will be required to clarify the mechanisms involved in nicotine relapse using these animal models now available.

3.9.4. Conclusions on experimental animals

Animal models to evaluate the rewarding and the reinforcing properties of nicotine, and the development of nicotine tolerance and dependence, are available. The models most currently used to evaluate nicotine addictiveness are based on its rewarding/reinforcing properties, are well established and have been widely used for other drugs of abuse to determine their addictive potential. Among these models, the operant self-administration paradigm is particularly useful considering its high predictive value for the abuse liability of a drug and therefore also possibly for its addictive potential in humans. A response easy to evaluate in the self-administration paradigm that has been related to the addictive potential is the break point (highest number of responses that the animal accomplishes to obtain a single delivery of a drug). A higher break point represents a direct measure of the motivation of the animal to obtain the drug and is often taken to imply an increase in the addictive potency of the drug. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently, although these new models can only be applied for some particular drugs and experimental conditions at the present moment.

3.10. Human studies of the role of additives in addictiveness and attractiveness of tobacco products

Tobacco addiction is maintained by nicotine, and tobacco products that do not deliver nicotine do not sustain addiction. However, it is important to distinguish between the stages of tobacco use, from early experimentation and initiation (prior to the development of dependence), through to regular use (and possible dependence) and possibly eventual cessation. Therefore, nicotine and additives may play different roles, or may differ in their relative importance during experimentation and initiation compared with the progression to regular use. In addition, the role of additives will differ according to whether the tobacco is delivered as a smoked or smokeless product.

Smoking and inhalation into the lungs, in particular, is a highly efficient form of nicotine administration, as the drug enters the circulation rapidly through the lungs and moves into the brain within seconds. This also allows precise dose titration, so a smoker may obtain the desired effects (Benowitz 2008). Therefore, additives and design characteristics which require the inhalation of tobacco smoke will be associated with increased dependence potential, and this will be particularly true when inhalation into the lungs (as opposed to the oral cavity only) is encouraged. In addition, various tobacco additives and flavourings can modulate the impact of nicotine, including via administration and inhalation behaviour. The impact of these additives on the attractiveness and palatability of tobacco products, in particular in naive users, may influence initiation of use and progression to regular use, before dependence is established.

Tobacco dependence is operationalised in multiple ways, but all definitions share core features of tolerance and withdrawal symptoms in relation to tobacco use. Most studies use either the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for tobacco dependence, or a proxy measure such as the Fagerström Test for Nicotine Dependence (FTND), or the number of cigarettes smoked per day. However, the number of cigarettes smoked per day is often a poor measure of dependence, given the substantial inter-individual variability in the amount of nicotine extracted from a cigarette. The majority of the variance in scores on the FTND is accounted for by the first item ("How soon after you wake do you have your first cigarette?"), and it is likely that many dependent cigarette users can be identified by how soon after waking they smoke their first cigarette. This is most likely due to the short half-life of nicotine, which means that after a period of sleep most tobacco users have very low levels of circulating nicotine, resulting in withdrawal symptoms which are rectified by tobacco use.

Human behavioural studies require either subjective or objective measures of the effects of tobacco, and this allows a comparison of these effects between tobacco products which do and do not contain specific additives. Subjective measures include self-report measures of mood and craving, which may be as simple as single visual analogue scale measures of liking (e.g. “How much do you like the taste of this cigarette”), or include validated questionnaire measures (e.g. the Positive and Negative Affect Schedule). The latter refers to a range of laboratory assessments, including actual smoking behaviour through smoking topography measurement, which allows the detailed measurement of number of puffs taken per cigarette, depth of inhalation, inter-puff interval, and so on. This may also include self-administration or cigarette choice paradigms (e.g. presenting participants with two cigarettes, only one of which contains an additive of interest, to determine which is preferentially smoked), which are more closely comparable with paradigms used in animal studies.

These measures are generally impossible or impractical to collect in survey studies, although the rates of use of different tobacco products, containing different additives, may allow their attractiveness to certain sub-groups (e.g. defined by age or ethnicity) to be inferred. A further complication is the possibility that what constitutes an attractive or palatable product may be culturally or ethnically specific.

3.10.1. Experimental and observational studies

Cigarette smoking topography describes the pattern of smoking behaviour for an individual cigarette smoker, and includes measures of puff volume, puff duration, puff flow, interpuff interval, and number of puffs per cigarette. This technology can be used to assess the effects of product design characteristics and additives on smoking behaviour. There is good evidence, for example, that cigarette smokers partially compensate for the low nicotine delivery by low tar cigarettes, possibly by inhaling more deeply, taking more puffs per cigarette, and so on (Frost et al. 1995), so that the addictive potential of low tar cigarettes may not be substantially different from that of high tar cigarettes (see also section 3.5.5). Similar effects have been observed when comparing nicotised and denicotised cigarettes (Strasser et al. 2007).

Neurological techniques have also been used (e.g. by Philip Morris) to assess the effects of additives on smokers' central nervous system functioning. Electroencephalography (EEG), pattern reversal evoked potential (PREP), and chemosensory event-related potential (CSERP) were used to measure physiological, sensory, and cognitive changes related to nicotine, flavourings and other additives (Gullotta 1994).

Other chemicals (e.g. pyrazine, vanillin, and propylene glycol) appear to increase P1-N2 amplitudes (the first positive and second negative peaks in the EEG waveform elicited by a novel stimulus, corresponding to early sensory processing) (Philip Morris 1995), and different tobacco flavourings affect CSERPs (EEG waveforms elicited by olfactory or gustatory stimuli) differently, even when smokers were unable to discriminate these subjectively (Gullotta 1994). This indicates that objective measures may be more sensitive to the modulation of smoking behaviour by specific additives than subjective measures (see also section 3.8.1 and 3.8.2).

The sensory effects of tobacco smoke may themselves acquire reinforcing properties through their repeated association with the rewarding properties of tobacco. This has been shown in various studies where, for example, denicotised cigarettes continue to be smoked in the absence of nicotine reward but are not smoked as frequently when the upper airway is anaesthetised to block the sensory effects of the tobacco smoke (Rose et al. 1985). This may explain in part the loyalty to specific brands shown by tobacco users, since the exact sensory properties of individual brands will differ. It is also possible that extended product characteristics (e.g. pack designs) may acquire reinforcing properties through similar processes, although this has not yet been investigated systematically.

A variety of product design strategies (e.g. ventilation holes, see section 3.5) and application of additives (e.g. ammonia or ammonia-derivatives, see section 3.8.1.3 and

3.8.3.2) may play important roles either via smoking behaviour such as puffing characteristics (see section 3.5.5), or via more direct biological effects such as nicotine bioavailability. These may in turn influence addictive effects and appeal to the user (Baker et al. 2004c, Djordjevic and Doran 2009). As reported by the tobacco industry, approximately 600 substances are used as cigarette additives, but among the most commonly used products only one additive (menthol) is widely advertised by the industry (Ahijevych and Garrett 2004).

3.10.2. Target groups (age, ethnicity, gender, socioeconomic position)

Internal tobacco industry documents illustrate that additives and technical characteristics have been extensively evaluated in relation to their appeal to specific target groups and markets (Carpenter et al. 2007). Some of this evidence relates to the US experience, in particular with respect to ethnicity, and it is not clear whether these results will generalise, in full or in part, to the European situation. However, some findings, such as those relating to younger age groups and gender, are more likely to generalise.

3.10.2.1. Age groups

There is evidence from tobacco industry documents that flavourings have been used to target younger smokers: “[U]se the FLITE technology to inject various flavours into the blend. These flavours would be new and unconventional. Two flavours which were discussed as options were Root Beer and Brazilian Fruit Juice, both of which *tend to appeal to the younger generation* while being rejected by their parents” (BAT 1997). This may act as a gateway to subsequent tobacco use in adulthood.

A survey in the US showed that 17 year old smokers are three times as likely to use flavoured cigarettes as are smokers over the age of 25 (Klein et al. 2008). Therefore, the addition of exotic flavours may be used to increase the appeal of tobacco products (including smokeless products), and in particular their appeal to naive users and younger age groups. Dutch survey data indicate that taste and smell are important determinants of brand preference among young smokers aged 10-18 years, with brands with light or mild taste regarded as less unhealthy (Talhout et al. 2009).

3.10.2.2. Ethnicity

In the USA, there is a striking difference in the use of mentholated cigarettes among African Americans and European Americans, with the prevalence of mentholated cigarette smoking much higher in the former group. Menthol is the most widely-studied additive, and therefore provides a case-study for some of the behavioural consequences of tobacco additives. This suggests that specific additives may be used to improve the attractiveness of tobacco products to specific populations or target groups.

Internal tobacco industry documents, available under the Freedom of Information Act in the USA, describe the relationships between sensory perception and the attitudes, preferences, and patterns of cigarette use among menthol smokers. Two unique types of menthol smoker are described: those who cannot tolerate the harshness and irritation associated with smoking non-menthol cigarettes, and those who seek out the specific menthol flavour and associated physical sensation (Kreslake et al. 2008b).

Additives also contribute to the effects of other tobacco products with either marginal or region-specific use. For example, clove cigarettes, used predominantly by East Asian populations, are composed of a mixture of tobacco (60–80%) and ground clove buds (20–40%), available with or without filters. Eugenol, an analgesic, is naturally occurring in cloves, and is present in milligram quantities in the clove cigarette filler. Like menthol, eugenol diminishes the harshness of the tobacco smoke (Djordjevic and Doran 2009).

3.10.2.3. Gender

While the targeting of specific groups and populations (e.g. young people, women, ethnic groups) is primarily through advertising campaigns for tobacco products, this targeting can also include the development of specific tobacco products, and the use of specific additives in these products. For example, cigarettes with perfumed scents and labelled as “slim” or “light” brands have been marketed to women. This is reflected in evidence that more women than men smoke light and ultra-light cigarettes (ONS 2007).

3.10.2.4. Socioeconomic position

Tobacco use is heavily socially patterned in developed countries, with prevalence of use being higher in lower income groups compared to higher income groups (Eek et al. 2010, Main et al. 2008). While tobacco use in general, and cigarette use in particular, has declined dramatically in wealthier socioeconomic groups over the last few decades, the decline in less wealthy socioeconomic groups has been much less pronounced. In particular, in the most economically disadvantaged groups, tobacco use prevalence has remained almost unchanged over this period. As a result, tobacco use is one of the largest causes of health disparities between socioeconomic groups in European countries. However, there is a lack of data to show if changing patterns of use in Europe are influenced by tobacco industry's targeting of certain socioeconomic groups.

3.10.3. Emotional/subjective effects

Flavours impart a specific taste or aroma to a product, while other additives may be used for a specific technological purpose in the manufacture of tobacco products (Baker et al. 2004b). Both flavours and other additives can confer emotional and subjective effects. The term “impact” is widely used in tobacco industry research and documents, and is a tobacco industry term for smokers’ subjective awareness of the drug effects of nicotine.

Organic acids have been used since the 1950s to improve “smoothness” of cigarettes. For example, Philip Morris found that lactic acid decreased subjective ratings of harshness and bitterness, and produced a sweeter flavour. Citric additives have been used not only for reduced harshness and flavour modification, but also to modify smoke pH, to neutralize nicotine “impact” (an industry term denoting the organoleptic sensation caused by nicotine; smokers often describe this as “throat catch” or “throat hit”). Tartaric and lactic acids likewise modify the pH of smoke. All of these organic acids increased smoothness and are associated with a decrease in nicotine “impact” (Philip Morris 1989, see also section 3.8.2.2). However, it is unclear whether these effects are due directly to pH modification.

Unregulated botanical and chemical additives might have “multiple-use” purposes, such as enhancing flavour and producing “smoother” cigarette smoke, as well as potentially preventing or masking symptoms associated with smoking-related illnesses (Rabinoff et al. 2007).

3.10.4. Conclusions

A wide range of subjective and behavioural effects of tobacco additives have been reported in humans, but there are relatively few studies published in the scientific literature, with much information having been obtained from tobacco industry documents under freedom of information legislation. In principle, similar methods to many of those used in experimental animal models may also be used in humans. However, there is greater variability in the specific methods employed, which include subjective reports of liking, behavioural measures of drug choice, neurobiological measures of drug effects (such as neuroimaging techniques), and direct measures of drug administration (such as cigarette smoking topography). The majority of additives used appear to be flavourings, and these may be used to target specific markets, such as young people, women, or ethnic groups. There is some evidence that these additives modify objective measures of

cigarette smoking behaviour (i.e. smoking topography), but this is somewhat inconsistent.

3.11. Effects of additives on nicotine-addictive properties

3.11.1. Modification of the pharmacology and reinforcement properties of nicotine

3.11.1.1. Comparison of addictive properties of nicotine vs. whole tobacco and modification of reinforcing properties of nicotine

Acetaldehyde is formed in high concentrations when cigarette constituents, including sugars, are burned. Animal research conducted by Philip Morris demonstrated a synergistic interaction between nicotine and acetaldehyde, using a lever-pressing model of self-administration in rodents (Charles et al. 1983, DeNoble et al. 1997). Rats pressed a bar more for the combination of nicotine and acetaldehyde than for either substance alone. If these results apply to humans, smokers would puff more with the combination of nicotine and acetaldehyde. As described below, an inhibitory effect of acetaldehyde on MAO-A and B is one of the possible mechanisms that reinforce the properties of nicotine. It should be noted that the contribution of acetaldehyde in smoke to the level in blood is minimal compared to, for example, the effect of ethanol consumption (Chen et al. 2007b, McLaughlin et al. 1990). There are indications that users of smokeless tobacco do not have a reduced MAO activity, suggesting that constituents of the smoke such as aldehydes are needed to inhibit MAO activity (Berggren et al. 2007). In section 3.8.1.4 the action of acetaldehyde and other aldehydes is described in more detail.

Tobacco is a potent reinforcing agent in humans, and nicotine is generally considered to be the major compound responsible for its addictive properties (Balfour et al. 2000, Dani et al. 1996, Di Chiara 2000). However, animal experiments indicate some discrepancies between the effects of nicotine and those of other drugs of abuse. For example, the capacity of repeated nicotine administration to elevate dopamine levels in the nucleus accumbens is controversial (Balfour et al. 1998, Di Chiara 2000, Vezina et al. 1992) and repeated nicotine treatments in rats induce a behavioural sensitisation which vanishes more quickly than that for other drugs of abuse (Ksir et al. 1985, Villégier et al. 2003). Furthermore, with the exception of ethanol which possesses potent sedative effects, most drugs of abuse, such as psychostimulants and opiates, induce a substantial locomotor hyperactivity both in rats and mice. Nicotine, however, is a weak locomotor stimulant in rats and generally fails to induce locomotor hyperactivity in mice at any dose (Marks et al. 1983, Sparks and Pauly 1999). Nevertheless, when animals are pretreated with an inhibitor of monoamine oxidases, nicotine is able to induce a potent locomotor hyperactivity, even in mice (Villégier et al. 2006a). These differences could suggest that the addictive effects of tobacco are not only due to nicotine and that monoamine oxidase inhibitors have a critical effect.

In fact, tobacco and tobacco smoke are known to contain a number of compounds, among which monoamine oxidase (MAO) inhibitors, such as harman, norharman or acetaldehyde, have been the focus of special interest (Breyer-Pfaff et al. 1996, Gäddnäs et al. 2000, Rommelspacher et al. 2002). Monoamine oxidases exist under two forms; MAO-A and MAO-B. They are enzymes that degrade dopamine, serotonin and noradrenaline - three neurotransmitters involved in addiction. The inhibition of MAO increases levels of monoamines in the brain which decrease the sensitivity of their respective receptors. Human MAO-A and MAO-B genes isolated from X chromosome-specific libraries span at least 60 kilobases, consist of 15 exons, and exhibit identical exon-intron organisation (Grimsby et al. 1991). Inhibition of monoamine oxidases by tobacco smoke does not result from the actions of nicotine (Carr and Basham 1991), but from that of other compounds also present in other psychotropic plants (Uelbelack et al. 1998). It was shown that MAO inhibitor pre-treatment allows the

maintenance of behavioural sensitisation to nicotine in rats (Villégier et al. 2003), thus suggesting a role of MAO inhibitors in the addictive properties of tobacco. More recently, tranylcypromine, a cyclized amphetamine 5000 times as potent an MAO inhibitor as amphetamine (Zirkle and Kaiser 1964), was found to be able to trigger a locomotor response to nicotine in mice (Villégier et al. 2006a) and nicotine self-administration in rats (Guillem et al. 2005, Villégier et al. 2006a). Moreover, increases in extracellular 5-HT levels induced by monoamine oxidase inhibitors appeared to be crucial for these effects (Villégier et al. 2006b).

Nicotine is commonly considered as a monoamine releaser (Summers and Giacobini 1995, Summers et al. 1996) that increases serotonergic neurons firing (Li et al. 1998, Marubio et al. 1999, Olausson et al. 2001a, Olausson et al. 2001b, Olausson et al. 2002). This increased release of 5-HT, in absence of MAO inhibitors, is however transient. Indeed, an immediate inhibitory retro-control blocking the firing of serotonergic raphe neurons through the stimulation of somato-dendritic 5-HT_{1A} receptors has been described (Engberg et al. 2000, Li et al. 1998, Mihailescu et al. 1998). It has therefore been proposed that MAO inhibitors, because of their enhancing effects on extracellular 5-HT levels, compensate the consequences of the indirect inhibition of serotonergic cells by nicotine, thus suggesting a mechanism by which MAO inhibitors contained in tobacco smoke could act in synergy with nicotine to induce addiction (Tassin 2008). Very recent experiments using 5-HT_{1A} agonists and antagonists have indicated that MAO inhibitors contained in tobacco desensitize 5-HT_{1A} autoreceptors to trigger the strong addictive properties of tobacco (Lanteri et al. 2009).

In humans, nicotine replacement therapies are the most widely used form of pharmacological intervention, but have proven to be remarkably unsuccessful (Medioni et al. 2005, Silagy et al. 2004). Interestingly, most tobacco smokers (> 80%) relapse after a few weeks withdrawal, i.e. when inhibition of MAO activity by tobacco and tobacco smoke is likely to have disappeared. It has also been argued that the lack of efficacy of nicotine replacement therapies was due to the continuous delivery of nicotine by gums or patches. It was indeed believed that peaks of nicotine occur in the brain after each puff of tobacco smoke. Very recent experiments, performed with positron emission tomography (PET) and ¹¹C-nicotine, indicate that these peaks exist only in the arterial blood of smokers and do not appear in the brain (Rose et al. 2010a). The half-life of nicotine in the human brain is 13 minutes, which is much longer than the ~45 seconds which separates two successive puffs. Indeed, brain nicotine levels increase regularly along with the cigarette consumption (Rose et al. 2010a).

The role of tobacco smoke on MAO is even more important than originally thought. A substantial inhibition of MAO-A has been found by neuroimaging in chronic smokers (Leroy et al. 2009). Another study has shown that smokers have the methylation frequency of their MAO-B gene promoter markedly lower ($P < 0.0001$) than non-smokers, thus inducing a higher quantity of MAO-B in smokers (Launay et al. 2009). Interestingly, this is also true for smokers who have quit for about 10 years. This was explained by showing that cigarette smoke induces an increase of nucleic acid demethylase activity and an epigenetic regulation of MAO-B. Altogether, these authors have shown that metabolism of 5-HT is modified in smokers but that it is also true for those who have stopped smoking for a long time (over 10 years) (Launay et al. 2009).

It seems therefore that MAO inhibitors, or any compound able to modify 5-HT metabolism and desensitize 5-HT_{1A} autoreceptors, may provide a more complete scheme of the addictive properties of tobacco in experimental models of reward.

3.11.2. Conclusions on effects of additives on nicotine addictive properties

There is evidence that nicotine cannot, by itself, explain the high addictive potential of tobacco and tobacco smoke. The increase of nicotine in the brain resulting from smoking a single cigarette is extremely rapid due to the absorption of smoke inhaled into the

lungs but the peak observed in arterial blood after a puff is not reflected in the brain where the half-life of nicotine is much higher than in blood. Converging data indicate that MAO (monoamine oxidase) inhibitors contained in tobacco and tobacco smoke act synergistically with nicotine to enhance addiction potential. Smokers have reduced levels of MAO in the brain. Among MAO inhibitors, compounds resulting from sugar combustion, such as acetaldehyde and more complex aldehydes, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT_{1A} autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive. As yet, data about the role of acetaldehyde are the only ones available. They are inconclusive and further investigation about other aldehydes is needed before the role of sugars as indirectly addictive compounds can be confirmed.

3.12. Methods to assess attractiveness

3.12.1. Introduction

According to the World Health Organisation (WHO), the terms “attractiveness” or “consumer appeal” refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use (WHO 2007b).

Overall, attractiveness is likely to be influenced by a subtle array and interaction of any number of these factors, although at certain times individual factors may take precedence (e.g. price, particularly during a recession). In addition, certain factors might be essential for enduring attractiveness (e.g. the presence and ease of delivery of nicotine).

The factors influencing attractiveness can be broadly divided into: extrinsic factors (e.g. marketing, packaging, pricing); and intrinsic factors (e.g. taste, smell, sensory attributes, and pharmacological factors). Additives play a role mainly in the intrinsic factor category, but marketing and packaging can also reflect the presence of additives in a way to attract and maintain customers (e.g. by signalling that the tobacco product contains menthol). Given the subtle interactions between different factors however, identifying and measuring the influence of individual additives on attractiveness of products is difficult. Separating the role of additives in enhancing addictiveness, from their role in enhancing other attractive attributes of a tobacco product is also complex.

3.12.2. Measuring attractiveness

There are two main ways of examining the influence of additives on the attractiveness of a product. Firstly, one can assess individual tobacco products, and compare their attractiveness on a number of scales/dimensions, against other tobacco products. By then examining what is known about the additive content of these products, indirect inferences can be made as to the role of individual additives in the overall attractiveness of the product, although there are important limitations to studies of this kind. Secondly, one can examine the influence of individual additives on attractiveness of a tobacco product, along a number of scales, by experimentally adjusting tobacco products to include or exclude individual additives and testing responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed. However, in practice this may be difficult to achieve by research groups outside of the tobacco industry, who are likely to lack the resources to manipulate additive content in this way.

Tobacco industry documents show that the tobacco companies frequently tested human smokers on their reaction to different cigarettes using focus groups, market testing, human smoking behaviour studies or consumer panels. For example, one study carried out by British American Tobacco in 1980 exposed a panel of smokers, trained to be objective in their evaluation of cigarettes, to different conditions wherein brand

identification was either masked or visible, in order to understand how brand identification and imagery affected subjective evaluation of cigarettes (Ferris 1980).

The difficulties with this type of research are that ethical restrictions will usually preclude human testing of different tobacco products, particularly among non-users or children. In addition, there are technical constraints on the ability to manipulate the presence or absence of specific additives in tobacco products. While the tobacco industry may be able to achieve this, such manipulations may be beyond the resources of independent academic research groups.

Both the main methods have advantages and disadvantages and should be seen as complementary. Ideally, a variety of methods and tests would be utilised and assessors would be looking for overall consistency in the findings, in order to conclude that an additive affected attractiveness.

3.12.2.1. Measuring attractiveness of different brands

Actual brand choices

Assessing actual brand use gives an overall indicator of attractiveness of products which reflects all the factors listed at the outset of this section covering both extrinsic and intrinsic variables, of which additive content is only one factor. A major difficulty of this approach will therefore be separating the influence of these factors. The largest influence is likely to be the marketing budget. For example, the popularity of Marlboro worldwide is likely due to the substantial funding spent on its advertising and promotion. A further complication with interpretation of brand preference data over time is that the tobacco industry has been expanding the number of variants of existing brands; since 1998 brand families have increased by more than 50%. For example, Benson & Hedges increased the number of brands from four in 1998 to 12 by 2008 (ASH 2010).

Brand choices can be examined cross-sectionally in populations (nationally and globally) but longitudinal data enable trends in brand preferences to be examined over time and in relation to changing product make up (content and design) as well as tobacco control policies and other factors. Brand preferences should be examined in subpopulations such as by gender, age, and sociodemographic factors, which might reflect targeting by tobacco companies. Brand preferences in younger age groups (e.g. 11-16 year olds) are especially important to identify as these can enable an assessment of attractiveness and appeal to children. In particular, it is important to assess which brands are used initially by children, followed by those that they progress onto over time. Products that attract children to smoking have been referred to in the literature as “starter products”. This refers to two main types of products: confectionary products which are made and packaged to look like cigarettes, thereby enabling children to imitate smoking (e.g. candy cigarettes, not discussed further here), and tobacco products which are made to look like confectionary (e.g. candy-flavoured cigarettes), thought particularly to appeal to children and ethnic minorities (Connolly 2004).

Comprehensive sources of data on brand preferences at country level broken down by socio-demographics are not readily accessible. As an example, we have selected data from the UK which suggest that brand preferences of children and adults can be quite similar. The top five brands in 2009 were identified as: Lambert & Butler King Size, Mayfair King Size, Marlboro King Size Gold, Benson & Hedges King Size Gold and Richmond King Size (Hegarty 2010). Comparable data are not available for youth from 2009 but in 2006, the most popular brands with 11-16 year olds were: Mayfair (58%), Lambert & Butler (56%), Richmond (45%), Benson & Hedges (28%) and Sovereign (23%) (Amos and Hastings 2009). Four of the brands were common to both adults and youth, and for each age group there was a dominance of economy brands. Trends over time indicate increasing popularity of economy over premium brands suggesting price may be playing a key role in current brand choices. As indicated in section 3.13.2, there

may be a trend in the UK for preferring brands marketed as containing no additives, but this observation needs confirmation.

Careful monitoring of brand preferences over time will be important for future research, as will disclosure by the tobacco industry of detailed product content information for all brands on the market.

Perceived brand preferences

By showing different brands to consumers, assessments can be made about how attractive the products are perceived to be. For non-tobacco users, responses will largely reflect extrinsic factors such as the packaging, but will also reflect their knowledge of experiences of others with the products. For users, such assessments also reflect knowledge and experience of using the products in addition. The role of additives therefore will need to be assessed and inferred alongside these other factors, assuming that differences in additives between the different brands are known. As stated above, this research involves examining the look of a pack, and its design and packaging.

Packages can be digitally altered experimentally to test the responses of the presence or absence of attributes (e.g. whether listing an additive such as menthol alters how people respond to the product). However, studies have shown that colours of packs quickly become associated with certain attributes; for example, one study in New Zealand found that green colouring indicated the presence of menthol (Peace et al. 2007). In these types of studies, different population groups should be compared to test if some products are more appealing than others. For example, one experimental study indicated that some adolescents had more favourable impressions of tobacco brands that featured cherry flavouring in the packaging (Manning et al. 2009).

This type of research has now been carried out in a variety of settings (e.g. internet, supermarket, and mall intercept studies) and using a variety of qualitative and quantitative research techniques (Hammond et al. 2009a, Hammond and Parkinson 2009b, Manning et al. 2009). The products have been assessed along several attributes including their perceived attractiveness, harmfulness, ease of initiation or cessation. Standardised designs, methodologies and questions therefore exist which can be utilised to facilitate comparative analysis.

Sensory attributes to users and others

Consumer perceptions of sensory attributes such as taste or palatability, smoke irritation and odour, can also be useful for indicating differences in brands. Although there is likely to be some impact of packaging and design on expectations of sensory effects, this area of testing will be more focused on attributes of the content and emissions of the product itself. This research can be done in two main ways:

- a) Through surveys of smokers in which questions cover reasons for selecting the brands they smoke and the role of sensory attributes.
- b) Experimentally, using panels of test subjects trying products and expressing preferences using, for example, visual analogue scales (see section 3.10). However, whilst perceived responses to these attributes are important, it is also useful to see how sensory differences translate into topography measurements and the presence of biomarkers, such as cotinine (see below).

These factors could be attractive to a smoker as they make it less troublesome for others in their presence, who are then less likely to complain about their smoking. The sensory attributes to be measured here would include smoke irritation, smoke odour, and visibility of sidestream and mainstream smoke. These assessments can be made as described above, but of non-smokers who live, work or are in the presence of smokers.

3.12.3. Conclusions on methods to assess attractiveness

Attractiveness depends on multiple factors that combine to stimulate use. These include extrinsic factors such as marketing, packaging and price, and intrinsic factors such as taste and smell. It is very difficult to identify the role of individual additives in enhancing addictiveness or enhancing other attractive attributes of tobacco products. The attractiveness of a product may be assessed by the direct comparison of different products by surveys, experimental measures or human testing.

Another way to examine the attractiveness of individual additives is to test a certain tobacco product by introducing the additive in different doses. When additives are thought to act in synergy, they may be tested together. In practice, however, overall attractiveness is assessed by comparison of brand choice in subpopulations according to gender, age and sociodemographic factors. By showing different brands to consumers, assessments can be made about their perceived attractiveness.

Sensory attributes such as taste, irritation etc. may be tested by surveys of users or experimentally on panels of test subjects. In general, methods similar to those described in section 3.10 may be used.

The main disadvantage of using any of the data described above is the lack of detailed information available on additive content of different brands and the extent to which additives contribute to any differences observed, over and above other factors intrinsic to the brand, and the price and marketing of the brands.

3.13. Tobacco use in the European Union

Manufactured cigarettes are by far the most preferred tobacco products in the 27 Member States of the European Union. Cigarettes constitute well over 90% of the tobacco sold whereas tobacco used in pipes and for RYO cigarettes (roll your own) amounts to about 5%. In most Western EU countries, smoking prevalence among men and women has in general stabilised or is decreasing. The number of smokers has also started to decrease in some countries in the eastern part of EU, although generally it is only stabilizing among men, with no clear overall trends, and in some cases a slight rise in prevalence among women is being recorded. In the EU as a whole the situation has been stable over the last decade (WHO 2007a).

The use of smokeless tobacco (snus) is common among males in Sweden. The sale of snus is banned in all other countries in the EU but other oral tobacco products may be sold. In the United Kingdom, both male and female migrants from the Indian subcontinent use a wide variety of smokeless tobacco products. Elsewhere, smokeless tobacco use is rare but a wide variety of tobacco products do find their way to Europe through immigration (SCENIHR 2008). Similarly, waterpipe smoking is spreading through cultural influence, mainly by migrants from the Middle East. However, during recent years, waterpipe use has become increasingly popular among teenagers in the general population.

The latest comprehensive data from the 27 Member States were collected for 2006, (WHO 2009). Where data are missing or misleading (Cyprus and Poland) other sources have been used.

3.13.1. EU adult smoking rates 2006

The overall adult daily estimated smoking prevalence (population-weighted) has stabilised at around 27.5% in the EU. The estimated average smoking prevalence among males is 33.2%: in 11 (mostly Eastern European) countries the rate of male smoking is higher, while in 11 (mostly Western European) countries the male smoking prevalence is below 30% (see figure 4). The estimated average female smoking prevalence in the EU is 21.8%. In 10 (mostly Western European) countries the prevalence rate is higher, while in only three countries it is 15% or less (see figure 5).

3.13.1.1. Gender differences

In all but one country (Sweden), smoking prevalence is higher among men than among women. Data from Latvia show the widest gender gap of 29%. A small difference between male and female smoking prevalence of less than 10% can be found in 11 (mostly Western European) countries.

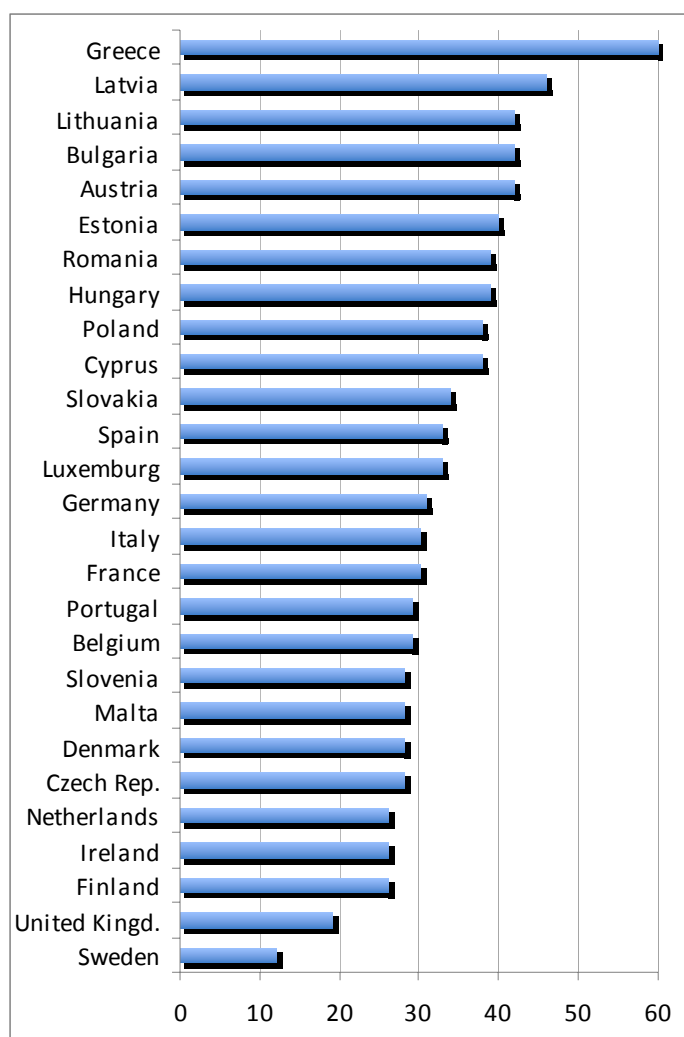


Figure 4: 2006 Rates of daily smokers among males in EU countries (WHO 2009)

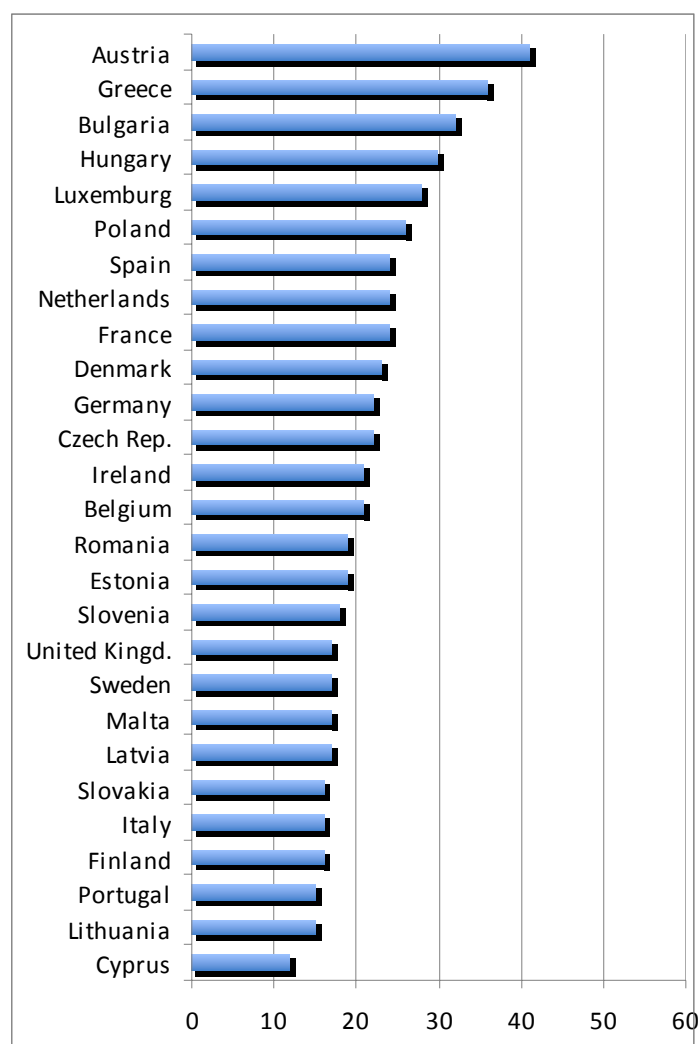


Figure 5: 2006 Rates of daily smokers among females in EU countries (WHO 2009)

3.13.1.2. Changes in smoking prevalence

Estimates for male and female smoking prevalence for 2002 and 2005 are available for 24 of the 27 EU countries. Only relative differences of more than $\pm 10\%$ have been taken into account as noteworthy changes when comparing data for these two years.

Since the 2002 European report on tobacco control policy, smoking prevalence among the male population has in general stabilised across the EU. A notable decrease has been reported for Sweden (16.5% to 14.1%), but in most countries in the EU male smoking prevalence did not show a significant change between 2002 and 2005. There was no significant change in female smoking prevalence although slight increases were observed in many countries.

In May 2010, near completion of the present report, the Special Eurobarometer 332/72.3 was published (EC 2010). This Eurobarometer, performed upon request of Directorate General Health and Consumers (SANCO) of the European Commission, reports on the results of an EU-wide telephone survey on tobacco conducted in late 2009. The survey method is standardised but the results are not directly comparable to the WHO reports quoted above. Furthermore, they are not comparable to an earlier Eurobarometer published in 2006 (EC 2006) due to changes of design (EC 2006, EC 2007a). Still, some additional information can be extracted. The Eurobarometer (EC 2010) reports the proportion of smokers as 29% (males 35%, females 25%) but does not distinguish between daily and non-daily smokers. It is not possible to ascertain whether this

represents a further drop in adult daily smoking rates compared to the WHO report from 2009 (figures 4 and 5) showing data collected in 2006.

However, Eurobarometer (EC 2010) provides other data of interest. The average number of cigarettes consumed is 14.4/day, ranging from 22 in Cyprus to 10 in Sweden. Men smoke, on average, three cigarettes/day more than women. When asked to single out the most important reason for choice of brand, taste is most important for 22% of smokers in the EU 27 while price is most important for 6%. The package scored 0%. One out of 10 smokers in the EU believes that a less harmful cigarette can be identified by taste (ranging from 27% in Hungary to 3% in Denmark). Unique for the Eurobarometer (EC 2010) is the data on waterpipe smoking. On average, 11% of EU adults have tested or use a waterpipe occasionally, whereas 1% smoke it daily. Differences of use vary between countries but being a young adult appears to increase the probability of use.

3.13.1.3. Conclusions on tobacco use in different EU countries

Manufactured cigarettes are by far the most preferred tobacco products in the 27 countries of the European Union and constitute well over 90% of smoked tobacco. The overall adult daily estimated smoking prevalence (population-weighted) has stabilised at around 27.5% in 2006 (males 33.2%, females 21.8%) but higher rates are found mainly in Eastern European countries. Smoking rates have not changed significantly between 2002 and 2006. Smokeless tobacco is used by over 10% of the population in Sweden but its use is rare in other EU countries.

3.13.2. Brand preference, use of additives and consumption patterns

The cigarette market in the UK (and Ireland and Malta) is quite divergent from the continental European countries. This is mainly because in the UK some typical “English” brands are popular and have a large market share. Some quite surprising observations can be made when looking at the top-10 brands marketed in the UK (Hegarty 2010):

1. Of the top-10 brands (according to market share), three brands (Lambert & Butler King Size, Richmond King Size and Richmond Superkings) contain no additives (water is not considered as an additive).
2. Five brands contain up to 10 additives.
3. Two brands (Marlboro King Size Gold of PMI and Royals King Size Red of JTI) both contain over a dozen additives.
4. Lambert & Butler King Size is by far the most sold cigarette brand. Brands without additives have a market share of 42%, whereas those with 1 to 10 additives have a market share of 48%. Brands containing over a dozen additives have a market share of only 10%.

The “taste” of a tobacco product is not only defined by additives but also by blend-selection. English brands i.e. the typical UK brands are made predominantly from flue-cured Virginia tobacco, which contains relatively high amounts of sugars. Marlboro for instance uses the “American blend” (a mixture of Virginia, Burley and Oriental tobaccos) as a base to which many compounds are added during the manufacture.

By blending, it is possible to manufacture cigarettes with a characteristic taste, without using additives. Imperial Tobacco has thus succeeded in producing a typical brand (Lambert & Butler King Size) via the blending approach. In addition, cigarettes marketed as “additive free”, may appeal to smokers that prefer “natural products”.

In Canada, the cigarette market consists almost exclusively of Virginia tobacco which is considered to contain relatively few additives. It should be noted, however, that domestically manufactured “Virginia flue-cured cigarettes” from Canada are by no means “additive-free” (Hammond and O'Connor 2008).

Tobacco products in Central and Eastern European countries before and after 1990

Before 1990, the tobacco used for making cigarettes was usually domestic black shag, and most cigarettes were made with low amounts of additives. Cigarettes were sold without filters and tar levels of 20 to 30 mg per cigarette have been reported. The average nicotine content in Poland in the 1980s was 2 mg per cigarette implying that levels were 1.5 to 2 times the level in Western Europe. After 1990, the large international tobacco companies quickly took over and cigarettes were manufactured in Central and Eastern Europe according to international standards. Most cigarettes are manufactured from light tobacco and the proportion of filter cigarettes rose to 90%. The properties of cigarettes, additives and taste enhancers are now similar to those used in Western Europe and follow the European Union requirements (Zatonski 2008). Availability, marketing, trends, taste, and attractiveness are all factors that may have contributed to the rapid market change.

3.13.3. Smoking prevalence among young people/Target Groups

The analysis of smoking prevalence among young people is from the European Tobacco Control Report 2007 based on the WHO Health Behaviour in School-aged Children (HBSC) study, a cross-national research study conducted every four years: 1993/1994, 1997/1998 and 2001/2002 (WHO 2007a). The 2005/2006 survey was launched in 41 countries and regions and no comparable data are yet available. Information based on a second survey instrument, the Global Youth Tobacco Survey (GYTS) was also used (GYTS Collaborative Group 2002). The GYTS was developed by the United States Centers for Disease Prevention and Control (CDC) and WHO and has been carried out in a large number of countries in the European Region (see table 5). With more and more countries carrying out and repeating the GYTS, comparisons should be possible in the coming years.

3.13.3.1. Current status

According to the HBSC study, weekly smoking prevalence rates were on average 2% among 11-year-olds, 8% among 13-year-olds, and 24% among 15-year-olds. In general, smoking prevalence rates increased more steeply between the ages of 11 and 13 years than between 13 and 15 years. The results of the HBSC and GYTS studies show that weekly smoking prevalence rates in 15-year-old boys were especially high (>30%) in some Eastern European countries (Estonia, Latvia and Slovakia). The highest smoking prevalence rates (>30%) among 15-year-old girls were found mostly in Western European countries such as Austria, the Czech Republic, Finland and Spain. The lowest smoking prevalence rates among 15-year-old boys (<15%) were in Greece and Sweden. Smoking prevalence rates among girls were below 10% only in Greece. An overview of smoking prevalence rates among young people in the EU obtained by the HBSC and GYTS studies is provided in table 5.

Table 5 Weekly smoking rates among boys and girls in EU countries (WHO 2007a)

Country	HBSC				GYTS		
	1997-1998		2001-2002		2001/2004		
	Boys	Girls	Boys	Girls	Year	Boys	Girls
Austria	30	36	26.1	37.1			
Belgium	28	28	21.3	23.5			
Bulgaria					2002	28.7	26.4
Czech Rep.	22	18	28.7	30.6	2002	29.9	32.8
Denmark	20	28	16.7	21			
Estonia	24	12	30.4	18.2	2002-3	31.8	23.0
Finland	25	29	28.3	32.2			
France	28	31	26.0	26.7			
Greece	18	19	13.5	14.1	2003	16.3	9.5
Hungary	36	28	28.2	25.8	2003	24.1	27.4
Ireland	25	25	19.5	20.5			
Italy			21.8	24.9			
Latvia	37	19	28.9	21.1	2002	30.2	22.1
Lithuania	24	10	34.9	17.9	2001	29.0	20.5
Malta			16.9	17.4			
Netherlands			22.5	24.3			
Poland	27	20	26.3	17.0	2003	20.8	14.3
Portugal	19	14	17.6	26.2			
Romania					2004	16.8	12.8
Slovakia	28	18			2003	31.3	28.8
Slovenia			29.5	29.7	2003	24.2	28.8
Spain			23.6	32.3			
Sweden	18	24	11.1	19.0			
United Kingdom	25	33	21.1	27.9			

3.13.3.2. Gender differences

The prevalence of weekly smoking among 15-year-old girls was higher than that of 15-year-old boys in 16 mainly Western European countries of those that implemented the HBSC study in 2001/2002 (Austria, Belgium, the Czech Republic, Denmark, Finland, France, Greece, Ireland, Italy, Malta, the Netherlands, Portugal, Slovenia, Spain, Sweden and the United Kingdom). In Austria, Belgium, Sweden and the United Kingdom, this difference was even greater than in the late 1990s. In the remaining (mainly Eastern European) countries (Estonia, Hungary, Latvia, Lithuania, Poland), smoking prevalence in girls was lower, but in many of these 10 countries, it was catching up and, in two countries (Czech Republic and Hungary), even overtaking smoking prevalence in boys. The GYTS data in general confirmed the pattern of higher rates of smoking prevalence among boys than girls in Eastern Europe.

3.13.3.3. Changes in smoking prevalence

Sixteen countries implemented the HBSC survey both in 1997/1998 and 2001/2002.

A comparison of the results from these two surveys shows that weekly smoking prevalence rates in 15-year-old boys decreased in 11 (mostly Western European) countries of the 16 countries, increased in four countries and remained stable in one. The picture among 15-year-old girls is quite similar: weekly smoking prevalence rates decreased in nine out of the 16 countries, and increased in seven.

A calculation of the averages from these two HBSC surveys shows that the average weekly smoking prevalence among 15-year-old boys and girls did not change significantly between the two periods, although a slight downward trend in boys and a slight upward trend in girls can be observed.

3.13.3.4. Conclusions on smoking according to different groups of young people

Weekly smoking rates among children and adolescents living in the European Union increase four-fold from about 2% at age 11 to 8% at age 13, and another 3-fold increase to 24% at age 15. The highest rates among boys are found in some Eastern EU countries whereas the highest rates among girls are seen in some Western EU countries. From the year 2000, non-significant trends towards decreased smoking among boys and increased smoking among girls have been observed. Smokeless tobacco use is common among adolescent boys in the Nordic countries but rare elsewhere.

Referring to section 3.12 it is clear that the tobacco industry not only has aimed to target different groups of users through advertising and promotion. They have also manipulated the cigarettes themselves. We have very limited data on market share by brand. Top ten lists have only been found from the UK (2009) and Germany (2007). Detailed information on annual cigarette sales in individual EU countries can be purchased from commercial sources, but the price is quite high.

However, even in those publications no data on brand preferences according to gender, age, ethnicity or culture/region are presented. Again, referring to section 3.12 it is conceivable that such information is collected by the manufacturers but treated as trade secrets.

Information about top selling individual brands in EU countries is available from commercial sources. In the public domain, only limited data are available. Data on brand preferences according to gender, age, ethnicity or culture/region are almost non-existent with a couple of limited reports from the UK being the exception. Referring to section 3.12 it is conceivable that such information is collected by the tobacco companies but treated as trade secrets.

3.13.4. Conclusions on EU

European Union tobacco smokers prefer manufactured cigarettes. The overall adult daily estimated smoking prevalence (population-weighted) had stabilised at around 27.5% in 2006 (males 33.2%, females 21.8%) but higher rates were found mainly in Eastern European countries. Smoking rates had not changed significantly between 2002 and 2005. The prevalence of weekly smoking among 15-year-old girls was higher than that of 15-year-old boys in 16 mainly Western European countries whereas the opposite was found in most Eastern European countries. In some countries (e.g. the UK) a large proportion of smokers preferred cigarettes marketed as “additive free”. Significant use of smokeless tobacco was seen only in Sweden and the UK.

3.14. Gaps of knowledge

In a number of areas, it was noted that insufficient information was available concerning tobacco additives and their roles for addiction and attractiveness. These areas include:

- Smoke composition of tobacco products other than cigarettes (cigars, cigarillos, and waterpipes).
- The neurophysiological basis of nicotine addiction.
- Importance of the level of different sugars for the addictive potency of tobacco products.
- Objective measures for attractiveness of tobacco products and additives.
- Information about which brands are preferred by new smokers and the reasons for brand choice

These areas are elaborated upon in the following section.

3.15. Research Recommendations

Although proposals for research recommendations were not part of the mandate of SCENIHR, we have found it useful to indicate some of the topics that were identified during the work on the opinion. It is evident that advanced studies on the action of nicotine and tobacco additives need considerable financial resources that are generally not available in public laboratories. Technological advances have been made in recent years that permit new information to be obtained, for instance on smoke composition and neural networks (functional neuroimaging). We propose either calls for European collaborative projects addressing questions about tobacco and additives or the creation of a European Institute for research on drugs of abuse. An improved knowledge in these areas would allow evidence-based regulation of the manufacture and marketing of tobacco products to be established. Among the proposed research areas are the following:

Smoke composition of tobacco products other than cigarettes

Very little is presently known regarding the composition of smoke emanating from cigars, cigarillos and waterpipes. Thus, knowledge necessary for assessing the potential of smoke constituents to facilitate inhalation of tobacco smoke and also the possibility for smoke to increase addiction is sparse.

The neurophysiological basis of tobacco addiction

It is suggested to determine, by neuroimaging studies, whether nicotine alone (e.g. given as pills) induces signals in the brain of dependent smokers that are different from non-smokers.

Furthermore, to perform experimental studies *in vivo* and *in vitro* (e.g. by neuroimaging, microelectrode arrays, neurochemical, and behavioural approaches) that investigate the influence of different tobacco additives on the addictive potential of nicotine. These studies are crucial to define the exact role of the multiple tobacco additives in the final high addictive potential of tobacco (in humans).

Importance of the level of different sugars for the addictive potency of tobacco products

Sugars are present in significant amounts in Virginia tobacco or are added in high quantities to Burley based tobacco products. It has been established that a high sugar content increases the attractiveness of tobacco products, but it has not yet been clearly demonstrated that sugars increase the addictive potency of tobacco products. It is, however, known that sugars generate numerous aldehydes upon heating, and scientific data indicate that these aldehydes increase the addictive potency of tobacco.

Studies both in animals and humans are therefore required to establish whether the sugar content is related to the addictive potency of tobacco products.

Topics to be studied: (1) Relative efficacy of various sugars to generate aldehydes; (2) Mode of action; and (3) The capacity of different tobaccos (i.e. plain Burley tobacco, Burley tobacco enriched with sugars vs. Virginia tobacco) to form aldehydes and inhibit MAO in situ. Other compounds besides aldehydes and possible mechanisms other than MAO inhibition should also be considered to explain the high addictive potential of tobacco products.

Objective measures for attractiveness of tobacco products and additive

It is necessary to perform innovative techniques such as neuroimaging to assess the attractiveness of tobacco additives objectively. The methods should be sufficiently sensitive to detect the contribution of a single additive added to a tobacco product.

Information on brands preferred by new smokers and the reasons for brand choice, focussed on tobacco additives

Certain typical UK brands are popular although no additives have been used in their manufacture, whereas popular continental brands contain many additives. The differences between these two types with respect to addictive potency should be investigated in comparable user groups. Comparison of use patterns across countries are not likely to give relevant information about the role of additives.

Topics of research: (1) Comparison of the sugar content in different brands; (2) Comparison of the potency to generate aldehydes; and (3) Comparison of the addictive potency (measure the consumption pattern at individual level).

The outcome provides the scientific rationale for the option to control sugars as tobacco additives and to limit their content in tobacco products.

It is also deemed necessary to determine what makes a specific brand attractive for new smokers. Is it the image, popularity, peer influence, taste, or other factors? In this context, epidemiological/sociological studies on trends (studies designed to evaluate the effect of additives on smoking behaviour and their role for initiation) can provide information on what can determine attractiveness.

3.16. Conclusions

In the present report, the available scientific evidence for the role of additives in the addictiveness and attractiveness of tobacco products has been evaluated. The main addictive substance in tobacco leaves is nicotine. However, the abuse liability of pure nicotine in animal studies is low, and great variations are found between individual animals. In contrast, the addictive potential of tobacco products in humans is high; therefore other substances in the final tobacco must play a role in addiction. The vast majority of tobacco products are consumed as cigarettes, and they typically contain around 10% additives by weight; mainly sugars, humectants and various flavours. It has not been possible to define specific additives that influence the addictiveness of tobacco products, but the possible importance of aldehydes formed by combustion of sugar and polysaccharides has been underlined. Sugars and flavours are important for the attractiveness of tobacco products, but it is difficult to distinguish the effects of these additives from indirect effects such as the marketing towards specific groups. Various gaps of knowledge have been indicated and some recommendations for research are made. In the following chapter (section 4), the scientific evidence detailed in the previous sections is summarised in order to answer the questions concerning the contribution of additives to addictiveness and attractiveness of tobacco products.

4. OPINION

In the light of the most recent scientific information, the Scientific Committee is requested to answer the following questions:

1. Which are the criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product?

In human studies there are clinical criteria for dependence (e.g. diagnostic and statistical manual of mental disorders (DSM), difficulty in quitting), laboratory measures of self-administration (e.g. neurobiological measures) and smoking frequency and depth of inhalation, as well as preference studies. These criteria indicate that tobacco in humans has a high addictive potential, but they have limitations when assessing the addictiveness of individual additives in the final tobacco product. There is no widely-agreed universal standard for human studies and as a result various possible endpoints exist. An addicted individual can be considered as someone who is suffering from a specific set of chronic conditions related to a modification of the regulation of their neural networks. It is the potential to induce these modifications which should be the criteria used to define the addictive potency of a product.

In animal studies the reinforcing potency of a drug is used as a criterion for addictive potential. Self-administration studies indicate that the abuse liability of pure nicotine is weaker than the addictive potential of tobacco products in humans. At present it is not possible to evaluate whether additives increase the addictive potency of the final tobacco product. Drugs of abuse such as nicotine induce different types of behavioural and neurochemical dysregulations in animal studies but no consensus about which of those are directly related to the addiction process in humans has yet been attained among scientists.

In conclusion, the criteria for defining dependence indicate that tobacco is highly addictive in humans. Animal studies that use intravenous administration indicate that the abuse liability of pure nicotine is weaker than the addictive potential of tobacco products in humans. In contrast to additives, the combustion-product acetaldehyde has been widely investigated in animals.

2. What are the methods currently used for assessing the addictive potency of a substance and are they considered adequate?

Many different methods are used in humans, but there is a lack of consistency between these methods. Human studies have many limitations in design (e.g. the use of conditioned cues and the need to work with smokers). Furthermore, ethical issues may arise when testing substances in humans.

There is currently no animal model to assess the addictive potency of the final tobacco product; however, pure nicotine has been studied extensively.

The methods currently used in animals to evaluate the addictiveness of any drug of abuse, including nicotine, are mainly based on the evaluation of the re-inforcing properties of the drug. These experimental animal models are mainly based on self-administration protocols in rodents, usually rats. The model with the highest predictive validity is the operant self-administration paradigm. A response which is easy to evaluate is the break point. This is defined as the highest number of responses that the animal completes in order to obtain a single delivery of a drug. A higher break point represents a direct measure of the motivation of the animal to obtain the drug and is often taken to imply an increase in the addictive potency of the drug.

Other models have also been used, such as the intracranial self-stimulation and the conditioned place preference paradigms. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently, although these new models can only be applied for some particular drugs and

experimental conditions at the present moment. The methods have additional limitations as in animal studies pure nicotine is injected intravenously and shows only a weak addictive potential whereas in humans tobacco is used differently (e.g. inhalation, oral consumption). The operant self-administration paradigm has been widely accepted as a reliable animal model with high predictive value for the abuse liability of a drug and therefore, possibly also for its addictive potential in humans. However, a consensus between scientists has not yet been attained on whether this method, which is appropriate to define the abuse liability, would also be the most suitable method to define the addictive potential of a drug.

In conclusion, there are many methods for assessing the addictive potency of a substance in humans, but they have limitations in design and ethical issues may arise. Animal studies using self-administration protocols evaluate the reinforcing properties after intravenous injection of the drugs but there is no consensus concerning the most suitable method for defining the addictive potential. The current methods can thus not be considered adequate.

3. Is the development of nicotine addictiveness dose-dependent?

In humans, there are little data available on pure nicotine use. However, when consumed in tobacco, frequency of use (number of cigarettes smoked per day) is positively correlated with dependence. This suggests that individuals who maintain higher nicotine levels in blood are more dependent than individuals who maintain low levels.

Based on the criteria described in Question 1, dose-dependency appears to have been shown in animal studies. In general, an inverted U-shaped dose-response has been revealed in animals, suggesting that the addictiveness of nicotine is not directly linear with the dose. In addition, pure nicotine is only weakly addictive in some animal studies.

There is substantial variation in response to nicotine and addictive potential in both animals and humans, and genetic factors probably play an important role.

4. Which additives are addictive by themselves in tobacco products?

No tobacco additives which are addictive by themselves have so far been identified. However, sugars, polysaccharides and cellulose fibres, which are naturally present in tobacco, or sugars added in high quantities to most tobacco products, give rise by pyrolysis to aldehydes, such as acetaldehyde, which is self-administered by animals and may thus be considered as potentially addictive.

However, experiments using denicotinised cigarettes show that besides nicotine, a mixture of factors in cigarette smoke probably plays an important role in craving and reinforcement. Although these factors do not have pharmacological effects similar to nicotine, they play a role in smoking behaviour.

5. Which additives enhance the addictiveness of nicotine and how?

A large percentage of the additives found in tobacco are sugars, or their derivatives, that by pyrolysis produce numerous toxic substances, including different combinations of aldehydes, one of which is acetaldehyde. Acetaldehyde injected into experimental animals enhances the addictiveness of nicotine, probably by inhibiting monoamine oxidases (MAO) in the brain. Smokers have indeed decreased levels of MAO in the brain. However, there is no proof that acetaldehyde in the smoke contributes significantly to blood levels of acetaldehyde, and it is likely those aldehydes other than acetaldehyde intervene directly or through the generation of new compounds in the smoke in the inhibition of MAO.

Additives that facilitate deeper inhalation (e.g. menthol) may enhance the addictiveness of nicotine indirectly. Other substances may enhance the addictiveness of nicotine by inhibiting its metabolism. Substances such as ammonia that increase the pH of the tobacco (and the smoke) result in higher amounts of uncharged nicotine, that is more

easily absorbed by the cells. However, due to the high buffer capacity of the lining fluid in the lungs it is uncertain if more nicotine is absorbed with higher smoke pH. It is unlikely that additives in smoked tobacco would increase nicotine blood levels sufficiently to enhance the addictive potential of the tobacco product. For smokeless tobacco it has been shown that more nicotine is absorbed in the mouth when the pH of the product is increased.

In conclusion, apart from the possible action of combustion products of sugars (acetaldehyde and similar compounds that enhance the action of nicotine by inhibition of MAO), there is no evidence as yet that additives enhance the addictiveness of nicotine and therefore of tobacco.

6. Which are the methods used to quantify the potency of additives in enhancing the addictiveness of nicotine and are they considered adequate?

The methods used to quantify the potency of additives to enhance the addictiveness of nicotine or tobacco, are described in the answer to question 2. The limitations of these methods arise from technical challenges in experimentally manipulating the presence or absence of an additive in the tobacco products used in these experiments. Such experiments have probably been carried out by the tobacco industry for some additives, especially sugars and their derivatives, but they require technical and financial resources that are not generally available except to the tobacco industry. In addition, there are ethical issues if testing in humans is considered.

In conclusion, the methods used to quantify the potency of additives in humans or animals have limitations, and the available methodologies are thus not considered adequate for a reliable quantification.

7. Which technical characteristics enhance the addictive potential of tobacco products?

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and carbon monoxide) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Smokers usually compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. In order to achieve the desired level of nicotine impact many smokers apparently take more puffs and inhale deeper when smoking low nicotine cigarettes.

A change of the technical characteristics of cigarettes may affect the mean particle size and, therefore, the distribution of the smoke aerosol. However, based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol.

In conclusion, technical characteristics can modulate smoking behaviour and thereby exposure to nicotine. However, there is no clear evidence that technical characteristics per se modulate the addictive potential of tobacco products.

8. Which are the criteria based on which an additive or a combination of additives can be considered (classified) attractive?

The criterion of attractiveness is the stimulation to use the product.

Attractiveness of additives refers to factors such as taste, smell and other sensory attributes. In addition, a number of external factors (e.g. ease of use, flexibility of the dosing system, cost etc.) contribute to the attractiveness of the product.

The attractiveness of tobacco products may be increased by a number of additives. Many different additives are used to create a specific taste/flavour in order to attract certain target groups. An attractive effect may be obtained by changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant

experience of smoking. The sweetness of the smoke is an important characteristic for certain users. Finally, in order to make smoking more acceptable to other people nearby, some additives have the function of reducing lingering odour or side-stream smoke visibility.

In conclusion, many different factors influence the attractiveness of tobacco products, not only the additives used but also a number of external factors.

9. What are the methods currently used for assessing attractiveness and are they considered adequate?

Animal models do not currently exist to allow the assessment of attractiveness.

There are two main ways of examining the influence of additives on the attractiveness of a product which have largely been conducted by tobacco industry.

The first is to assess individual tobacco products and compare their attractiveness against other tobacco products on a number of scales/dimensions. By then examining what is known about the additive content of these products, judgements can be made as to the role of individual additives in the overall attractiveness of the product. This can be done using a variety of research methods, such as panel studies and surveys, experimental measures and human testing.

The second is to examine the influence of individual additives or combination of additives on attractiveness of a tobacco product, along a number of scales, by experimentally adjusting tobacco products to include or exclude individual additives and testing responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed.

The difficulties with this type of research include ethical considerations that will usually preclude human testing of different tobacco products, particularly among non-users or children.

In conclusion, it is only possible to assess attractiveness in humans, and this may be done by comparison of different products used or by adjusting tobacco products experimentally. However, such studies in human subjects are difficult to carry out due to ethical considerations and the current methods are thus not considered adequate for a reliable quantification of attractiveness in humans.

10. Which additives increase attractiveness of tobacco products?

Numerous additives are used in order to increase the attractiveness of tobacco products.

Various sugars constitute a large proportion of additives, and the sweetness of the smoke is an important characteristic of the product.

Some additives are used to attract certain target groups, because they give the product a specific taste/flavour particularly appreciated by the target group. The best known example is menthol (African Americans) and the use of fruit and candy flavours in high amounts to favour smoking initiation by young people.

A number of additives decrease the harshness and increase the smoothness of the smoke. As a result the smoke inhaled is less aversive, cooler and milder, which improves the experience of smoking and promotes smoking initiation. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by additives such as propylene glycol and glycyrrhizin, a substance in liquorice. Menthol, due to its local anaesthetic effect may enable a deeper inhalation of the smoke. It also acts on sensory nerve endings, resulting in a cooling effect appreciated by smokers.

For cigarettes, certain additives yield a full and white smoke (for example, magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate, calcium carbonate). Other additives reduce the lingering odour of the smoke in order to favour the acceptability of

smoking to people around (for example, acetylpyrazine, anethole, limonene, vanillin, benzaldehyde).

In conclusion, many different additives have been used to increase the attractiveness of tobacco products but it is very difficult to identify the role of individual additives in enhancing attractiveness. In several countries there is a growing trend of using “natural” tobacco products advertised as containing no additives.

11. What is the association between additives and tobacco consumption (independent of any addictive potential they might have)? Which additives are used to target specific groups?

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products, although it is difficult to disentangle the direct effects of additives from indirect effects such as the marketing of specific products at specific groups. For example, the consumption of menthol cigarettes is much higher among African Americans in the USA than among other populations, while flavourings (e.g. fruit and candy) appear to be targeted at young people.

It is notable that waterpipe smoking is becoming increasingly popular in some EU countries (and elsewhere), potentially due to the flavoured tobaccos used and the mild smoke, which facilitate the inhalation of large volumes into the lungs. Smokeless tobacco products have gained increased interest from the tobacco industry because they may be used in places where smoking is prohibited.

Additives and design characteristics may modify consumption patterns, theoretically in a way which may impact on uptake of tobacco use and/or the development of dependence. However, in spite of the many additives commonly used, tobacco products openly marketed as containing specific additives (e.g. menthol cigarettes) command a relatively small market share in EU countries and in some markets so-called natural tobacco products are becoming popular.

In conclusion, additives have been used largely by the tobacco industry to target specific groups. However, the effect of marketing is probably very important and there is currently a trend in several countries to use products labelled “without additives”.

Research Recommendations

A number of knowledge gaps of importance for determining the effects of tobacco additives on addiction and attractiveness have been identified. Some of these gaps have been translated into specific research recommendations that are summarised here.

In addition, it has been noted that due to financial constraints, it is not possible to perform many of the suggested research recommendations at ordinary public research laboratories. Instead, there is a need for European collaborative projects addressing questions about tobacco and additives or even the creation of a European Institute for research on drugs of abuse, in which questions pertaining to tobacco use should also be investigated. An improved knowledge of the roles for tobacco additives in addiction and attractiveness would allow evidence-based regulation of the manufacture and marketing of tobacco products to be established.

Specific areas where research is strongly needed include:

Smoke composition of tobacco products other than cigarettes

The knowledge necessary for assessing the potential of smoke constituents to facilitate inhalation of tobacco smoke and also the possibility for smoke to increase addiction is sparse.

The neurophysiological basis of nicotine addiction

This includes studies of effects on the brain due to nicotine ingestion in both smokers and non-smokers, as well as experimental studies on the influence of additives on the addictive potential of nicotine.

Importance of the level of different sugars for the addictive potency of tobacco products

Although it has been established that a high sugar content increases the attractiveness of tobacco products, it has not yet been clearly demonstrated that sugars increase the addictive potency of tobacco products. Studies, both in animals and humans, are therefore required to establish whether the sugar content is related to the addictive potency of tobacco products.

Objective measures for attractiveness of tobacco products and additives

It is necessary to perform experimental studies to assess the attractiveness of tobacco additives objectively. The methods should be sufficiently sensitive to detect the contribution of a single additive added to a tobacco product.

Information about which brands are preferred by new smokers and the reasons for brand choice

It is deemed necessary to determine what makes a specific brand attractive for new smokers. In this context, epidemiological/sociological studies on trends (studies designed to evaluate the effect of additives on smoking behaviour and their role for initiation) can provide information on what can determine attractiveness.

5. COMMENTS RECEIVED DURING THE PUBLIC CONSULTATION

Information about the public consultation has been broadly communicated to national authorities, international organisations, and other stakeholders. The website opened for comments the 9th of July 2010 and the deadline for submission was the 5th of September 2010. The number of responses submitted by the website was 31; 22 contributions were from organisations, and nine were from individuals. Of the organisations, three were non-governmental, nine business, four public authorities, two academic institutions and four other institutes. In evaluating the responses from the consultation, submitted material has only been considered for revision of the opinion if:

1. It directly refers to the content of the report and relates to the issues that the report addresses;
2. It contains specific comments and suggestions on the scientific basis of the opinion;
3. It refers to peer-reviewed literature published in English, the working language of the SCENIHR and the working group. In some cases, however, other documents have also been considered, especially technical reports from the tobacco industry (further explained in the methodology section 3.2);
4. It has the potential to add to the preliminary opinion of SCENIHR.

Each submission which meets these criteria has been carefully considered by the Working Group. Overall, many of the comments were relevant and of good quality and the opinion has been partly revised based on these comments. Several of the submissions repeated arguments included in the response to the call for information in November 2009. The literature has been updated with relevant publications up to September 2010.

In the following section the comments and revisions to each of the 11 questions to the committee are considered. For all of the questions the majority of the submissions agreed or mostly agreed with the response given by the committee

1. Criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product

One comment addressed the clinical criteria. As mentioned already in the text, the clinical criteria follow the ICD-10 classification and are also in accordance with definitions made by WHO expert groups. The expression “weak” addictive potential of nicotine has been criticised by some stakeholders and the meaning has been clarified in several places by using a statement such as the following: “self-administration studies in animals indicate that the abuse liability of pure nicotine is weaker than the addictive potential of tobacco products in humans”. In order to document that tobacco is highly addictive in humans, a new table (table 1) summarising epidemiological and other studies has been inserted in section 3.1.

2. Methods currently used for assessing the addictive potency of a substance

Some stakeholders criticised the use of animal models. However, for all the questions we have evaluated data available from animal models in parallel with data from human studies. *In vitro* methods do not presently allow responding to questions that involve complex interactions in the body. Self-administration methods are commonly used in animals to test the reinforcing properties of different products. It has been found that the reinforcing effect of a compound presents a high predictive value for the abuse liability in humans. Therefore, although these methods are not directly measuring the addictive effects of a compound, there is a consensus of the scientific community about the interest of such animal models in the study of addiction.

3. Dose-dependency of development of nicotine addictiveness

Some comments question the dose-dependency of nicotine. The dose dependency is certainly not linear, but we maintain that the pharmacological effects of nicotine, including its reinforcing effects, are dose-dependent. Similarly to other drugs of abuse, an inverted U-shape dose-response curve has been reported for the reinforcing effects of nicotine. This dose-response curve reveals:

- a) The reinforcing effects of nicotine are directly dependent on the dose until a maximum level is reached.
- b) After reaching this level, the reinforcing effects also decrease in a dose-dependent manner, probably due to the appearance of other pharmacological responses that include aversive effects. This decrease of the reinforcing effects has been also reported when using high doses of all the other drugs of abuse (i.e. cocaine, amphetamine, heroin, morphine, alcohol, cannabinoids and others).

Some stakeholders emphasize the lack of accumulation of nicotine in dependent smokers. However, the lack of accumulation has no relationship with the dose-dependence of pharmacological effects. Concerning the well-reported desensitization of the nicotinic binding sites after repeated nicotine administration, this is not related to the presence or absence of dose-dependence in the pharmacological effects of nicotine. Indeed, other drugs of abuse that induce dose-dependent pharmacological effects also produce receptor desensitization (opioids, psychostimulants, alcohol, cannabinoids and others), which has been mostly related to the development of tolerance to their pharmacological responses.

Concerning the genetics of nicotinic acetylcholine receptors, several recent articles mentioned in the opinion reveal an association between genetic variations of such receptors and nicotine dependence.

One stakeholder mentions that smokers and non-smokers have a similar absorption and metabolism of nicotine and this would suggest a possible absence of pharmacokinetic tolerance. However, it would not be related to the dose-dependence of the pharmacological effects of nicotine. On the other hand, the development of physical dependence (presence of withdrawal symptoms) has been well reported in animal and human studies to be directly dependent on the dose of nicotine.

4. Additives in tobacco products which are addictive by themselves

Some comments addressed the sugar content of tobacco. It should be underlined that some tobaccos (e.g. Burley tobacco) initially have a low content of sugars due to curing methods. However, sugars are added during the processing so that the final product has a sugar content comparable to that of other tobaccos. This proves the importance of added sugars/inherent sugars for the flavour of the product. This final composition also explains why there is no significant difference between sugar-related pyrolytic end-products (mainly aldehydes) in the smoke from American blend and Virginia cigarettes. We agree that the sentence indicating that “sugars, added in high quantities in most tobacco products”, is not correct because some tobaccos such as Virginia tobacco have a high natural content of sugars. The text has thus been changed in several places.

As mentioned in the opinion, acetaldehyde which is formed by combustion of sugars and polysaccharides, is self-administered in animals and may thus be addictive in itself. There is no proof that acetaldehyde from tobacco smoke enters the brain, but many other aldehydes are formed that may have the same effect, see also the comments below on the increased addictiveness of nicotine caused by aldehydes.

Apart from sugar, no other additives have been found to be addictive by themselves in the doses used in tobacco products.

5. Additives that enhance the addictiveness of nicotine

As indicated above, aldehydes are formed by burning of sugars (and polysaccharides), not only formaldehyde and acetaldehyde but also more complex aldehydes which are probably not degraded by aldehyde dehydrogenase. Many comments indicate that there is little scientific evidence that acetaldehyde present in tobacco or tobacco smoke and produced by sugar pyrolysis is responsible for an increased addictiveness of nicotine through an inhibition of monoamine oxidases. In animal studies, infusion of acetaldehyde potently increases the rate of nicotine self-administration and inhibits monoamine oxidases. Although acetaldehyde is formed in tobacco smoke from sugar combustion, we agree that it is not demonstrated that acetaldehyde in tobacco smoke enters the brain through the smoke inhaled. It is however clear that tobacco smoke contains compounds which inhibit monoamine oxidases. Aldehydes are intermediary products formed by monoamine oxidases which transform monoamines to organic acids and are therefore potent inhibitors of monoamine oxidases. Aldehydes are also "alcohol dehydrogenated" compounds and can be formed through the combustion of poly-alcohols such as sugars. It is thus very likely that acetaldehyde is not the only aldehyde obtained following sugar combustion and these more complex aldehydes may also inhibit monoamine oxidases. Moreover, their complexity would protect them from the action of the aldehyde dehydrogenase which oxidizes acetaldehyde. In addition, some of the added flavours are complex aldehydes. The possible role of complex aldehydes arising from sugar combustion is now emphasised in the report.

Two comments indicate that Berlin and Anthenelli acknowledge, in their 2001 paper, that it is a "hypothesis" that chronic habitual smoking can be understood in terms of reduced MAO activity. Berlin and Anthenelli also acknowledge that their conclusion regarding MAO inhibition by compounds found in tobacco smoke or tobacco can potentiate nicotine's effect is "speculation". Although we agree with this quotation we would like to note that Berlin and Anthenelli wrote this comment in 2001 and since this date many studies have indicated the fundamental role of inhibitors of monoamine oxidases in the effects of tobacco. All the references are quoted in the opinion. Briefly, it has been shown that irreversible mixed A and B monoamine oxidases increase the serotonin cerebral extracellular levels and induce a desensitization of 5-HT_{1A} receptors. This desensitization of raphe nucleus 5-HT_{1A} receptors allows nicotine: (i) to induce locomotor activity in mice; (ii) to be readily self-administered by rats; and (iii) to uncouple noradrenergic and serotonergic neurons in mice. This latter finding indicates that a synergy between nicotine and inhibitors of monoamine oxidases can occur even if the pharmacokinetics of each compound is entirely different.

As mentioned in the opinion, there is no proof that ammonia in tobacco enhances the nicotine uptake due to increased pH in cigarette smoke. Note that although ammonia is not on the common list of ingredients submitted to the European Commission, ammonium salts and other buffering salts are used as additives.

Concerning mentholated cigarettes, the contradictory results from studies on puffing intensity and human smoking behaviour has been presented already in the opinion. The same is true for the biomarker studies: the results for COHb, NNAL, nicotine equivalents have been described. The CYP2A6 polymorphism has been addressed elsewhere (3.6.1.5). One comment confirmed the statement about the function of TRPM8 and the interaction of menthol with this receptor. Further statements about the lack of interaction with other receptors seem not to be relevant for the opinion. One comment made reference to the recent NCI bibliography on menthol and tobacco with 340 references. In the opinion only the most relevant references have been used. The inhibition of nicotine metabolism by menthol was illustrated with results of the Benowitz et al. (2004) study. This study had been performed in a clinical setting with i.v. application of deuterated nicotine and deuterated cotinine. Therefore the possibility of inhibiting influences could be determined more precisely than in field studies comparing nicotine metabolite excretion from smokers of mentholated and non-mentholated cigarettes (e.g. Wang et al. 2010).

Some comments addressed smokeless tobacco and pointed out that the additives used were not all found in the same product. This is correct and we have clarified the appropriate text sections accordingly.

6. Methods to quantify the potency of additives in enhancing the addictiveness of nicotine

Some comments criticised the use of animal models. As indicated in question 2, we have evaluated data available from animal models in parallel with data from human studies. Self-administration methods are commonly used in animals to test the reinforcing properties of different products. The reinforcing effect of a compound in animal studies presents a high predictive value for the abuse liability in humans. Therefore, although these methods are not directly measuring the addictive effects of a compound, there is a consensus of the scientific community about the interest of such animal models in the study of addiction.

7. Technical characteristics that enhance the addictive potential of tobacco products

Few comments addressed this point specifically. Changes in technical characteristics may influence smoking behaviour, but it is not certain whether this leads to a higher risk for addiction.

8. Criteria for considering an additive or a combination of additives as attractive

In the definition of attractiveness, we have used the criteria employed in the WHO report (WHO 2007b). The broadness of the term “attractiveness” is acknowledged in the report. Industry terms such as “acceptability” and “preference” have more specific meaning, but capture only part of what constitutes the attractiveness of a product. The role of sensory and environmental cues is already acknowledged. It is acknowledged in the opinion that there is a lack of evidence regarding the specific impact of menthol on smoking behaviour. Although some smokers are not particularly attracted by the sweetness, it is generally admitted that the sweetness is an important characteristic of tobacco. The tobaccos that do not naturally have a high sugar content are treated with casing to increase the sugar content and add various flavours (see section 3.4).

9. Methods currently used for assessing attractiveness

We agree with the comments that there is a lack of standardised methods for assessing attractiveness in the context of tobacco products. This is already acknowledged in the report, although the basic principles of how to assess subjective ratings are well established in behavioural science. Greater consistency across studies which investigate these factors would be welcome. Survey and market data may provide useful information, but any relationships between, for example, market share and the presence or absence of additives will be minimally informative due to the potential for multiple confounding influences, and cross-country differences in “national taste” etc. Some comments addressed the question of ethics of panel testing of tobacco products. While studies of the presence or absence of additives on smoking behaviour in established smokers may be ethically acceptable, these will be minimally informative with respect to effects on initiation. It would generally be regarded to be unacceptable to present tobacco (with or without additives), a harmful product with high addiction liability, to tobacco naive participants.

10. Additives that increase attractiveness of tobacco products

It is acknowledged in the report and has been acknowledged for a long time by the tobacco industry that a large number of additives seeking to increase the attractiveness of their products are used. However, bearing in mind the broad meaning of “attractiveness”, the report does not provide clear evidence that a specific additive

affects the attractiveness of tobacco products intended for smoking. For smokeless tobacco there were some comments on possible harm reduction and use of smokeless tobacco as cessation aid. These questions have been treated in detail in the SCENIHR report from 2008 and we have now inserted some remarks in the text. In brief, there is no evidence that promotion of smokeless tobacco will result in substantial public health benefits due to dual use of cigarettes and smokeless tobacco. There is also no evidence that smokeless tobacco is more efficient as a smoking cessation aid than established therapies.

11. Association between additives and tobacco consumption – target groups

A number of comments addressed this point. The potential for menthol and ammonia to influence smoking initiation and behaviour is discussed in the report but the data are inconclusive.

Gaps of knowledge and Research recommendations

A number of comments proposed to have more precise indications about knowledge gaps and research recommendations. Although this was not part of the mandate, this section has now been rewritten in order to explain better the considerations of the working group and the Scientific Committee.

6. MINORITY OPINION

None

7. LIST OF ABBREVIATIONS

ACh	Acetylcholine
AM251	N-(piperidin-1-yl)-5-(4-iodophonyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APA	American Psychiatric Association
ASH	Action on Smoking and Health
BAT	British American Tobacco
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment)
BN	Bates Number
BZgA	Bundeszentrale für gesundheitliche Aufklärung (Federal Centre for Health Education)
CAS	Chemical Abstracts Service
CB1	Cannabinoid receptor 1
CDC	Centers for Disease Prevention and Control
CLD	Cased Leaf Dryer
CMD	Count median diameter
CNRS	Centre national de la recherche scientifique (French National Center for Scientific Research)
CNS	Central nervous system
CO	Carbon monoxide
COHb	Carboxyhaemoglobin
CSERP	Chemosensory event-related potential
CYP	Cytochrome P450 monooxygenase
DA	Dopamine
DAP	Diammonium hydrogen phosphate
DKFZ	Deutsches Krebsforschungszentrum (German Cancer Research Center)
DNA	Deoxyribonucleic acid
DSM (-IV)	Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition)
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EEG	Electroencephalography/Electroencephalogram
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
FDA	(United States) Food and Drug Administration
FTND	Fagerström Test for Nicotine Dependence

GABA	Gamma (γ)-Aminobutyric acid
Glu	Glutamate
GM	Genetically modified
GYTS	Global Youth Tobacco Survey
HBSC	Health Behaviour in School-aged Children
5-HT	5-Hydroxytryptamine
IARC	International Agency for Research on Cancer
IC₅₀	The half-maximal inhibitory concentration
ICD	International Classification of Diseases
ICRP	International Commission on Radiological Protection
i.v.	Intravenous
JTI	Japan Tobacco Inc.
LD₅₀	Median lethal dose
MAO	Monoamine oxidase
mGlu5	Metabotropic glutamate 5
mGlu2/3	Metabotropic glutamate 2/3
MMD	Mass median diameter
mRNA	Messenger ribonucleic acid
NAc	Nucleus accumbens
nAChR	Nicotine acetylcholine receptor
NCI	National Cancer Institute
NMDA	N-Methyl-D-aspartate
NNAL	4-N-(Nitrosomethylamino)-1-(3-pyridyl)-1-butanol
NNAL-Gluc	NNAL-Glucuronide
NNK	4-N-(Nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NRT	Nicotine replacement therapy
ONS	Office for National Statistics
PET	Positron emission tomography
pH	Measure of acidity or basicity of a solution
pKa	Dissociation constant – measure of the strength of an acid or a base
PMI	Philip Morris International
ppm	parts per million
PPTg	Pedunculo pontine tegmental nucleus
PREP	Pattern reversal evoked potential
QNE	Quantity not exceeded
RECON	Reconstituted tobacco
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The Netherlands National Institute for Public Health and the Environment)
RYO	Roll your own
SCCS	Scientific Committee on Consumer Safety

SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
STP	Smokeless tobacco products
STRATUS	Studies with Rimonabant and Tobacco Use
T_{1/2}	Half-life
TNCO	Tar, nicotine and carbon monoxide
TRPM8	Transient receptor potential channel
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet
VTA	Ventral tegmental area
WHO	World Health Organization

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9. GLOSSARY

Abuse liability	Abuse liability of a drug is the likelihood that its use will result in addiction (dependence) and it can be assessed in laboratories by methods referred to as abuse liability testing.
Additives	The present report uses the term additives for added ingredients or substances. Additives are defined as any substance that is added, except water, during the course of manufacture of a tobacco product, including preservatives, humectants, flavours, and processing aids.
Addiction	Addiction is the commonly used term referring to what is technically known as “dependence” and is widely employed to connote severe substance dependence.
Addictiveness	Addictiveness refers to the pharmacological potential of a substance to cause addiction.
Agonist	A ligand for a receptor which induces a response, identical or partial, to the response obtained with the endogenous ligand.
Attractiveness	The terms “attractiveness” or “consumer appeal” refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use.
Break point	Highest number of responses that the animal accomplishes to obtain a single delivery of a drug.
Bronchodilator	A substance that dilates the bronchi and bronchioles.
Casing	Casing refers to the sauce composed of a variety of ingredients such as humectants, sugars, cocoa, liquorice and fruit extracts which is applied to tobacco during the manufacturing process.
Conditioned cue	Neutral stimulus that associates with a reward. Used in abuse liability testing.
Curing	Curing is the process for drying freshly harvested tobacco with partially or fully controlled temperature and moisture schedules.
CYP2A6	It is an abbreviation of Cytochrome P-450 2A6 (family 2, subfamily A, polypeptide 6), a constituent of the endoplasmic reticulum P-450 mixed function oxidase system. CYP2A6 is the main enzyme system involved in the oxidative metabolism of nicotine and cotinine, as well as many other xenobiotics and pharmaceuticals. A significant interindividual variability in CYP2A6 and mRNA levels has been observed in humans and other mammals.
Denicotinised	The removal or reduction in the nicotine content of tobacco, for example by means of blending genetically-modified tobacco which has been engineered to lack nicotine.
DSM	Diagnostic and Statistical Manual of Mental Disorders. Published by the American Psychiatric Association (USA) which provides standard criteria for the classification of mental disorders. It is used in the United States and in varying degrees around the world. It is not exempt from scientific criticism in many countries.
EEG	Electroencephalogram.

GABA receptor	An oligomeric class of neuron membrane receptors to which the γ -aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, binds.
Harman	A beta-carboline that is formed in smoke by interaction between acetaldehyde and tryptophan. It inhibits the enzyme monoamine oxidase (MAO).
Harshness	A chemically induced physical effect associated with a roughness, rawness experience generally localised in the mouth, and to a lesser degree, in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. It can cause a drying, rasping, coarse, astringent sensation.
Hyperlocomotive effect	Increase in locomotor activity usually recorded in rodents.
IC ₅₀	Inhibitory concentration 50. The concentration of a compound that inhibits 50% a given maximal response (biological, biochemical, etc.).
Ingredients	see Additives. The present report uses the term additives for added ingredients or substances.
LD ₅₀	Lethal dose 50. Dose of a compound that kills 50% of a group of administered animals (it represents a probabilistic concept).
Manipulandum	Device used in experimental settings in order to transmit an active response. In the present report the device is used to measure self-administration of drugs in experimental animals.
MAO	Monoamine oxidases exist in two forms, A and B. They metabolize monoamines such as noradrenaline, dopamine and serotonin.
Metabolism	The chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. In metabolism some substances are broken down to yield energy while other substances are synthesized.
Narghile or shisha	Expressions for the Oriental waterpipe.
Norharman	Condensation product in smoke that inhibits the enzyme monoamine oxidase (MAO). See also harman.
pH	Measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14.
P450 enzyme system	The cytochromes P450 are hemoproteins and important constituents of the so-called monooxygenase system.
Pyrolysis	Chemical decomposition of condensed substances that occurs spontaneously at high enough temperatures.
Receptor	Protein or protein complexes present in the cell membranes (plasmatic, endoplasmic or nuclear) or the cytoplasm to which physiological signaling molecules, e.g. neurotransmitters, hormones, etc., drugs and xenobiotics specifically, bind.
Reinforcement	Ability of a stimulus to promote behavioural responses in order to obtain (positive reinforcement) or to avoid (negative reinforcement) such a stimulus.
Rewarding	Stimuli that have appetitive (desirable) consequences and/or produce a hypothetical pleasurable internal state (hedonia).
Self-administration	Experimental procedure that allows the animals/humans to administer a drug to themselves. Self-administration methods

	are widely used to directly evaluate the reinforcing properties of a drug.
Smoothness	Reduction in the harsh irritation of nicotine-containing tobacco smoke.
Uncharged	Used e.g. for nicotine to describe the free base, that under acidic conditions (lower pH) may be charged (protonated) with one or two protons.



**Scientific Committee on Health, Environmental and Emerging Risks
SCHEER**

**Opinion on
Additives used in tobacco products
(Opinion 2)
Tobacco Additives II**



The SCHEER approved this Opinion by written procedure on 16 December 2016.

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Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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In addition, the Commission relies upon the work of other Union bodies, such as the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHEER

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- complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health, for example antimicrobial resistance, nanotechnologies, medical devices and physical hazards such as noise and electromagnetic fields.

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ABSTRACT

The Commission has established a priority list of 15 additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations, based on a scientific Opinion (Tobacco Additives I) of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The EU Tobacco Product Directive (TPD) prescribes that Member States shall require manufacturers and importers of tobacco products to carry out comprehensive studies on these additives.

The European Commission requested the SCHEER to provide guidance on the type and criteria for comprehensive studies, and on the most suitable methodologies to be used on the first 15 tobacco additives and for additives on future updated lists. The SCHEER has provided such guidance together with a reporting template in Annex I.

As tobacco additives have no benefits for health, but rather may promote use of and addiction to an extremely toxic product, a risk-benefit analysis is not the appropriate paradigm for assessing the additive. When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies, the SCHEER recommends that the assessors consider the worst-case evaluation.

In the first part, the SCHEER proposes a step-wise strategy as the most pragmatic and efficient way to assess the toxic and addictive effects and the characterising flavour and facilitating inhalation properties as potentially contributing to the attractive effects of tobacco additives. The proposed strategy ensures that testing is minimised, including the possibility to evaluate groups of additives having similar structures and properties. In step 1, the collection and then the evaluation of the available data on toxicity, addictiveness, characterising flavour and facilitating inhalation properties of the additive need to be carried out by applying a Weight of Evidence approach (step 1). In step 2, collection/evaluation of data is extended to the additive's pyrolysis products; if no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions. Here, it is important to note that no validated methods exist for the determination of pyrolysis products from tobacco additives, but some indications are given in the Opinion.

In case data retrieved in Step 1 and 2 are not sufficient or robust enough to make the evaluation possible, non-testing methods, such as quantitative structure–activity relationship (QSAR) and read across, are proposed, followed by *in vitro* approaches, addressing the different endpoints to be considered (Step 3).

Regarding types of effects, unless the previous step highlighted some concern for a specific end-point, toxicity should be assessed first, as accepted methods and evaluation frameworks are available, followed by assessing whether a product contains a characterising flavour. Next, addictiveness should be assessed, an effect for which no validated tests are available, although some mechanisms underlying addictiveness are known. The issue related to interaction of the additive with other additives/ingredients is also considered.

In addition to proposing specific steps and tests to be considered by industry, some general criteria were also identified. Most importantly, the test outcomes should be

relevant for tobacco smoking. This implies that they should be related to actual levels of human exposure and to tobacco-induced diseases. Furthermore, comparative toxicity testing strategies, where differences in the effect of the tobacco product with and without the additive are evaluated, are not considered suitable to address the properties outlined in the Terms of Reference with the currently available methodology. Indeed, at present, these studies lack discriminative power due to the high background toxicity of tobacco products and their results cannot be generalised to all products and brands, having a different composition with respect to tobacco type, blend and additives. Here, the effects of the pure additive, and its pyrolysis products, are considered in order to evaluate their contribution to tobacco product toxicity. Comparative studies are also not endorsed to study the effect of additives on addictiveness with animal models, for the same reasons. In human studies, there are two exceptions on this general rule: characterising flavour testing and inhalation facilitation or nicotine uptake. For ethical reasons, the performance of new animal studies is not endorsed to assess the contribution of an additive to the tobacco product toxicity. Therefore, as a principle, only *in silico* and *in vitro* studies should be considered for new testing in Step 3, following the EU policy to ban animal studies for chemicals to be used in voluntary products. Human studies are generally discouraged; they may be used (e.g. in case of flavour assessment), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products.

The data gaps already identified in the Opinion on Tobacco Additives I for the 15 additives included in the EU Commission priority list have been now analysed and the activities to be performed upfront have been described. In general, important data gaps for the 15 priority additives are information on addictiveness, inhalation facilitation and characterising flavour, as well as on the identity of the pyrolysis products.

Keywords: tobacco, additives, combustion products, cigarettes, roll-your-own, smoking, toxicity, addictiveness, attractiveness, characterising flavour, facilitated inhalation.

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1 BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The new Tobacco Products Directive 2014/40/EU strengthens the rules regarding the reporting and composition of tobacco products. In addition to tightening the obligations of manufacturers to report on ingredients¹ contained in tobacco products, the Directive regulates permissible additives (or levels thereof) in order to improve the functioning of the internal market whilst guaranteeing a high level of public health.

A) Article 7 of Directive 2014/40/EU foresees in particular the prohibition of the following:

- 1) tobacco products with a characterising flavour (Art 7(1))
- 2) tobacco products containing the following additives² (Art 7(6)):
 - a) vitamins or other additives that create the impression that a tobacco product has a health benefit or presents reduced health risks;
 - b) caffeine or taurine or other additives and stimulant compounds that are associated with energy and vitality;
 - c) additives having colouring properties for emissions;
 - d) for tobacco products for smoking, additives that facilitate inhalation or nicotine uptake; and
 - e) additives that have CMR³ properties in unburnt form.
- 3) tobacco products containing flavourings in any of their components such as filters, papers, packages, capsules or any technical features allowing modification of the smell or taste of the tobacco products concerned or their smoke intensity. Filters, papers and capsules shall not contain tobacco or nicotine. (Art 7(7))
- 4) tobacco products containing additives in quantities that increase the toxic or addictive effect, or the CMR properties of a tobacco product at the stage of consumption to a significant or measureable degree. (Art 7(9))

The provisions outlined above shall apply in the first stage to cigarettes and roll-your-own tobacco. The exemption for other product categories may be removed under certain conditions.

B) Moreover, in line with Article 6 the Commission has to develop and update a **priority list of at least 15 additives** contained in cigarettes and roll-your-own tobacco by May 2016. This list shall contain additives

- 1) for which initial indications, research, or regulation in other jurisdictions exist suggesting that they have one of the following properties:
 - a) contributes to the toxicity or addictiveness of the products concerned / increases the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
 - b) results in a characterising flavour⁴;

¹ 'ingredient' means tobacco, an additive, as well as any substance or element present in a finished tobacco product or related products, including paper, filter, ink, capsules and adhesives (TPD 2014/40/EU)

² 'additive' means a substance, other than tobacco, that is added to a tobacco product, a unit packet or to any outside packaging (TPD 2014/40/EU)

³ CMR - carcinogenic, mutagenic or toxic for reproduction

- c) facilitates inhalation or nicotine uptake; or
 - d) leads to the formation of substances that have CMR properties / increases the CMR properties in any of the products concerned to a significant or measurable degree; and
- 2) which are amongst the most commonly used additives by weight or number according to the reporting of ingredients.

For these priority additives, enhanced reporting obligations will apply in the form of comprehensive studies which shall examine for each additive whether it has any of the properties 1 a) to d) specified above. Those studies shall take into account the intended use of the products concerned and examine in particular the emissions resulting from the combustion process involving the additive concerned. The studies shall also examine the interaction of that additive with other ingredients contained in the products concerned. The results of these studies shall assist Member States and the Commission in their enforcement efforts regarding Art. 7.

The SCENIHR published a scientific Opinion on the attractiveness and addictiveness of additives in 2010⁵. In light of the time that has passed since then and the need to address the current regulatory requirements, the SCENIHR has been asked to address the questions outlined in the Terms of Reference below.

⁴ 'characterising flavour' means a clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herbs, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product (TPD 2014/40/EU)

⁵http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_031.pdf

2 TERMS OF REFERENCE

The main purpose of the requested scientific Opinion is to assist the Commission in identifying the additives that should be put on the priority list. The scientific Opinion can, however, also provide useful input for Member States and the Commission in their broader regulatory/enforcement activities (e.g. setting thresholds/banning of additives), in particular in areas where the knowledge base may currently still be limited. In particular, the Committee is asked the following:

Opinion 1:

1. Based on scientific evidence (including a review of relevant scientific data) and other relevant information currently available (initial indications, regulation in other jurisdictions), the Committee is asked to identify - for each category separately - those additives that fall/are suspected to fall within the scope of the following categories:

- a. Contributing to the toxicity or addictiveness of the products concerned / increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree;⁶

The assessment should include for each of the additives identified a comprehensive description of the type of information supporting its identification as well as a description and quantification of the strength of the observed characteristic and the strength of the available evidence supporting this finding⁷. If the Committee identifies more than 20 additives for a category, the Committee is entitled to prioritise in the light of the criteria set out in this section. In this case, the description is limited to the top 20 additives per category, whilst the other additives can be listed without description.

The Committee is asked to also consider in its assessment the interaction with other ingredients contained in the products concerned and the emissions resulting from the combustion process involving the additive concerned as well as the intended use of the products. Relevant knowledge gaps should be identified.

As far as relevant information is available, the Scientific Committee is asked to identify within its assessment the most commonly used additives by weight or number. If additives belong to a single group of substances with identical or very similar properties, both the group of substances and the list of substances falling into that group shall be presented and the most relevant substance(s) within that group identified.

6 If an additive is included in Annex VI of Regulation (EC) No 1272/2008, its CMR-classification should be provided and considered as appropriate. Additives that have CMR properties in unburnt form should be identified/listed, but do not require a comprehensive description.

7 Registrations/assessments of relevant substances under Regulation (EC) No 1907/2006 should be provided and considered as appropriate.

When examining the composition of tobacco products and the use of individual substances, the Scientific Committee is invited to consult the data on additives reported by the tobacco industry under the Tobacco Products Directive 2001/37/EC, but may also consider additional data sources. Furthermore, the Committee is invited to consider during their assessment the lists of additives permitted/prohibited for use in tobacco products as implemented by certain Member States.

2. Based on its assessment in point 1, the Committee is asked to establish a list of a minimum of 20 and maximum of 30 additives that are suitable/recommended to be added to the priority list of additives in line with Article 6 of TPD 2014/40/EU. When establishing the list, the Committee shall consider the public health risks associated with the additives (actual or suspected), strength of the available evidence and to the extent possible, the frequency of use of the additives in tobacco products. The Committee should indicate as far as possible rankings of additives in light of the above and provide an explanation for its ranking⁸.

Opinion 2:

3. Furthermore, the Committee is asked to advise the Commission on the type and criteria for comprehensive studies that should be requested from manufacturers to assess the relevance of the individual additives, considering inter alia the knowledge gaps identified in point 1 above and the interaction of the additive with other additives/ingredients. Advice is also sought on the most suitable methodologies to be used (including a structure of the reports that can be peer reviewed).

⁸Substances belonging to the same group of identical/very similar substances should be considered jointly.

3 SCIENTIFIC RATIONAL

3.1 Introduction

In response to the Commission's requests, the SCENIHR adopted Opinion I (Tobacco Additives I), in which 48 single chemicals were listed as priority additives, which met the 30 entries maximum limit because some chemicals with very similar structures (i.e. aliphatic gamma-lactones, including 8 chemicals) and/or properties (e.g. weak acids, including 8 group members) were grouped together. They were selected on the basis of two initial criteria: the frequency of use in different brands and the amounts used in cigarettes, then further screened based on their hazardous properties, because they have been or are suspected of having one or more of the following properties:

- a. Contributing to the toxicity or addictiveness of the products concerned / increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree.

On the basis of these criteria:

- 17 substances were identified because they fall or are suspected to fall in the category: toxic in unburnt form, among which 6 are suspected of CMR potential, which were ranked highest on the suggested list because the Tobacco Products Directive foresees the prohibition of additives that have CMR properties in unburnt form.
- 20 substances were identified because they are known or suspected of forming irritant, toxic and/or CMR chemicals after combustion including sugars, sugar-containing additives and cellulose.
- 14 substances were identified because they are suspected of facilitating inhalation or of increasing nicotine uptake.
- 19 substances were identified because they show a characterising flavour, a factor potentially contributing to attractiveness.

Since the SCENIHR was asked to prioritise the selected chemicals to the best of its ability, three groups were identified. In addition to the 6 chemicals suspected of CMR potential, menthol was included in the 'highest priority group'.

A second group was identified based on the possibility of forming CMR compounds after combustion.

All the remaining identified additives are categorised in the third group, although it was not possible to rank them on the basis of their specific hazard profile and the only possibility was to use content/frequency ranking as a possible criteria for prioritisation or a combination of more than one of four characteristics provided for in Article 6.

On May 18, 2016, the Commission adopted the Commission Implementing Decision (EU) 2016/787 laying down a priority list of additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations⁹, identifying 15 chemicals among those listed in the SCENIHR Opinion (Additives used in tobacco products; Tobacco Opinion 1) adopted in January 2016.

In this Opinion 2, on the basis of the knowledge gaps mentioned in the next section, and after revising the available open literature and approaches taken by International Agencies, the SCHEER provides advice to the Commission on the type and criteria for comprehensive studies that should be requested by Member States from manufacturers to assess the contribution of the individual additives used in cigarette and roll-your-own tobacco to tobacco product toxicity, addictiveness¹⁰, adding to a characterising flavour or facilitating inhalation.. A step-wise strategy was proposed (Section 3.4)¹¹. The issue related to interaction of the additive with other additives/ingredients is also considered.

It should be noted that, by contrast to adding them to medicines or food, for example, additives in tobacco products have no health benefits for the consumer. On the contrary, by making smoking more attractive, they promote an extremely unhealthy behaviour. Therefore, a risk-benefit analysis is not the appropriate paradigm for assessing the additive. When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies, it is a SCHEER recommendation that the assessors consider the worst-case evaluation.

The same reasoning applies to the addictive effects, inhalation facilitation and characterising flavour of tobacco additives, as they will indirectly lead to adverse health consequences by increasing consumption of the product.

In addition to the general strategy, the major data gaps already identified in Tobacco Opinion 1 have been analysed to determine the most appropriate steps (and end-points) to be carried out and then used for the evaluation (Section 3.5), in order to speed up the process, making possible testing feasible within the 18-month time-frame. To give an example, for the 6 chemicals for which a genotoxic potential could not be ruled out for the unburnt form, the first step will be to evaluate their genotoxicity: if the results were positive, no other testing would be necessary, since according to the TPD (Article 7) they would automatically be banned for use as tobacco additives. In case of negative results, they would enter the general strategy of testing and be considered as would any other compound.

⁹<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016D0787&from=EN>

¹⁰ [For the concepts of addiction \(or "dependence"\), this Opinion refers to the definitions given in the previous evaluation \(SCENIHR, 2010, 2016\). Addictiveness refers to the pharmacological potential of a substance to cause addiction, in line with the TPD definition as 'the pharmacological potential of a substance to cause addiction, a state that affects an individual's ability to control his or her behaviour, typically by instilling a reward or a relief from withdrawal symptoms, or both'. In addition to the neurobiological characteristics of the substance itself, dependence potential is related to the dose, speed of absorption, metabolism, and the physical and chemical features of the formulation \(WHO, 2007\).](#)

¹¹ [SCHEER was not requested to give detailed protocols for specific studies and whenever possible referred to test guidelines or other approaches already adopted in areas other than tobacco products.](#)

3.2 Knowledge gaps identified in Opinion 1

There was generally scant toxicological information regarding tobacco additives analysed for Opinion 1, and the available information was often limited to the oral route of exposure, especially for flavouring substances that are used by the food industry, or, to a lesser degree, to the dermal route, for substances that are also commonly used in cosmetic products. Data on the effects of additives in tobacco following inhalation is generally scant, although this is the most relevant exposure route. Indeed, the additives are either transferred to inhaled smoke in pure form, or are combusted and converted via pyrolysis into potentially toxic products. Because there was also little data on their kinetic behaviour, it was difficult, if not impossible, to make route-to-route extrapolation for additives.

A general scarcity of information was observed regarding the actual level of exposure to additives both in the unburnt form in tobacco products and resulting from combustion – including data on pyrolysis. This is particularly relevant since toxic combustion products generated upon pyrolysis of additives have the potential to increase the exposure to toxic substances and thus increase the health hazard associated with cigarette smoking (National Institute for Public Health and the Environment, 2012). The fate of the additive depends on its physico-chemical properties such as its volatility and reactivity, the design of the cigarette and the smoking topography of the user. The additive may be distilled from the tobacco rod, and end up in smoke intact, or it may be (partly) combusted. In case of (partial) pyrolysis, not only the unburnt additive is relevant, as the smoker will be exposed to the pyrolysis products as well. In the tobacco matrix, either the intact additive or its pyrolysis products may react with other additives, tobacco- or smoke components (pyrosynthesis). For instance, only minor amounts of the non-volatile sugars in tobacco (approximately 0.5% of glucose and sucrose) are transferred unchanged into the mainstream smoke, whereas the major part will combust, pyrolyse or participate in pyrosynthesis processes (Talhout *et al.*, 2006).

Although for most tobacco additives, direct information about their possible contribution to addictiveness and characterising flavours does not exist, information can be derived from the mode of action of the additive (e.g. addictiveness can be related to increased nicotine bioavailability or to local anaesthetic effects facilitating the inhalation of tobacco smoke).

Generally speaking, the scarcity of information on exposure and on toxic effects as described in Opinion 1 made risk assessment for most additives difficult, if not impossible.

3.3 Methodology

3.3.1 Development of the general approach to assess the effects of tobacco additives

Given the fact that additives in tobacco products have no health benefits for the consumer, but rather promote an extremely risky behaviour, risk-benefit evaluations are not appropriate. Based on evaluation of approaches for regulation of other types of components, the SCHEER concluded that a step-wise approach is the most pragmatic and efficient way to proceed in the assessment of the toxic, addictive and attractive

effects of tobacco additives. The tiered approach proposed by DKFZ (DKFZ, 2010) was used as a starting point, but was then further developed and adapted to include the evaluation of the additives in view of their role in creating a characterising flavour, facilitating inhalation and making tobacco products more addictive. The order of the steps has been proposed in such a way to minimise testing. First, an evaluation of the available data is proposed for both the additive in unburnt form (Step 1) and its pyrolysis products (Step 2); if no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions. Next, in Step 3 non-testing methods, such as quantitative structure–activity relationship (QSAR) and read across, are employed, followed by *in vitro* approaches. Each step represents a possible decision point, when data allow a proper evaluation based on a WoE approach: if it could be unequivocally demonstrated that the additive falls in one of the four categories of Article 6 of the TPD, no further testing is needed. Regarding types of effects, toxicity is assessed first, as CMR chemicals are not allowed as additives (Article 7), and accepted methods and evaluation frameworks are available for toxicity testing, followed by characterising flavours, because accepted methods and evaluation frameworks are available. Finally, addictiveness is assessed, and since no validated tests are available here, the assessment can be guided by the available knowledge of the mode of action.

3.3.2 Addressing the data gaps identified in Opinion I for the priority list additives

The data gaps already identified in Tobacco Opinion 1 for the 15 additives included in Commission Implementing Decision (EU) 2016/787 have been analysed. Based on the data gaps described in the 'Rationale for inclusion' in Opinion 1, the activities to be performed upfront have been described. Then on the basis of the obtained results, if the additive does not meet the criteria for exclusion as an additive listed in art. 7 of the TPD, it can be subject to the general evaluation step-wise procedure described in the Opinion.

3.3.3 Information collection

Information on guidance for the data collection and tests to be performed in the different steps of the step-wise approach was collected by the SCHEER on available open literature/websites and from documents by other Committees or International Organisations (e.g. WHO, EPA, EFSA, JECFA)

3.3.4 Information evaluation

For this Opinion on tobacco additives, the available information was analysed by the SCHEER to identify tests and testing structures that are appropriate for the assessment of the toxic and addictive effects, facilitation of inhalation and characterising flavours of tobacco additives.

3.4 Step-wise approach to assess the toxic and addictive effects, inhalation facilitation and characterizing flavour properties of tobacco additives

A pragmatic and efficient step-wise approach is suggested, in order to assess the toxic and addictive effects as well as the additives' properties that facilitate inhalation and lend a characterising flavour to cigarettes and roll-your-own tobacco. The tobacco industry has the burden of proof that an additive does not fall within the scope of the four categories mentioned in the terms of reference and it is tobacco industry's responsibility to deliver data. The data need to be evaluated by independent scientific bodies with expertise in risk assessment of the toxic, addictive, inhalation facilitation and characterising flavour properties of chemicals.

The formation of consortia and joint reports by industry is endorsed in order to limit the financial and administrative burden for industry and authorities, as well as the amount of literature evaluation and testing by industry, and subsequent evaluation of the submitted reports by independent institutes. For the toxicological evaluation of additives in tobacco products, the tiered approach proposed by DKFZ (DKFZ, 2010) has been taken as a starting point. This approach has been adapted to the SCHEER purposes and widened to allow for the evaluation of the addictive effects, inhalation facilitation and characterising flavour of additives (see Figure 1). This is because apart from toxicity, tobacco additives may indirectly increase tobacco-related harm by increasing the consumption rate of tobacco products, either by making the product more attractive to the consumer (e.g. by resulting in a characterising flavour and by facilitating inhalation), or by enhancing its addictiveness (National Institute for Public Health and the Environment, 2012). As far as possible, this possibility has to be considered. Although a standardised methodology is not available, it is possible to derive information from the mode of action of the additive (e.g. addictiveness can be related to increased nicotine bioavailability or to local anaesthetic effects facilitating the inhalation of tobacco smoke; see the SCENIHR Opinion, 2010).

Whenever the evaluation of the additive in the unburnt form gives rise to any concern in relation to art 7 of the TPD (e.g. foreseeing the prohibition of additives having CMR properties) based on data collected in Step 1, the evaluation is stopped, meaning that the additive does not meet the requirement of the TPD. The same rule is applied to Step 2 for the pyrolysis products. In these cases, industry can proceed to step 4 in their reporting.

In case data are not available, or are not sufficient or robust enough to make the evaluation possible, the procedure should go to the next step.

In case of high uncertainty about the evaluation based on available data, there are two possible options:

- Application of the 'worst-case' evaluation
- Delivering of additional data (i.e. via Step 3) by tobacco industry

Step 2 is analogous to Step 1 but related to the pyrolysis products; the two steps can take place concurrently, if this is more efficient and saves time. The collection of available data is mandatory in order to priorities the most appropriate end-point(s) to be assessed in Step 3, to limit useless testing.

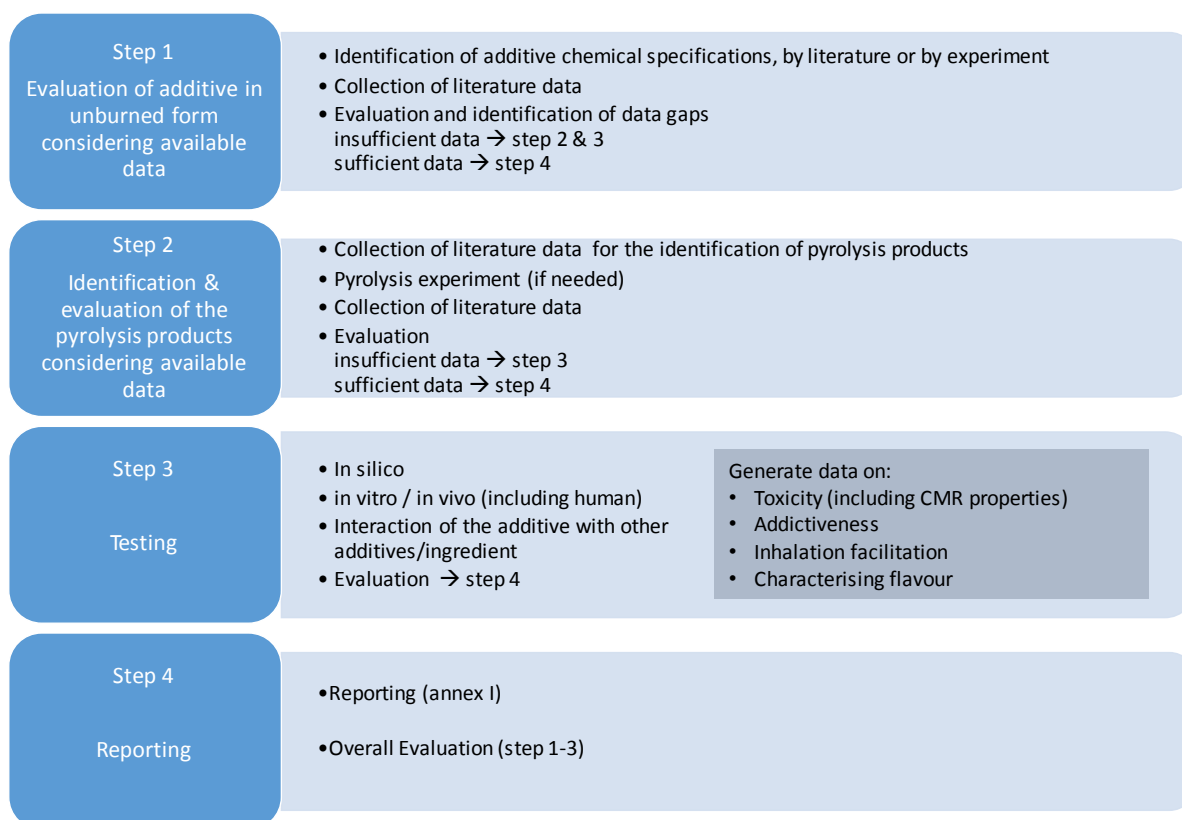


Figure 1. Step-wise approach to be applied to the assessment of the toxic, addictive, inhalation facilitation and characterising flavour properties of tobacco additives. For terminology, please refer to the text. Please note that in vivo tests may only be considered in exceptional cases.

This procedure could be applied either to individual additives or to groups of additives. Additives could be indeed grouped, following rules previously established in other fora to evaluate e.g. groups of food flavouring at EFSA¹² or groups of chemicals in Regulation (EC) No 1907/2006 i.e. REACH (to apply the read-across principles)¹³ in order to limit the use of animal testing (as requested in art. 13). The ECHA provides practical guidance on the issue (available at the ECHA website link); however, to this aim, the approach described in the OECD GUIDANCE ON GROUPING OF CHEMICALS No. 194¹⁴ is recommended.

The approach described in the OECD guidance document (GD) is to consider closely related chemicals as a group, or category, rather than as individual chemicals, for assessing the hazards of chemical substances, increasing efficiency and improving animal welfare. Since the technique of assessing groups of substances is an evolving

¹² <https://www.efsa.europa.eu/en/topics/topic/flavourings>

¹³ <http://echa.europa.eu/support/grouping-of-substances-and-read-across>

¹⁴ GUIDANCE ON GROUPING OF CHEMICALS, SECOND EDITION Series on Testing & Assessment No. 194 (2014) available at [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)

science, the GD is revised periodically and it is therefore compulsory that the tobacco industry follows the most updated version when applying it. As it is recommended by the GD itself, early consultations between industry and authorities are recommended to ensure that any regulatory requirements are fulfilled.

The GD outlines a process for grouping chemicals to include the identification of analogues/members of categories, the mechanistic basis for using analogues or chemical categories and the robustness of both approaches. The GD also describes the use of (Q)SARs for data evaluation and data-gap filling (read-across, trend analysis and (Q)SARs).

3.4.1 Step 1: Evaluation of the additive in unburnt form

The first step starts with the identification of the additive chemical specifications, by literature or by experiment (for the physico-chemical characterisation, if not available, data can be obtained following the OECD or ISO test guidelines to this purpose). This initial step is absolutely necessary in order to identify the nature of the additives and comprises also qualifying and quantifying of any impurity present. CAS numbers need to be provided for all relevant chemicals (additives and impurities). The chemistry and specification of a substance (or mixture of substances), in terms of chemical structure(s) and physico-chemical properties is also asked for in other legislations, e.g. for food additives.

It may not always be possible to fully characterise natural extracts, but as much information as possible is required to understand the extent to which variability in composition is controlled during manufacture. Data on the chemical composition of a natural extract additive should be provided by industry with emphasis on the concentrations of constituents of relevance; this includes the concentrations of compounds classified according to their chemical structure (e.g. flavonoids, terpenoids, alkaloids, etc.), constituents being characteristic for tobacco additives (chemical fingerprint, markers). Information on maximum levels for microorganisms and possible contaminants, including e.g. heavy metals, mycotoxins, pesticide residues and polycyclic aromatic hydrocarbon (PAH) residues, should be provided (EFSA, 2012).

Then, all available information on the additive in unburnt form is collected and evaluated. This includes open literature on peer-review journals as well as grey literature (e.g. unpublished reports of studies used for regulatory purposes), including JECFA, EFSA and FEMA documents or data coming from any other regulatory request, in case the additive is used in other contexts.

This step allows the collection of available information on the additive in its unburnt form, useful for its risk assessment. In addition, it allows the identification of the major data gaps (if any) to be addressed in Step 3, with regard to toxicity and addictiveness data, as well as inhalation facilitation or increase nicotine uptake properties or resulting in characterising flavour.

For future reference by the regulator, industry is also asked to indicate which additives are closely related regarding chemical structure, functions, purpose and effects.

3.4.1.1 Collection of available data

Whenever possible, all information already available on the toxicity of the additive (obtained with different experimental models, i.e. *in vivo*, *in vitro*, *in silico* and human data) should be collected, used and evaluated before any testing is initiated (in Step 3). Some knowledge on the toxicity of tobacco additives exists; much less is known on their addictiveness or on how they facilitate inhalation and increase nicotine uptake properties or result in characterising flavour. Open literature as well as grey literature (e.g. unpublished reports of in-house performed studies with regulatory purposes) should be included. If studies have been already performed in view of seeking approval of the same chemical for uses other than tobacco additive by Applicants other than Tobacco Industry, a letter of access should be acquired, in order to avoid repeating the same tests.

Initial electronic literature searches with appropriate key words/dates should be a starting point for data gathering. The databases and search engines used may include for example PubMed, Web of Science, Scopus, Toxline, Chemical and Biological Abstracts, and Google Scholar. The data search methods will identify many papers that potentially could be used. A first screening is then needed in order to focus on those relevant for the specific purposes, using appropriate inclusion/exclusion criteria. Articles that do not appear to meet the inclusion criteria should be excluded from further analysis. To apply a standardised methodology it is recommended that the literature search strategy and selection criteria (inclusion/exclusion) for the review are based on the EFSA Systematic Review Guidance (EFSA Journal 2010).

In order to collect data on addictiveness and on properties that facilitate inhalation or result in characterising flavour, all investigations on possible related mode of action or mechanisms should be considered. In this respect, although never used in this context, an emerging approach is the adverse outcome pathway (AOP) – a framework designed to conceptually link a molecular initiating event to an adverse outcome of relevance to risk assessment (Ankley *et al.*, 2010). The AOP framework allows for a better understanding of the mechanistic linkages between cellular responses and downstream impacts on apical outcomes that are of concern within a regulatory context (Villeneuve and Garcia-Reyero, 2011). Potential practical uses of AOPs also include the above-mentioned grouping of common chemicals for read across (not only based on chemical structures but on biological activity), identification of research and data gaps, serving as a framework for regulatory priority setting, and informing hazard characterization and risk assessment (Becker *et al.*, 2015). AOP methodology may be useful in elucidation of molecular basis for addictiveness of tobacco products e.g. role of pH changes on nicotine absorption, MAO-A inhibition, Dopamine (DA) release and turn over, CYP metabolism and inhibition (for details see paragraph 3.4.3.5). Accordingly, the same applies to investigation on properties facilitating inhalation or resulting in characterising flavour (for details see paragraph 3.4.3.6). OECD developed a guidance document outlining methods and best practices for creating and assessing AOPs, in which it calls for the assessment of an AOP's weight of evidence (OECD, 2013; AOP-Wiki, 2014). AOP wiki represents a joint effort between the European Commission – DG Joint Research Centre (JRC) and the U.S. Environmental Protection Agency (EPA). This serves as one component of a larger OECD-sponsored AOP Knowledge Base effort and represents the central repository for all AOPs developed as part of the OECD AOP Development Effort by the Extended Advisory Group on Molecular Screening and Toxicogenomics.

3.4.1.2 Evaluation

Collected data give information regarding the possibility for the additive used in cigarette/roll-your-own to fall into one or more of the four categories:

- a) Contributing to the toxicity or addictiveness of the products concerned/increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b) Resulting in a characterising flavour;
- c) Facilitating inhalation or nicotine uptake;
- d) Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/RYO) to a significant or measurable degree.

The methodological quality of the selected papers should also be addressed, including the design, execution, analysis and reporting of the study. Expert judgement is vital in the assessment of the quality and the interpretation of data, therefore the appropriate identification and selection of relevant publications/reports is extremely important. When possible (e.g. for toxicity studies) this screening should be based on Klimisch scoring¹⁵.

The acceptance of each publication/report that is considered to be relevant should be based on the quality and relevance criteria summarised in by SCENIHR (2012).

All selected documents of potential importance should be subject to similar treatment in the evaluation process. Positive and negative studies should be evaluated using similar procedures and criteria and considered of similar importance if the quality is judged to be comparable. In positive studies the evaluation needs to consider both causal and non-causal explanations of the results. For example, one key question would be "with what degree of certainty can one rule out the possibility that the observed positive result is produced by bias, e.g. confounding or selection bias, or chance?". In the case of negative studies, it is necessary to assess the certainty with which it can be ruled out that the lack of an observed effect constitutes evidence against a hazard or whether it could result from (masking) bias, e.g., too small exposure contrasts, too crude exposure measurements, too small exposure groups/populations, or chance. Consideration should also be given to the possibility of a publication bias i.e. that positive findings are more likely to be published than negative findings.

It is recommended that the whole data set, judged as relevant, reliable, and of good quality, should be used for the (risk) assessment of the tobacco additive and its pyrolysis products, if any. Different approaches for assessment of whole data sets, referred to as weight of evidence (WoE) evaluation or systematic review (often used interchangeably), have been promoted (Koustas *et al.*, 2014; Rooney *et al.*, 2014; European Food Safety Authority, 2010; IARC, 2006). In general terms, these approaches are processes of summarising, synthesising and interpreting a body of evidence to draw conclusions, e.g. regarding the relationship between a chemical exposure and an adverse health effect. The WoE approach promotes the use and integration of information from all available evidence.

¹⁵H.J. Klimisch, M. Andreae and U. Tillmann (1997): *A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data*, [Regulatory Toxicology and Pharmacology](#) Vol 25, pp 1–5

Unfortunately, formal procedures and consistent terminology for WoE processes are lacking, although a WoE evaluation is mentioned in the REACH regulation, the Biocides directive, the Cosmetics regulation, and the regulation for Classification, Labelling and Packaging (CLP). Some guidance documents are only available for industrial chemicals or contaminants in food (Ågerstrand and Beronius, 2015). However, a number of organisations have established their own frameworks for assessing/evaluating evidence, including the SCENIHR (2012), and the work is still in progress in both the SCHEER and EFSA. Since the approach is rapidly evolving, it is compulsory that in applying it, the Tobacco Industry follows the most updated version.

As indicated above, it is possible to apply substance grouping of read-across principles: this approach uses relevant information from analogous ('source') substances to predict the properties of 'target' substances. The application and reporting of this approach as described in section 3.4 is recommended; if applied correctly, there is no need to have specific information on every additive.

Whenever the evaluation based on the WoE approach of the available data on the additive in its unburnt form unequivocally identifies no concern for any health effect, and it also falls in one of the 4 categories with no data gaps, there is no need to go for further testing in Step 3. On the contrary, whenever the evaluation based on the WoE approach of the available data on the additive in its unburnt form give rise to any concern regarding CMR properties, the evaluation is stopped, meaning that the additive does not meet the requirement of TPD art. 7. In both cases it is possible to directly proceed to Step 4.

In case data are unavailable, insufficient or not robust enough to make any evaluation possible, the procedure should go to Step 3,

3.4.2 Step 2: Evaluation of the pyrolysis products

In the second step, information available on the identification of pyrolysis products of additives must be collected and evaluated. This can be done on the basis of available data (see section 3.4.1.1 for criteria), but in case no sufficient data (in quantitative or qualitative terms) are available, the second step foresees that pyrolysis studies need to be performed in realistic, standardised experimental conditions (see section 3.4.2.2). Then available open and grey literature data on the toxicological profile, addictiveness or properties facilitating inhalation or resulting in characterising flavour on the identified pyrolysis products should be collected, as described in Step 1 for the additive in the unburnt form.

3.4.2.1 Collection of available data

Available data on the pyrolysis products of additives is collected in the same way as described in Step 1 (see 3.4.1.1).

3.4.2.2 Pyrolysis studies(if needed)

To identify the compounds formed during the combustion process of a tobacco additive, tobacco industry in general performs smoke chemistry studies on a comparative basis where a research cigarette is machine smoked with and without the additive present (Talhout *et al.*, 2006). Burning (smoking) the tobacco that contains a specific amount of the additive and subsequent analysis of selected smoke components is described for

many different additives (Baker *et al.*, 2004a; Baker *et al.*, 2004b, c; Carmines, 2002; Rustemeier *et al.*, 2002). However, subtle differences between the selected smoke components will not be noticeable, and it is not feasible to screen the effect on all 6000 known smoke components, hence usually only the so-called Hoffmann analytes are screened. Given the complexity of cigarette smoke, it is difficult to identify individual materials that may result from the pyrolysis of ingredient mixtures, unless radioactively labelled additives are used, but that method is sophisticated and expensive. Furthermore, this method cannot determine whether the additive is a precursor or a catalyst for the formation of a certain smoke component (Torikai *et al.*, 2005).

Pyrolysis, on the other hand, is a useful technique for evaluating materials used at low levels, where it is unlikely that smoke chemistry assays could detect a change. Therefore, combustion processes in a burning cigarette have also been simulated with pyrolysis methods (Baker *et al.*, 2004b; Busch *et al.*, 2012; Lee *et al.*, 2007).

This technique is useful as a first screening of potential pyrolysis products, their thermal stability and the temperature at which they are formed (Baker and Bishop, 2004). However, the pyrolysis conditions only approximate the burning cigarette with regard to temperature and atmosphere and make no allowance for the presence of other tobacco and/or smoke components that may interact with the additives. Pyrosynthesis processes related to the tobacco matrix will not occur when the additive is pyrolysed as a single component outside of the tobacco matrix. When it is suspected that such reactions will occur, one may consider pyrolysing a simple mixture containing the additive together with the component with which reaction is foreseen (either with the component itself or with its pyrolysis products. For instance, micro-vial pyrolysis of a glucose/proline mixture resulted in formation of Amadori intermediates, important in the formation of (Maillard) products that influence the aroma (Mitsui *et al.*, 2015). Pyrolysis was performed at 700 °C, approximating the temperature of the pyrolysis zone of a burning cigarette, for 10 s under atmospheric conditions (headspace gas in vial not replaced by an inert gas).

Pyrolysis studies can be performed under a given set of experimental conditions that need to resemble processes in a burning cigarette in terms of e.g. temperature, rate of temperature change, and atmosphere (amount of oxygen). During the cigarette-burning process, the temperature of the tobacco and the burning cone can range from room temperature up to 900 °C, and the amount of oxygen can range from 0 to 18%. It is important that the design of the pyrolysis study reflects the conditions of burning cigarettes with oxygen levels ranging from 0% to 14% and the temperature in the burning zone ranging from ambient temperature to 900 °C (Baker and Bishop, 2004; Stotesbury *et al.*, 1999; Torikai *et al.*, 2004).

Many studies tried to simulate the processes during smouldering and combustion. Stotesbury performed pyrolysis at 14 sets of pyrolysis conditions: temperatures between 200 °C and 700 °C in 2 % and 10 % oxygen, and at 800 °C and 900 °C in 2 % oxygen. Baker used an atmosphere of 9% oxygen in nitrogen, arguing that this is the average amount throughout the pyrolysis/distillation zone inside the burning cigarette during a puff. From an initial temperature of 300 °C, to simulate the smouldering before taking a puff, the sample is heated at 30 °C s⁻¹ to 900 °C, and kept for 5 seconds, to simulate the maximum duration of the high-burning zone temperature during puff under extreme human smoking conditions. According to Baker, 30 °C s⁻¹ is the approximate mean heating rate throughout the pyrolysis/distillation zone during a puff. This seems rather

slow, as that would imply it would take 20 seconds before the maximum temperature is reached, whereas a human puff only takes one or two second. However, most studies are performed with a similar heating rate (Torikai *et al.*, 2004). Purkis *et al.* programmed the temperature from 300 to 900 at 25 °C per second to reflect cigarette smoking and give an appropriate set of conditions to limit artefact formation (Purkis *et al.*, 2011). It is important that the reaction vial is not closed, so that the additive can distil away at lower temperatures.

Flash pyrolysis is performed when the sample is rapidly inserted in a pre-heated furnace that is already at the highest temperature, for instance at the temperature range of 200–300 °C to simulate cigarette smouldering (Zhou *et al.*, 2011). Time of flight spectroscopy allows for almost real time sampling, enabling identification of reactive compounds before being degraded (Hertz-Schunemann *et al.*, 2015) (Busch *et al.*, 2012).

Taking into account the studies described above, the SCHEER recommends the following experimental design in most cases performed by tobacco industry:

Thermal degradation (pyrolysis, pyrosynthesis and combustion products) of each additive is to be studied under different reaction regimes (inert and 2-14% oxygen) over the temperature range 200–900 °C. The thermal degradation products of two different pyrolysis conditions should be identified:

- (1) upon gradually heating the sample from 200–900 °C and
- (2) conventional pyrolysis, in which a new sample is pyrolysed at minimally 3 different temperatures (~ 300°C, 600°C and 900°C).

Pyrolysis experiments should be carried out at least in triplicate. Chemical analysis of the components in the pyrolysate needs to be performed with state of the art techniques in the field of GC-MS and LC-MS, as appropriate for the specific additive. The World Health Organization in its report to the Sixth Conference of the Parties¹⁶, identified eight non-exhaustive lists of toxicants: Health Canada, RIVM, USA FDA, Counts, Dybing and Fowles, Hoffman analytes, Philip Morris-Australian brands, and Philip Morris-Canadian brands. These toxicants need to be identified and quantified, if present, using analytical reference standards. Tobacco-specific components, such as nitrosamines and alkaloids, are not expected to be present.

For additional components, not on these lists, the following procedure is advised. For identification purposes, library software can be used, such as the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software. Components with a peak-to-peak signal-to-noise ratio below three can be discarded. Also, components with a probability of correct identification below 70% can be excluded.

If components with a toxicological hazard are identified, their identification needs to be confirmed and their amount needs to be quantified using analytical reference standards.

Apart from components that may increase the toxicity, specific attention needs to be given to components that have addictiveness-enhancing properties, flavouring

¹⁶http://apps.who.int/gb/fctc/PDF/cop6/FCTC_COP6_14-en.pdf

properties, or inhalation facilitation properties (e.g. anaesthetic and/or bronchodilating properties) (SCENIHR, 2010).

3.4.2.3 Evaluation of data

The collected data will be evaluated according to a WoE approach (see 3.4.1.2). Again, when the available information is considered reliable and robust enough concerning both the identification of pyrolysis products and on their toxicological and addiction profile or on properties facilitating inhalation, increasing nicotine uptake or resulting in characterising flavour, a solid evaluation can be possible based on the WoE approach, on the basis of which a decision (positive or negative) can be taken. For instance, if the data set unequivocally identifies the additive as not falling into any of the four categories and not posing any concern for any health effect, with no data gaps, there is no need to go for further testing in Step 3. On the contrary, if it is demonstrated that compounds proven to have carcinogenic or mutagenic properties are generated from pyrolysis of an additive, this additive will contribute to the toxicity of the product concerned, considering the stochastic nature of the carcinogenic effect.

When data are unavailable, insufficient or not robust enough to make any evaluation possible, the procedure should go to Step 3.

3.4.3 Step 3: Testing and evaluation of results

The third step is related to the testing of additives or their pyrolysis products, whenever a data gap is identified in Step 1 and 2, or uncertainties have to be reduced, according to methods accepted by other regulations. The outcomes of tests must be related to actual human exposure and tobacco-induced diseases, and be relevant not only for subchronic, but also for chronic exposure in intermittent use sessions (Johnson *et al.*, 2009).

A relevant test design will not only consider methods to investigate toxicity, but also characterising flavour, facilitating inhalation and addictiveness. Therefore information should be collected if it is related to the known mechanisms that contribute to addictiveness or on properties facilitating inhalation, increasing nicotine uptake or resulting in characterising flavour.

Based on expert judgement of the major data gaps with regard to toxicity, characterising flavour, facilitating inhalation properties and addictiveness data identified in Step 1, it must be decided which endpoint to start with. This will generally be the endpoint for which most evidence is available of a potential concern. If no priority concerns have been identified, it is advised to start with toxicity, as in that case, accepted *in vitro* tests are available and there are frameworks for interpreting the results.

This step will also address the possible interactions, at chemical level (e.g. pyrolysis) and for the toxicological part based on the MeA/MoA.

It should be noted that whenever there is a good scientifically-based reason for asking for an exception for presenting data related to a specific end-point, a detailed justification reporting the rationale for the derogation must be provided.

Once data are collected by applying testing in Step 3, they should be evaluated together with data collected in Step 1 and 2 by using a WoE approach. If uncertainties cannot be

reduced by results coming from Step 3 testing, the SCHEER recommends that the assessors consider the 'worst-case' evaluation.

3.4.3.1 Comparative paradigms are not endorsed

In order to provide a relevant outcome to the question of whether an additive contributes to the toxicity, addictiveness and properties facilitating inhalation or resulting in characterising flavour of the products concerned (i.e. cigarette and roll-your-own tobacco; Article 6(2) of the TPD), the study design must adhere to some methodological criteria.

Strictly speaking, the only way to comply with Art.6 TPD2 (A6), to assess whether additives increase "toxicity or addictiveness ... to a significant or measurable degree" should be comparative testing (Kienhuis *et al.*, 2016). However, it must be noted that comparative testing strategies, where differences in effect of the tobacco product with and without the additive are evaluated, at the moment are not considered suitable, given the current toxicity tests and available methodology. Indeed, the emissions of tobacco products are highly toxic, in particular regarding cigarette smoke (Kienhuis *et al.*, 2016). Due to the high intrinsic toxicity of tobacco products, it is challenging to demonstrate any differences, whether they be increases or decreases, induced by an additive.

Due to the high toxicological activity of both the test product (tobacco product with additive) and the control (tobacco product without additive) in comparative testing strategies, the discriminatory power that can be obtained in toxicity assays may not be sufficient (COT/COM/COT, 2009; DKFZ, 2010; Oldham *et al.*, 2012).

Very sensitive tests would be required, with a clear dose-response relationship, in order to show any differences from these high background effects. As such tests are not currently available, no new comparative studies (tobacco product with and without additives) will be considered, since these studies lack discriminative power. Studies that are already available could be presented, but the relevance of their results has to be evaluated in a WoE approach based on these considerations.

In line with this, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC, 2009) concluded: "*The Committee considered that the available studies used to assess the contribution of individual or mixed ingredients or additives to the overall toxicity of tobacco products are inadequate to assess the risks posed by conventional cigarettes, so it is not possible to assess the modulation of that risk resulting from inclusion of additives. The relationship between effect (an increase in biomarker) and exposure is also poorly understood. Furthermore, it is possible that additives might alter smoker behaviour, such as to increase product use; this increased exposure would be likely to result in an increased risk.*"

Furthermore, an international Working Group on Tobacco Additives (WG), assigned by the Brazilian regulatory agency ANVISA, assessed many industry-sponsored studies addressing the effects of mixtures of commonly used additives on cigarette smoke chemistry and toxicity. Although industry claimed that additives have no effect on the levels of chemical components of cigarette smoke and toxicity, the WG concluded that the available data were insufficient to accept the tobacco industry's claims that additives do not increase the inherent toxicity of tobacco smoke (Ferreira *et al.*, 2015; Working Group on Tobacco Additives, 2014): "*Given the current toxicity tests and test designs, it*

is not yet possible to determine whether or not addition of specific ingredients (tobacco additives) to tobacco products adds to tobacco mainstream smoke's inherent toxicity. This is because tobacco itself is already quite toxic, and any added toxicity is difficult to detect within the current test designs used by tobacco industry, i.e. combinations of in vitro testing and animal testing."

For the future, tests are needed that are sensitive enough to assess the toxicity attributed to additives above the overall toxicity of tobacco products, which can associate assay outcomes to human risk and exposure. In this respect, *in vitro* tests combined with toxicogenomics, using biomarkers of exposure and disease are the most promising (Kienhuis *et al.*, 2016). However, for the time being, no standardised methods have been validated to this purpose.

Another problem with comparative testing is the choice of the product to be tested, since the additional toxicity of the additive would differ between product types and brands. If an additive would be tested in the intended brand, the results (related to toxicity, addictiveness and attractiveness) could not necessarily be generalised to all products and brands, having a different composition. Therefore the obtained results may not lead to general prohibition/acceptance of specific additives but rather to prohibition/acceptance on a product-by-product basis (DKFZ, 2010).

When the addition of sugars is taken as an example, it will be very important whether a reference containing Burley tobacco, that does not contain natural sugars, or Virginia tobacco, with high sugar levels, is selected, or a blend of these tobacco types. This is even more important as cultural differences exist in the preference for Virginia-type cigarettes, American blend, or Burley. According to the TPD, the use of additives necessary for the manufacture of tobacco products should be allowed, as long as they do not result in a characterising flavour or increase the addictiveness, toxicity or CMR properties of the product. Thus, in this particular case, sugar addition to replace what is lost during the curing process, needs to be evaluated against the possibility of toxic and carcinogenic compounds forming following pyrolysis.

For similar reasons, comparative studies are in general also not endorsed for examining the effect of additives on addictiveness. In human studies, there are two exceptions to this general rule. The first is characterising flavour testing. By definition, this is a comparative testing paradigm, as clearly noticeable flavour is a property of a tobacco product, caused by the addition of flavour additives, and not a property of an additive itself (see section 3.4.3.6). In this experimental set-up, strong flavours other than that of tobacco can be distinguished, whereas more subtle flavours caused by an additive will not be consciously discriminated. The second includes additives that facilitate inhalation or nicotine uptake, and perhaps similar effects in humans, as e.g. reported for smokers of mentholated cigarettes vs non-mentholated cigarettes. In this case, large groups of test subjects are needed, and effects on e.g. sensory perception, smoking topography and biomarkers of exposure (e.g. nicotine in blood or cotinine in urine) can be discriminated. In most human studies, any effects of the additive will probably not be picked up in comparative testing designs, or at least be more prominent in non-comparative designs. For instance, when focusing on the dependence capacity of tobacco additives, studies on acute effects done within subject measurements (placebo vs. additive(s) of interest) are regarded as more valuable. This will improve the sensitivity to picking up small changes in neuronal activity caused by administration of the additive.

Instead of using a comparative study design, the effects of the pure additive and its pyrolysis products must be considered in a relevant testing strategy, such as the tiered approach proposed by the SCHEER (Fig.1).

3.4.3.2 The use of animal testing

So far, available inhalation or other animal studies have been used to assess the effect of tobacco additives. Whenever studies using animals are available, they should be evaluated in Step 1 and 2. However, it is ethically questionable to carry out any new animal studies to evaluate the 'safety' of a tobacco additive, as tobacco products are highly harmful with no benefits to individual or public health. Therefore, as a principle, only the use of *in silico* and *in vitro* studies applies to Step 3, following the EU policy recommending implementation of 3R methods for refinement, reduction, and replacement of animal models, leading to the ban of animal studies for chemicals to be used in voluntary products such as cosmetics (EU Regulation no. 1223/2009). Non testing or alternative testing approaches followed for the evaluation of cosmetic ingredients¹⁷, whenever relevant to tobacco products, could be considered.

Human studies are discouraged, although they may be used in specific case (e.g. for characterising flavour assessment or facilitating inhalation properties), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products. Generally and especially in those specific cases in which animals are proposed, early consultations are recommended between Receiving Competent Authorities at Member State level and tobacco industry, presenting a testing strategy including *in silico*, *in vitro* and only in exceptional cases *in vivo* tests. In order to limit the testing, formation of Consortia is recommended.

In the exceptional cases when animal testing would be deemed necessary, it is compulsory to be compliant with the Animal Welfare EU policy and to respect the Regulation on Animal Testing.

3.4.3.3 Quality system

In line with the provisions of other regulatory contexts, the SCHEER recommends the use of a Quality system (e.g. Good Laboratory Practice or ISO17025) for carrying out the pyrolysis or other physico-chemical studies as well as pre-clinical toxicity studies (including those to assess the mode of action underlying possible contribution to addictiveness and characterising flavour, increasing nicotine uptake or facilitating inhalation).

In case the principle of the Mutual Acceptance of Data is applied (again to limit the testing) the quality system of choice should be the GLP, following the application of the GLP OECD principles, to which all the National Monitoring Authorities in the different OECD Member States make reference. Human studies should follow the appropriate Quality System, since GLP are usually not applied to this kind of testing. For the physico-chemical studies, GLP compliance is not always requested and the ISO17025 could be chosen.

¹⁷http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

3.4.3.4 Toxicity testing

In silico

If toxicological data on the additives are not available or are limited, they can be produced using *in silico* approaches. As a first step, QSAR methods are encouraged to identify alerts for genotoxicity, carcinogenicity and reproductive toxicity, to get information of potential CMR properties of the additive or of its identified pyrolysis products: this could also take advantage of similarities with other chemicals by applying the read-across methodology.

Non-test information about the biological activity of a substance can be derived in a variety of ways, ranging from simple inspection of the chemical structure through various read-across techniques, the use of expert systems, metabolic simulators, to global or local (Q)SARs. The usefulness of such techniques varies with the amount and nature of information available, as well as with the specific regulatory questions under consideration.

Models for the identification for alert of genotoxicity and carcinogenicity have a long tradition; (Q)SAR models for mutagenicity can apply to a limited set of congeneric substances (local models) or to a wide variety of non-congeneric substances (global models).

Many global models for mutagenicity are commercial and some of the suppliers of these global models consider the data in their modelling sets to be proprietary. Proprietary means that the training set data used to develop the (Q)SAR model is hidden from the user. In other cases it means that it may not be distributed beyond use by regulatory authorities.

There are hundreds of (Q)SAR models available in the literature for predicting test results for genotoxic endpoints for closely related structures (Naven *et al.*, 2012; Bakhtyari *et al.*, 2013). These are known as local (Q)SARs. However, quality of reporting varies from model to model and predictivity must be assessed case-by-case on the basis of clear documentation.

In case of robust data on the identification of structural alert or based on read across indication of CMR properties, it is not necessary to go further, since according to the TPD no CMR substance can be used as a tobacco additive. In case there are doubts, *in vitro* testing can be conducted (see below).

Regarding the other toxicological properties, other QSAR tools are available. Some of them are briefly described in the following:

OECD built an open software application (<http://www.qsartoolbox.org/>), named QSAR Toolbox. The Toolbox and guidance on its use are freely available. The OECD QSAR Toolbox facilitates the practical application of grouping and read-across approaches to fill gaps in (eco-)toxicity data, including but not limited to genotoxicity and carcinogenicity, for chemical hazard assessment. The Toolbox incorporates information and tools from various sources, into a logical workflow. Crucial characteristic of the workflow is the grouping of chemicals into categories (group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties

are likely to be similar or follow a regular pattern, usually as a result of structural similarity). The most important features are:

1. Identification of relevant structural characteristics and potential mechanisms or mode of action of a target chemical.
2. Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action.
3. Use of existing experimental data to fill the data gap(s).

The Joint Research Centre of the EU provides several tools for modelling for the safety assessment of chemicals. They offer the following computational tools (freely downloadable or accessible from their webpages):

- JRC QSAR Model Database: database hosting structured and peer-reviewed information on QSAR Models (<https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database>);
- Toxtree, software tool to generate prediction(s) on mechanisms of action or toxicological effects, the tool is based on a decision-tree approaches (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxtree);
- Dart, (Decision Analysis by Ranking Techniques) a software tool designed to rank chemicals according to environmental and toxicological concerns (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/DART);
- Toxmatch, a flexible application for grouping chemicals based on chemical similarity designed to be helpful in read-across (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch).
- Stat4tox, a tool which carries out concentration-response analysis for *in vitro* experiments (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/stat4tox).

ECHA provides a detailed overview on non-testing methods in sub-section R.7.7.3.1 of Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance (see http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf), in particular with regard to the prediction models for mutagenicity and the OECD QSAR toolbox.

A list of the available (free and commercial) predictive software for ecotoxicological, toxicological and environmental endpoints, including mutagenicity models, has been compiled within the frame of the EU project Antares (<http://www.antares-life.eu/>). For example, the Danish EPA and the Danish QSAR group at DTU Food (National Food Institute at the Technical University of Denmark) have developed a (Q)SAR database that contains predictions from a number of mutagenicity models. The database is freely accessible via <http://qsar.food.dtu.dk>. The online database contains predictions for over 166,000 substances and includes a flexible system for chemical structure and parameter searching. A user manual with information on the individual models including training set

information and validation results is available at the website. The database is also integrated into the OECD (Q)SAR Toolbox.

Another example of a database with predictions on mutagenicity is the Enhanced NCI Database Browser (<http://cactus.nci.nih.gov>) sponsored by the U.S. National Cancer Institute. It contains predictions for over 250,000 substances for mutagenicity as well as other non-mutagenic endpoints, some of which may provide valuable mechanistic information (for example alkylating ability or microtubule formation inhibition). It is also searchable by a wide range of parameters and structure combinations.

Use of harmonised templates, such as the QSAR Model Reporting Format (QMRF) and the QSAR Prediction Reporting Format (QPRF) developed by the Joint Research Centre (JRC) of the European Commission (http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools/QRF), can help to ensure consistency in summarising and reporting key information on (Q)SAR models and substance specific predictions generated by (Q)SAR models. The JRC website also hosts the JRC (Q)SAR Model Inventory, which is an inventory of information on the validity of (Q)SAR models that have been submitted to the JRC (http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsarinventory).

If the exposure could be well characterised without uncertainties, the application of the Threshold of Toxicological Concern (TTC) concept could be foreseen providing the applicability domain will be expanded to include sufficient data coming from inhalation studies. Its use has been proposed, but never applied in regulatory context in relation to the inhalation route (Costigan and Meredith, 2015). In addition, the concept of the 'internal TTC', independent on the route of exposure, is a promising approach that is also starting to be considered in the area of cosmetic products (SCCS, 2016).

In vitro

There are a number of *in vitro* tests that can be used to assess many different toxicological end-points. *In vitro* toxicity tests are applicable to a wide variety of test materials including ingredients added to tobacco, tobacco extracts, tobacco smoke condensates, and whole or vapour phase smoke. These assays may also be used to explore interactions between components of cigarette smoke. The test item should be the additive in its unburnt form and relevant pyrolysis products (in case information coming from Step 1 and 2, as well as from non-testing methods in Step 3 give rise to insufficient data or high uncertainties in the evaluations) as well as to tobacco smoke particulate and whole or vapour phase smoke, when relevant.

The first choice has to be given to tests already adopted at international levels (e.g. OECD Test Guidelines¹⁸, or ISO methods) or tests validated by EURL-ECVAM. The OECD test guidelines (TGs) describe the applicability domain, the principles of methods, the test procedure and they also address reporting.

In case a non-TG *in vitro* method is used (e.g. a test validated by EURL-ECVAM), the reporting should be appropriate, following what it is indicated in the OECD Guidance

¹⁸<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>

Document for describing Non-Guideline *in vitro* test methods No.211 (2014)¹⁹. The application of integrated approaches on testing and assessment (IATA) is highly recommended, as described in documents adopted by both EURL-ECVAM and OECD²⁰.

Since the acceptance at regulatory levels is evolving, the TG as well as the GDs are periodically revised, therefore it is compulsory that in choosing the appropriate test, the tobacco industry follows the most updated version. Indeed, as requested by EU regulation 1223/2009 on cosmetics, the EU Commission has to report every year to the European Parliament as well as to the EU Council on the progress related to the development, validation and regulatory acceptance of alternative methods as communicated by a Report from EURL-ECVAM²¹.

If information gathered through *in silico* methods are not conclusive and there are doubts related to genotoxicity potential, an *in vitro* genotoxicity test battery or *in vitro* transformation test for carcinogenicity can be applied to clarify these end-points. The *in vitro* genotoxicity testing methodologies are well described in the several adopted OECD TG. Please refer to the OECD Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines²² or to the OECD web site for the last update on adopted TG. In the battery, it would be necessary to include tests able to identify point mutations in prokaryotic and mammalian cells as well as chromosomal aberrations or *in vitro* micronucleus assay and DNA damage and repair (for details, see also the SCCS Note for Guidance 9th Revision, 2016)²³.

In vitro methods to address local toxicity (i.e. phototoxicity, skin corrosion and irritation, eye irritation, skin sensitisation) are available (see table 1) and should be performed, whenever relevant, using the air-liquid interface.

IT is more difficult, however, to address systemic toxicity by means of *in vitro* testing only, since at the moment no adopted TGs are available.

For carcinogenicity, two cell transformation assays have been included in OECD guidance Documents (table 1), which - using an IATA with *in silico* and read across data - could give sufficiently robust information.

Only a few *in vitro* studies are available to address only very specific reactions possibly leading to reproductive problems, such as *in vitro* binding to estrogen and androgen receptors (see the OECD conceptual framework to evaluate endocrine disrupting chemicals). Nevertheless, they can be included, and evaluation should be carried out also considering the indication coming from QSAR and read across in a WoE approach.

¹⁹GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE *IN VITRO* TEST METHODSSeries on Testing and Assessment No. 211

²⁰<http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96418/eurl%20ecvam%20toxicokinetics%20strategy.pdf><http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282015%2922&doclanguage=en>

²¹ http://ihcp.jrc.ec.europa.eu/our_lab/eurl-ecvam

²²<https://www.oecd.org/chemicalsafety/testing/Genetic%20Toxicology%20Guidance%20Document%20Aug%2031%202015.pdf>

²³http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

Cytotoxicity testing after repeated exposure (e.g. 14 days) is considered a possible end-point of choice (acute toxicity is not relevant for tobacco products, hence also for additives). Positive and negative controls should be used, cells of human origin have to be preferred; non-specific tests as well as organ specific cells should be used (e.g. cells coming from the lung or intestinal cells, accounting for inhalation and swallowing of smoke, but also cells representing CNS, cardiovascular system, etc. to be evaluated on a case-by-case basis). For a correct interpretation of results, it should be considered that whenever cell lines are used, they are generally characterised by a low and unbalanced metabolic capability, therefore at least one of them should maintain this function over the treatment period (e.g. HepaRG cells) or primary cells should be used. The use of primary cell lines can also account for the interindividual variability that in some cases is translated to differences in susceptibility to toxic effects.

The major endpoints evaluated in *in vitro* cytotoxicity assays include the effect of a substance on cell viability (survival) and growth rates, but other end-points (such as mitochondrial functionality, induction of apoptosis) can be included. Cytotoxicity testing is used in the area of medical devices: methods to be considered can be found within the harmonised European standard ISO 10993-1:2009 "Biological evaluation of medical devices Part 1: Evaluation and testing within a risk management process". The ISO 10993 – 5:2009 describes test methods to assess the *in vitro* cytotoxicity of medical devices.

However, the study design should take into account that the most relevant route of exposure for tobacco additives is inhalation.

There has been significant progress made in recent years in approaches to expose cells *in vitro* to chemicals that pose a toxicological concern via the inhalation route (Aufderheide *et al.*, 2011; Bakand and Hayes, 2010). A number of *in vitro* exposure systems have been developed (although not yet validated) to facilitate the study of the effects of the whole smoke mixture on both mammalian and bacterial cells, and this has been the subject of a recent review (Thorne and Adamson, 2013).

Current cell-based *in vitro* models of the respiratory tract consist mainly of 2D monolayers of primary tracheobronchial epithelial cells or an immortalised cell line cultured on a semipermeable membrane insert at an air-liquid interface to induce cell polarization, differentiation, and mucus production (Forbes *et al.*, 2005). A more sophisticated technique that enables the stable and reproducible exposure of cultivated cells to cigarette smoke at the air-liquid interface such as CULTEXW Radial Flow System (RFS) module has been proposed recently (Rach *et al.*, 2013).

It was documented that air-liquid interface culture played a significant role toward the *in vitro* recapitulation of the *in vivo* environment, presenting the cells with an apical side resembling the lumen of the respiratory tract and a basolateral side representing vascular supply of nutrients (Berube *et al.*, 2010), with increased expression of cilia in primary cells and differences in barrier and mucus-secreting properties of cell lines observed (de Jong *et al.*, 1994, Grainger *et al.*, 2006). However, the absence of an extracellular component with co-cultured cells in a 3D environment can result in an oversimplification of the airway barrier, lacking in physiological relevance. Therefore, more sophisticated models based on 3D human normal and diseased tissue are required to provide *in vitro* models that improve validity of tested compounds in humans. Much of respiratory tissue engineering research has seen a transition from single cell-type culture

on inserts toward co-culture and the inclusion of scaffold material. Accordingly, models, in which the epithelium is cultured at an air-liquid interface over a scaffold substrate embedded with co-cultured cells, are the subject of much interest and are even available now as commercial 3D research products, such as the MatTek EpiAirway-FT technology (Berube *et al.*, 2010). Indeed, there is an overall consensus in the literature that introducing an epithelial cell analogue into the co-culture environment, often through the use of a biomaterial scaffold, could enhance cell culture, cell-cell signalling, and functionality. A triple co-culture system, in which human bronchial epithelial cells A 549, human mesenchymal cells and dendritic cells were cultured in monolayers, has shown promise for studying immunological responses to inhaled particulates (Rothen-Rutishauser *et al.*, 2005, Herzog *et al.*, 2013). Co-culture of Calu-3 cells with Wi38 lung fibroblasts was achieved on the scaffold to create a submucosal tissue analogue of the upper respiratory tract, validating system as a platform to support co-culture and cellular organisation reminiscent of *in vivo* tissue architecture. These scaffolds were validated as a substrate to support functional mucus express from an airway epithelium. Calu-3 cells cultured on CHyA-B scaffolds also expressed the tight junction protein ZO-1 and F-actin, indicating the formation of an epithelial barrier layer on the constructs and differentiation of the Calu-3 cells. Recently, another *in vitro* model system using tissue-engineered constructs has been developed which might improve our understanding of epithelial tissue and disease and use for testing toxicity of different compounds (O'Leary *et al.*, 2016).

Tobacco smoke assessment *in vitro* has traditionally focused on the particulate phase captured on a Cambridge filter pad and eluted in DMSO (Crooks *et al.*, 2013) or bubbled through cell culture media or PBS (Andreoli *et al.*, 2003). Cell cultures are then exposed under submerged conditions to the particulate phase. The gas vapour phase of cigarette smoke has been also considered as described in Health Canada Official Method T-501, T-502, and T-503.

Submerged culture conditions and particulate-based exposures do not represent physiologically that of mainstream tobacco smoke exposure in the human lung. Furthermore, separating smoke fractions in this way could lead to alterations and chemical changes that may not be representative of the whole smoke aerosol. In order to address these challenges, whole smoke exposure systems have been developed. Whole smoke exposure systems offer many technical challenges, but represent a more physiologically relevant test system that captures the full interactions of both the particulate and vapour phases together (Fukano *et al.*, 2004). An additional advantage of these systems is that a multitude of different cell cultures can be exposed at the air-liquid interface (ALI) to whole smoke, better simulating human exposure (CORESTA, 2006).

Whole smoke exposure systems offer the advantage that all phases of smoke can be analysed together or independently depending on the experimental set-up. This has allowed researchers to tailor their experiments to investigate both phases of tobacco smoke, yielding useful information. There is a variety of whole smoke systems available and the majority of these systems can also be used to deliver individual aerosols or other complex aerosol mixtures to cell cultures. However, there are no defined regulatory protocols for tobacco whole smoke exposure systems, and so far there is no recognised approach to the measurement of dose. The vapour phase of cigarette smoke within these systems remains poorly understood. With the variety of exposure options

available to researchers and bespoke systems relatively easy to fabricate or replicate, dosimetry tools may bridge the gap and play an important role, not only in the measurement of actual cellular dose but also in the characterisation and validation of these systems (Thorne and Adamson, 2013).

There are continuous efforts to introduce existing testing methods into regulatory framework of tobacco products risk assessment: product testing protocols are being developed for assays such as Ames bacterial mutagenicity and Neutral Red Uptake (NRU) cytotoxicity (Adamson *et al.*, 2014).

The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA, 2004) *in vitro* Toxicology Task force of industry recommends using a test battery composed of the following assays:

1. A bacterial mutagenicity assay. The Ames Salmonella mutagenicity assay is recommended.
2. A mammalian cell assay for cytogenetics/mutation. The Task Force recommends the micronucleus assay, the chromosome aberration assay or the L5178Y mouse lymphoma assay.
3. A cytotoxicity assay conducted with an appropriate mammalian cell line. The Task Force recommends the neutral red cytotoxicity assay (CORESTA, 2004).

Analysis of the recent publications has shown that these recommendations are used increasingly in the toxicity assessment of different tobacco products (see e.g. Manuppello and Sullivan, 2015).

Considering non-cancer endpoints, a battery of *in vitro* tests have been proposed for assessing CVD risk associated with cigarette smoking (Fearon *et al.*, 2013). The battery is comprised of functional *in vitro* assays to model endothelial damage, angiogenesis, and migration of vascular smooth muscle cells as initial and subsequent events in CVD (Fearon *et al.*, 2013). Other tests, such as for oxidative stress or inflammatory response, may be conducted to assess the adverse effects of cigarette smoke *in vitro*.

However, it is the opinion of the SCHEER that the choice of the test battery should not be fixed *a priori*, and should be rather tailored on the basis of information coming from the *in silico* and read-across analysis (e.g. bridging *in vitro* studies can be necessary to support the read across).

Table 1 summarises the most recent, internationally accepted, validated *in vitro* methods, which may be used for the toxicity assessment of the tobacco products.

Modified after AltTox (<http://alttox.org/mapp/table-of-validated-and-accepted-alternative-methods>) and PISC (<http://www.piscld.org.uk/alternatives-approved-by-regulators/>).

Table 1: *IN VITRO* METHODS ADOPTED FOR REGULATORY USE

Toxicity endpoint	<i>In vitro</i> methods		Recommendations and standard methods (OECD)
ACUTE TOXICITY	3T3 neutral red uptake (NRU) cytotoxicity test to estimate starting doses for oral acute systemic toxicity		OECD GD 129, published in 2010
	NHT neutral red uptake (NRU) cytotoxicity test to estimate starting doses for oral acute systemic toxicity		OECD GD 129, published in 2010
ACUTE PHOTOTOXICITY	3T3 neutral red uptake (NRU) phototoxicity test		OECD TG 432, published in 2004
SKIN IRRITATION	Integrated approach on testing and assessment (IATA)		OECD GD 203, published in 2014
	Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 439, revised in 2015
		EpiDerm™ (MatTek, US)	OECD TG 439, revised in 2015
		SkinEthic™ (L'Oréal, France)	OECD TG 439, revised in 2015
		LabCyte EPI-Model (J-TEC, Japan)	OECD TG 439, revised in 2015
SKIN ABSORPTION/ PENETRATION	<i>In vitro</i> diffusion method		OECD TG 428, published in 2004
SKIN SENSITISATION	Adverse outcome pathway (AOP) for skin sensitisation		OECD series on testing and assessment 168, published in 2012
	Direct peptide reactivity assay (DPRA)		OECD TG 442C, published in 2015
	ARE-Nrf2 luciferase test method (e.g. KeratinoSens™ assay)		OECD TG 442D, published in 2015
	Human cell line activation test (h-CLAT)		OECD TG 442E, published in 2016
CARCINOGENICITY	Cell transformation assay (CTA) (Syrian hamster embryo cell transformation assay (SHE CTA))		OECD Guidance Document Env/JM/Mono(2015)18, May 2015
	<i>In vitro</i> cell transformation assays (CTA) Bhas 42 cell transformation assay (Bhas 42 CTA)		OECD Guidance Document Env/JM/Mono(2016) 1, January 2016

Besides these internationally validated methods and the ISO 10993 – 5:2009 adopted for medical devices, there is a great number of other methods employing technological innovations, such as reconstituted human tissue cultures, 3-D organotypic cultures comprised of differentiated human cells in co-cultures, air-liquid interface exposure systems, cell transformation assays and high content genomic analysis, that are used for tobacco product analysis.

3.4.3.5 Addictiveness testing

Tobacco comprises of thousands of substances, of which nicotine is the most characterising and most addictive component. Additives, as well as natural tobacco substances other than nicotine, may have addictive capacities themselves or can interact with nicotine and the nicotine receptor system, herewith enhancing the effects of nicotine. For example, these additives can have effects on nicotine bioavailability, duration, and concentration in the blood circulation or nicotine-dependent activation of mesolimbic pathways in the brain. The term 'dependence potential' is commonly used to describe addictive capacity.

Guidelines to assess the impact of tobacco product contents on dependence potential could be similar to those already established for testing the dependence potential of pharmaceutical products (the used methods are known as Abuse Liability Assessment). Special challenges include product complexity and the diverse range of tobacco products (Henningfield *et al.*, 2011; WHO Study Group on Tobacco Product Regulation TobReg, 2012). For example, the US FDA has issued guidance that covers dependence potential assessment for a range of different substances, formulations, and product types in which factors such as additives and product design features may act to either promote or deter dependence potential (U.S. Dept. of Health and Human Services, 2010, 2015).

Experimental testing of the dependence potential of tobacco additives is still limited due to the lack of validated administration models for the examined individual compound itself and in co-administration with other tobacco additives. In the proposed step-wise approach, the possibilities to experimentally quantify the dependence potential of tobacco additives (often) co-administered with nicotine is discussed.

In silico

Nicotinic acetylcholine receptor (nAChR) Computer models. nAChRs are ligand-gated cation channels found throughout the central and peripheral nervous systems (Gotti *et al.*, 2006; Jensen *et al.*, 2005). Neuronal nACh receptors participate in many neurological processes including cognition (Levin and Simon, 1998), pain sensation (Damaj *et al.*, 2000), and nicotine reward mechanisms (Dani and De Biasi, 2001; Pavlovicz *et al.*, 2011; Tapper *et al.*, 2004). In the past years several nAChR *in silico* models have been developed integrating protein (sub-) structures, dynamics and functional relationships. Among those, the most widely expressed nAChR subtype in the brain is the neuronal $\alpha 4 \beta 2$ nACh receptor (Haddadian *et al.*, 2008). The $\alpha 4 \beta 2$ nAChR comprises high-affinity nicotine-binding sites (Tapper *et al.*, 2004) but the mechanism how ligand binding leads to channel opening remains elusive. The quality of the current $\alpha 4 \beta 2$ nAChR model was evaluated using flexible docking of nicotine docking to the closed- and open-channel models. Besides the potential nicotine interactions with surrounding residues that could stabilize nicotine positions, a high degree of involvement of aromatic residues in the nicotine binding sites was also observed (Haddadian *et al.*,

2008). Further development of these models may provide information about how nicotine and other tobacco additives (ligands) regulate nAChR activation in smoking dependence.

Ligand-based Monoamine oxidase (MAO) models. The enzyme MAO plays an important role in the metabolism of several neurotransmitters by oxidative deamination. MAO-A inhibition is associated with enhanced dopamine activity leading to increased reinforcement behaviour. The combustion of, among others, natural or added (poly-)sugars in tobacco products result in acetaldehyde which reacts in the body with tryptophan and tryptamine. This reaction results in the formation of the beta-carbolines, harman and norharmane, which are MAO inhibitors (Herraiz and Chaparro, 2005; Talhout *et al.*, 2007). Other examples of MAO inhibitors isolated from tobacco leaves or present in tobacco smoke are 2,3,6-trimethyl-benzoquinone, 2-naphthylamine and a coffee-extracts and synthesized and modified natural coumarin derivatives (Fowler *et al.*, 2003; Gnerre *et al.*, 2000; He *et al.*, 2014; Herraiz and Chaparro, 2006).

Ligand-based models can provide new insights in enzyme selectivity, mechanisms of action and the relationship between the MAO inhibitory activity and the molecular structure of the different inhibitors (Vilar *et al.*, 2012). There are different types of ligand-based models which can be used, such as Quantitative Structure-Activity Relationship (QSAR) with 2D and 3D descriptors (Johnson, 1976; Vilar *et al.*, 2008; Winkler, 2002), 3D- Comparative Molecular Field Analysis (CoMFA) (Cramer *et al.*, 1988; Zhang *et al.*, 2011), 3D-pharmacophores (Langer and Hoffmann, 2006) or ligand-network models (Keiser *et al.*, 2007; Park and Kim, 2008). QSAR studies have become one of the most popular ligand-based approaches in modern chemistry (Shelke *et al.*, 2011; Vilar *et al.*, 2008; Vilar *et al.*, 2012) and can also be used to model ligand-based selectivity of different tobacco additives and the potency to inhibit MAO activity.

In vitro

Three-dimensional lung tissue constructs (3D lung-on-a chip) and mathematical computer models. These kinds of models are the results of the integration between *in vitro* models (the 3D organ-on-a-chip) and *in silico* models. It has been shown that inhalation during smoking results in a rapid brain increase of nicotine in the brain thereby contributing to nicotine dependence in smokers. Inhalation can be facilitated by certain additives leading to deeper and more frequent inhalation by the cigarette smoker resulting in an increase in lung exposure and nicotine uptake. Additives (e.g. menthol, theobromine and eucalyptol) can achieve this by enhancing sensory properties such as cooling effects or by having local anaesthetic and bronchodilating properties (Usmani *et al.*, 2005). Also, a change in the physical properties of tobacco (e.g. particle size) can be altered by certain additives to allow (nicotine) particles to enter deeper levels of the lungs (SCENIHR, 2010).

The efficiency of nicotine uptake and tobacco additives via the lung in the blood stream is difficult to measure. Engineered 3D lung tissue constructs and mathematical computer models can be used to provide predictive information on lung uptake and particle deposition (Asgharian *et al.*, 2012; Nichols *et al.*, 2014; Nichols *et al.*, 2013). These engineered 3D models of human tissue mimic *in vivo* conditions and allow for more natural and robust human *in vitro* respiratory tract models compared to multi-cell *in vitro* models. These constructs can be used to assess cell-based responses, physiologic functions, pathologic changes and even toxicity or responses to tobacco additives.

The (3D) airway models or lung-on-a-chip models can be also used to measure experimentally effects due to any additive in **altering nicotine uptake** such as alkalizing compounds or that result in easier absorption of nicotine by epithelial.

Capacity to change pH values. Additives that exert capacities to increase the pH values will result in higher amounts of uncharged nicotine (Hurt and Robertson, 1998; Wayne and Carpenter, 2009). This may result in more easily absorption of nicotine by the epithelial cells in the mouth and probably also in the lungs (Tomar and Henningfield, 1997). Opposing positions on smoke pH and its effect on unprotonated nicotine have been published and studies have been designed in an attempt to evaluate this effect empirically, but there is insufficient evidence available to prove or disprove this point. Although the tobacco industry stresses that the buffering capacity of the lung surface liquid (7mval/pH unit) at pH 7.4 is not changed by nicotine concentrations of 0.1 mg per puff (Holma and Hegg, 1989; Klus *et al.*, 2012), it is valuable to check additives or substances for their capacity to change the pH of the tobacco and the smoke.

(Inhibition of) The enzymatic activity of MAO. Additives may influence the dependence potential of nicotine by interacting with the neural responses to the drug. For example, MAO inhibitors that are not leading to dependence on their own slow the breakdown of monoamines such as DA, thereby affecting the overall motivational impact of nicotine. Inhibition of the enzymatic activity of MAO can be measured *in vitro* using peroxidase-linked spectrophotometric assay. Enzymes can be isolated from rat liver microsomes or by recombinant generated enzymes. Using recombinant human MAO-A and MAO-B, IC₅₀ values for enzyme inhibition can be experimentally determined (Lewis *et al.*, 2007). *In vivo* MAO activity can be analysed using PET (see paragraph 'neurobiological effects using imaging techniques' below).

CYP metabolism inhibitor ratio. The metabolism of nicotine is a complex pathway : as first step it is metabolized in the liver by cytochrome CYP2A6 and CYP2B6 enzymes, which can be induced or inhibited by some chemicals (Hukkanen *et al.*, 2005). Inhibition of nicotine metabolism enhances its bioavailability and alters the behavioural effects in mice (Alsharari *et al.*, 2014; Bagdas *et al.*, 2014). Additives modulating the activity of metabolic pathways are therefore likely to affect the dependence potential of nicotine. The effectiveness of an additive in inhibiting nicotine metabolism is expressed as relative CYP inhibitor ratio (Rahnasto *et al.*, 2008). The inhibitory concentration of human CYP2A6 can be tested in an *in vitro* assay using recombinant enzyme or human liver microsomal preparations (Rahnasto *et al.*, 2003). Examples of known tobacco additives that inhibit CYP2A6 enzymes are menthol, benzaldehydes and several lactones added to tobacco (Benowitz *et al.*, 2004; Kabbani, 2013; Kramlinger *et al.*, 2012; Rahnasto *et al.*, 2003).

In vivo (not recommended as the first choice)

Analysis of nicotine as Biomarker of exposure in humans. Nicotine bioavailability is defined by an optimal rate of adsorption and distribution from the lungs into the bloodstream. Upon uptake in the lungs, the bioavailability of nicotine in the body is determined by properties such as its hydrophobicity and solubility. It has been proposed that the use of alkalizing compounds (such as ammonia) as tobacco additive increases the absorption of nicotine in the lungs. A biomarker analysis of nicotine in blood samples from smokers of cigarettes with different ammonia yields was performed to evaluate the effects on nicotine bioavailability. Different ammonia yields in cigarettes did not increase

the rate or amount of nicotine absorption from the lungs to the arterial blood circulation (McKinney *et al.*, 2012; van Amsterdam *et al.*, 2011). It cannot be excluded from these studies that ingredients other than ammonium salts influence nicotine adsorption in a similar way.

Dopamine (DA) release and turn over. Activity of neurons in the mesolimbic DA brain area is not only measured by nACh receptor activation but also by measuring the result of this receptor activation, a change in the release or turnover of DA. DA release and turnover can be measured either *ex vivo* or *in vivo* via isolation of specific brain tissue or microdialysis. A study in mice showed up-regulation of nAChR subtypes in various brain regions upon exposure to nicotine and menthol using western blots. A significant increase in nicotine plasma levels was observed, which was accompanied by an increase of withdrawal intensity (Alsharari, 2015).

Neurobiological effects using imaging techniques. Exerting additive effects on nicotine dependent activation of the mesolimbic pathway can be studied *in vivo* using several neuroimaging techniques like functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) (Jasinska *et al.*, 2014). Each technique can be used to understand only some aspects of processes involved in tobacco consumption like brain structure (MRI), different aspects of brain function (PET, SPECT, fMRI, and ASL), and pharmacokinetics (PET, SPECT) in animals as well as humans (Kober and Deleone, 2011).

The neuronal activity upon exposure to nicotine and other tobacco additives can be measured by the activation of nACh receptors, neurotransmitter release and transcriptional activation of specific mRNAs (van de Nobelen *et al.*, 2016). Labelling and tracing of nicotine, MAO or nAChR can demonstrate nicotine occupancy at nAChRs, nAChR availability and upregulation of nAChRs induced by tobacco smoking (Brody *et al.*, 2014; Jasinska *et al.*, 2014; Volkow *et al.*, 1999). Substances that inhibit the nicotine degradation or metabolism or serve as a carrier for nicotine can be identified by these derivative endpoints.

PET. PET is a common functional neuroimaging technique which uses a short-lived radioactive tracer (incorporated into a biologically active molecule as glucose) infused into a living subject. As the tracer decays, the PET system detects pairs of gamma rays that are indirectly emitted in the process, and uses them to localise the tracer to a particular region in the brain. In this way, the concentration of tracer molecules can be estimated at different locations in the brain or other tissue. Several tracers are discussed below.

Radiotracers:

- **for nicotine.** A PET study with radiolabelled nicotine [¹¹C]nicotine demonstrated that a single puff leads to a rapid rise in brain nicotine concentration with a gradual wash-out period (Berridge *et al.*, 2010). In addition, the nicotine accumulation in the brain during smoking of one full cigarette increases in a linear fashion with successive puffs, rather than in puff-associated spikes and rapid wash-outs. Relating to this finding, the authors reported that dependent smokers showed a slower rate of brain nicotine accumulation than non-dependent smokers (Kober and Deleone, 2011; Rose *et al.*, 2010).

- **for $\alpha 4\beta 2$ nACh receptors.** Researchers have also developed brain-imaging radiotracers for $\alpha 4\beta 2$ nACh receptors with radiolabelled A-85380 compounds (Abreo *et al.*, 1996) or analogues (2-FA /6-FA for PET and 5-IA for SPECT), having the most widespread use (Koren *et al.*, 1998). However, the slow kinetics of these radio ligands restricts the large-scale use in experimental studies (Sabri *et al.*, 2015). New generation $\alpha 4\beta 2$ nAChR specific radioligands are now under development, like [^{18}F]Flubatine, [^{18}F]AZAN and [^{18}F]nifene, demonstrating faster kinetic properties in PET research (Brust *et al.*, 2008; Hillmer *et al.*, 2011; Hockley *et al.*, 2013; Kuwabara *et al.*, 2012; Wong *et al.*, 2013).

PET imaging studies demonstrated the effect of cigarette smoking on $\alpha 4\beta 2$ nAChR occupancy, showing that smoking causes displacement of 2-FA for prolonged time (at least several hours) (Brody *et al.*, 2006a). Dose-dependent reduction in 2-FA displacement was observed by both controlling the number of puffs smoked and the nicotine concentration smoked (Brody *et al.*, 2009). These findings suggest that nicotine mediates 2-FA displacement by occupying $\alpha 4\beta 2$ nAChRs. Several authors, using 5IA-SPECT and 2-FA-PET, have shown that habitual cigarette smoking is associated with up-regulation of $\alpha 4\beta 2$ nAChRs (Cosgrove *et al.*, 2009; Mamede *et al.*, 2007; Mukhin *et al.*, 2008; Staley *et al.*, 2006; Wullner *et al.*, 2008). The nAChR density returns to normal after a prolonged abstinence of weeks to months (Cosgrove *et al.*, 2009; Mamede *et al.*, 2007). In addition, exposure to nicotine from second-hand smoke resulted in substantial brain $\alpha 4\beta 2$ nAChR occupancy in smokers and non-smokers (Brody *et al.*, 2011). Taken together, these results suggest that exposure to cigarette smoke, most likely through the effects of nicotine, influences $\alpha 4\beta 2$ nAChR density in the human brain (Lotfipour *et al.*, 2011).

- **for Dopaminereceptor (DA).** With radiotracers such as [^{11}C]raclopride and [^{11}C]PHNO (PET) or [^{123}I]IBZM (SPECT), striatal DA release has been reliably measured using PET and SPECT imaging (Laruelle, 2000). An increase in DA competes with the radiotracer to bind at the dopamine receptor; resulting in a decrease in radiotracer binding compared to baseline. This allows calculation of the 'occupancy' of the receptors by DA or a change in binding potential and is an indirect measure of DA release based on the 'occupancy model' (Cosgrove *et al.*, 2015; Laruelle, 2000).

Many studies have examined nicotine and tobacco smoking-induced DA release in human subjects. All these studies confirm that smoking elicits ventral striatal dopamine release and is associated with a reduction of craving (Barrett *et al.*, 2004; Brody *et al.*, 2006b; Brody *et al.*, 2004; Montgomery *et al.*, 2007; Scott *et al.*, 2007; Takahashi *et al.*, 2008). In the most recent study, smokers were imaged with [^{11}C] PHNO before and after a cigarette (Le Foll *et al.*, 2014). Binding potential was reduced after smoking by 12 and 15 % in D2-rich and D3-rich regions, respectively.

A major concern with the existing studies is the timing of the dopamine response. The response to smoking a cigarette is a transient increase in DA. The analysis in these studies, however, use an average of all the data collected over 30 min to up to 2 h. This significantly dilutes measurement of a transient dopamine response (Sullivan *et al.*, 2013). Thus, analysis techniques with improved

temporal resolution may be better suited to more transient DA release (Cosgrove *et al.*, 2015).

- **for μ -opioid receptors.** There is strong evidence for a link between nicotine administration and endogenous μ -opioid mechanisms, mediating some of nicotine's addictive properties and distress during withdrawal (Nuechterlein *et al.*, 2016). Acute endogenous opioid release upon nicotine administration was demonstrated in animal and cell culture studies (Boyadjieva and Sarkar, 1997; Davenport *et al.*, 1990). However, in human studies the findings are inconsistent. The indirect measures of neurotransmitter release and μ -opioid receptor activation upon nicotine administration, as measured with PET, have shown both a reduction in binding potential (suggesting activation of neurotransmission) and an increase in binding potential (deactivation) or no significant change in different regions of the brain (Domino *et al.*, 2015; Kuwabara *et al.*, 2014; Ray *et al.*, 2011; Scott *et al.*, 2007). Measures at baseline have also shown either lower or no significant differences between smokers and non-smoking controls (Kuwabara *et al.*, 2014; Scott *et al.*, 2007). The μ -opioid system is suggested to be strongly influenced to placebo treatment (Nuechterlein *et al.*, 2016; Pecina *et al.*, 2015; Scott *et al.*, 2008; Zubieta *et al.*, 2005). Therefore, studying the opioid system does not appear to be the most sensitive and robust way to define tobacco dependence.

How the administration of tobacco additives changes these effects as measured is largely unknown. A recent PET study showed that in brains of female menthol cigarette smokers, nicotine accumulated faster thereby contributing to dependence. However a role of menthol in enhancing brain nicotine accumulation was not supported by this study (Zuo *et al.*, 2015). Another PET study using labelled nAChR subunits showed an upregulation of these receptors in the brain of menthol smokers, indicating a higher nicotine exposure in smokers of menthol cigarettes (Brody *et al.*, 2013). However, other mechanisms for menthol-influencing receptor density are possible. Analysis of nicotinic acetylcholine receptor activity *in vitro* shows that menthol inhibits nAChR subtypes in a non-competitive manner (Ashoor *et al.*, 2013; Hans *et al.*, 2012).

SPECT. In SPECT, the same mechanism is used as in PET, but differs in a way that the radioactive tracer directly emits a single gamma ray during decay. The nature of the signal allows for lower resolution images than PET as the SPECT tracers typically have a longer half-life, but scans are more easily performed. There are differences in the physics and chemistry used in PET versus SPECT, but the outcome measure of receptor availability is the same. Depending on the tracer used, PET and SPECT data can quantify regional brain activity (e.g. via glucose metabolism when the tracer is a modified sugar, as in ^{18}F -fluorodeoxyglucose), receptor occupancy (e.g. with ^{11}C -raclopride and dopamine receptors), and pharmacokinetics when multiple measurements are taken after drug consumption.

Functional Magnetic Resonance Imaging (fMRI). Besides PET, which is already an advanced technique in this field of research, fMRI is a promising and non-invasive upcoming technique. In fMRI, blood oxygen levels (brain activity) can be measured in the brain by use of strong magnetic fields. In the first fMRI study on the effect of acute nicotine administration, active smoking participants were injected with nicotine in

different concentrations. A dose- and time-dependent increased BOLD signal occurred in several cortical and subcortical regions, with prominent signal changes in the cingulate cortex, dorsolateral and medial orbitofrontal regions (Stein *et al.*, 1998) as well as the ventral striatum, amygdala, thalamus and insula (Menossi *et al.*, 2013). fMRI studies assessing tobacco additives with a (passive) inhalation tobacco devices can be used to identify brain areas involved in addiction. As there are no validated administration models for smoking or nicotine administration which can be used during scanning, this is an important limiting factor in fMRI research.

The majority of the above-mentioned imaging studies focus on chronic exposure in a cross sectional design (smokers vs. non-smokers) at a single point in time. When focusing on the dependence capacity of tobacco additives, studies on acute effects using within subject measurements (placebo vs. additive(s) of interest) is regarded as more valuable. This will improve the sensitivity to picking up small changes in neuronal activity caused by administration of the additive. Repeated exposure and repeated measurements can predict dependence capacity. Further improvement of the study protocol and development of even more efficient radio ligands may be beneficial to finding indications for tobacco additives that increase dependence.

Behavioural responses in rodents. Current animal models for tobacco product dependence are based on assessing nicotine dependence rather than dependence of tobacco additives or tobacco products as a whole. These models aim to deliver pure nicotine using an intravenous self-administration paradigm despite the fact that nicotine itself is regarded as a relatively weak reinforcer (Caille *et al.*, 2012). Current tests to analyse dependence potential can monitor self-administration, speed of acquisition, conditioned rewarding effects and drug discrimination (Hoffman and Evans, 2013; Wilkinson and Bevins, 2008; Yararbas *et al.*, 2010). Also severity of withdrawal can be measured (Bagdas *et al.*, 2014). Animal models also allow controlling of factors that can affect study outcome such as environmental factors, genetic background and prior drug exposure. The self-administration paradigm has been widely accepted as a reliable animal model with high predictive value for the dependence potential of a drug and can be used to support findings observed in humans. The current available models can possibly be adapted to assess the effect of (nicotine in combination with) other tobacco-related additives on dependence. A recent animal study showed that the sensory properties of menthol can serve as a conditioned reinforcer for nicotine (Wang *et al.*, 2014).

Behavioural outcome measures in human. Several behavioural measures can be used to assess dependence in human. Dependence for nicotine and smoke(less) tobacco can be self-assessed using the Fagerström Test for Nicotine Dependence (FTND) or the cigarette withdrawal scale (CWS-21) (Etter, 2005; Etter *et al.*, 2003; Fagerstrom, 2012). The FTND uses a twelve-item cigarette dependence scale that covers the main definitions of dependence: compulsion, withdrawal symptoms, loss of control, neglect of other activities, time allocation and persistence despite harm. The FTND can assess the degree or severity of tobacco dependence using a scale indicative for the level of dependence.

The cigarette withdrawal scale (CWS-21) is a 21-item multidimensional self-administered scale that measures withdrawal symptoms and predicts relapse to smoking (Etter, 2005). Recently, a revision for DSM-V was proposed in order to increase the predictive

value of these criteria for tobacco dependence assessment (American Psychiatric Association, 2013; Baker *et al.*, 2012).

Indicators of nicotine dependence were assessed in menthol and non-menthol cigarette smokers using the FTND. Differences were observed in time to first cigarette of the day (TTF) suggesting greater urgency to smoke but not on amount of cigarettes smoked on a day (CPD) (Collins and Moolchan, 2006; Hoffman and Simmons, 2011). An important limitation of these methods is that these tests are a diagnostic instrument for assessing dependence in people and not necessarily the dependence potential of the given substance or product type. However, these questionnaires can be adapted for the assessment of dependence to tobacco-related additives.

Recommendations

To accurately assess tobacco dependence potential for regulatory purposes, it is necessary to use multiple evaluation methods, whereby several factors associated with tobacco dependence are analysed. Combinations of techniques examining neurochemical physiological and behavioural changes in specific brain regions with nicotine dependence may provide sufficient and robust information. Correlations between responses and convergence of studies will lead to evidence-based conclusions. For regulatory purposes, consensus needs to be established on the (combination of) tests that are preferred. The SCHEER therefore proposes to use a step-wise approach of 1) *in silico*, 2) *in vitro*, 3) *ex vivo*, and 4) *in vivo* methods- only in exceptional cases, to be agreed with the Receiving Authority on a case-by-case basis. The use of *in vivo* studies is indeed questionable for ethical reasons, therefore the performance of new studies are only justified under exceptional circumstances. After negative results of testing the tobacco additive on dependence capacity in the first agreed appropriate method (*in silico*), the next step should be considered, and appropriate test(s) should be selected (*in vitro* models), and so on. It is strongly recommended that *in silico* and *in vitro* tests to assess additive-induced addictiveness are developed and validated in the future by independent organisations.

3.4.3.6 Characterising flavour and inhalation facilitation properties

This section will discuss a procedure to assess tobacco products with characterising flavours that are prohibited in the TPD, as well as some other mechanisms such as inhalation facilitation, that may contribute to additive-induced attractiveness.

Animal models do not currently exist for this kind of assessment. In humans, the attractiveness of individual tobacco products can be compared in panel studies, surveys and by experimental measures. To test the response to a specific additive, tobacco products can be produced to exclude or include individual additives. However, this type of research is difficult nowadays due to ethical considerations that will often preclude human testing (SCENIHR, 2010).

Characterising flavours

Over 80% of all Dutch cigarettes contain at least one flavour, and almost half of all additives in any tobacco product is added as a flavour (Pennings *et al.*, 2016). Flavours may be added to tobacco, cigarette paper, the filter or to the foil wrapper, in an attempt to enhance the tobacco flavour, mask unpleasant odour, and deliver a pleasant cigarette-pack aroma (WHO, 2007). Many different additives are used to create a specific taste/flavour in order to attract certain target groups. Regarding flavour, the EU

Tobacco Product Directive (TPD, Article 7) prohibits cigarettes and roll-your-own tobacco having a characterising flavour other than the of tobacco, as they could facilitate initiation of tobacco consumption or affect consumption patterns (European Union, 2014). A characterising flavour is defined as a '*clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herb, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product.*' The prohibition of tobacco products with characterising flavours does not preclude the use of individual additives outright, but it does oblige manufacturers to reduce the additive or the combination of additives to such an extent that the additives no longer result in a characterising flavour.

Talhout *et al.* (2016) published an inventory of methods suitable to assess additive-induced characterising flavours of tobacco products, and concluded that because flavour perception is subjective and requires human assessment, sensory analysis in consumer or expert panel studies is necessitated. They recommend developing validated tests for descriptive sensory analysis in combination with chemical-analytical measurements. Testing a broad range of brands, including those with quite subtle characterizing flavours, will provide the range of concentrations above which an additive will impart a characterising flavour (Talhout *et al.*, 2016).

The Commission has recently adopted two implementing acts establishing the rules and mechanism for determining products with characterising flavours²⁴.

The determination of such flavours can concern products before consumption (e.g. before combustion) as well as emissions resulting from normal use (direct and indirect) of the products. To develop a method for determining characterising flavours and to perform some pilot experiments, the Commission contracted the HETOC Consortium in August 2014²⁵. The HETOC-consortium carried out, as an external contractor, a study on the determination of characterising flavours. Sensory testing complemented by chemical analysis was concluded to be an appropriate method to determine characterising flavours. Their pilot had confirmed that an expert panel is a good approach, but the training phase needs to be more extensive when the real panel is set up. Smelling is the preferred starting point for determining characterising flavours and it was recommended to consider, as a future step, whether a smoking experiment was needed. It was concluded that specific reference spaces for cigarettes and RYO are needed.

Beside the characterising flavour features, other phenomena can contribute to make a tobacco product more 'attractive'. According to the partial guidelines for implementation

²⁴Commission Implementing Regulation (EU) 2016/779 of 18 May 2016 laying down uniform rules as regards the procedures for determining whether a tobacco product has a characterising flavour

http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0048.01.ENG&toc=OJ:L:2016:131:TOC

Commission Implementing Decision (EU) 2016/786 of 18 May 2016 laying down the procedure for the establishment and operation of an independent advisory panel assisting Member States and the Commission in determining whether tobacco products have a characterising flavour

http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0079.01.ENG&toc=OJ:L:2016:131:TOC

²⁵http://ec.europa.eu/health/tobacco/products/implementation/characterising_flavours_en.htm

of Articles 9 and 10 of the WHO framework convention on tobacco control, “attractiveness” refers to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use. Note that not all of these properties are related to additives. WHO-FCTC advises Parties to regulate, by prohibiting or restricting, ingredients that may be used to increase attractiveness of tobacco products (WHO, 2012). The FCTC guidelines in relation to the regulation of the contents of tobacco products and regulation of tobacco product disclosures call in particular for the removal of ingredients that increase palatability, create the impression that tobacco products have health benefits, are associated with energy and vitality or have colouring properties.

The TPD includes two references to attractiveness. In the introductory considerations, point 13, it is mentioned that *“In order to carry out their regulatory tasks, Member States and the Commission require comprehensive information on the ingredients and emissions from tobacco products to assess the attractiveness, addictiveness and toxicity of tobacco products and the health risks associated with the consumption of such products.”* In article 19, Notification of novel tobacco products, it is mentioned that manufacturers and importers of such a product shall provide *‘available scientific studies on toxicity, addictiveness and attractiveness of the novel tobacco product, in particular as regards its ingredients and emissions.’*

In the following, the possibility to assess characteristics other than the characterising flavour as contributors to attractiveness are briefly presented, some of which having the possibility of “Facilitating inhalation or nicotine uptake”, which is a criterion of the mandate (category c) included in the ToR).

Other sensory attributes which can facilitate inhalation or nicotine uptake

Low irritation to mouth, throat and chest and satisfaction can facilitate inhalation and possibly nicotine uptake (Jaffe and Glaros, 1986; Kochhar and Warburton, 1990). Mildness, a combination of improved aftertaste, less bitterness, improved mouth feeling and reduced irritation, is reported to be appreciated, especially by younger and beginner smokers, with their undeveloped tastes and a low tolerance for irritation from tobacco smoke (Carpenter *et al.*, 2007).

Additives that influence these sensory attributes, such as mildness, and a pleasant aftertaste, possibly facilitate smoking initiation. By reducing and changing the harshness of the smoke, special target groups may be reached (Carpenter *et al.*, 2005a, Carpenter *et al.*, 2005b, Cummings *et al.*, 2002, Klein *et al.*, 2008, Wayne and Connolly, 2002). A confidential tobacco industry document describes a class of casing materials referred to as ameliorants used to *“...smooth out harshness and bitterness and/or eliminate pungent aromas from tobaccos”* (Jenkins *et al.*, 1997). Examples of such ameliorants included sugars, cocoa and liquorice. Cocoa, also at levels that do not impart a characterising flavour, can alter cigarette flavour and improve product acceptability (Sokol *et al.*, 2014). Various sugars constitute a large proportion of additives, and the sweetness of the smoke resulting from its caramel combustion products is an important characteristic. Thus, product appeal for starters may be further diminished by regulating trigeminal attributes as well. Smoking panels can be used to assess sensory attributes like irritation, impact, flavour, aftertaste. For irritation, it may also be possible to use *in vitro* models.

Some additives have multiple chemosensory effects. Pyrazines, which are flavours resulting from pyrolysis of amines and sugars, are reported to induce chemosensory effects such as reducing the harshness and irritating effects of nicotine and other tobacco smoke constituents in the airways. In addition, they may reinforce the learned behaviour of smoking, enhance elasticity and help optimise nicotine dosing. Wayne and Henningfield also describe evidence from internal industry documents that “smokers develop a taste for specific flavors or characteristics of tobacco use other than nicotine, and come to associate use with these characteristics” (Wayne and Henningfield, 2008). Vanilla, for example, increases mildness, and smokers will switch to other vanilla-containing brands, but not to brands without vanilla taste. Menthol is also known for its taste, as well as inducing a “cooling” effect which masks the harshness and the taste of raw tobacco (Lawrence *et al.*, 2011).

Harshness and smoothness. According to the tobacco industry definition, harshness is a chemically-induced physical effect associated with a roughness, rawness experience generally localized in the mouth and to a lesser degree in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. Harshness can also cause a drying, rasping, coarse, astringent sensation usually associated with the smoke flavour of Virginia or air-cured type tobaccos. Harshness is classically measured in four degrees: (i) Free – an absence of harshness; (ii) Touching – a slight awareness of a sensation; (iii) Scratchy – some discomfort, a stinging effect; and (iv) Harsh – rough, raw, raspy, coarse, astringent, painful inhalation. Reducing the harshness of the smoke makes it possible to inhale deeper and increase the number of puffs, as physical barriers will be reduced (Wayne and Henningfield, 2008).

The harshness depends partly on the tar/nicotine ratio, but may also be decreased by certain additives such as propylene glycol or levulinates. Tar provides a strong flavour and mouth sensation, masking the harsher, bitter taste of nicotine which may be unpalatable to new smokers and uncomfortable to established smokers. Certain highly flavoured additives may also have the same properties to “smoothen” or reduce the harsh irritation of nicotine in tobacco smoke. In order to make the smoke less aversive and permit deeper inhalation, additives such as liquorice and menthol are used. Another approach is to use nicotine salts that do not cause the same irritation, but are still delivering nicotine or keeping the nicotine effect by means of a quicker absorption by ensuring larger amounts of free nicotine (SCENIHR 2010, Bates *et al.*, 1999, Keithly *et al.*, 2005). Finally, the addition of humectants such as glycerol, propylene glycol and sorbitol keep the humidity of the tobacco product at a desired level; dry tobacco generates an unpleasant harsh smoke.

Impact and smoothness. The term “impact” is widely used in tobacco industry research and documents, and is a tobacco industry term for smokers’ subjective awareness of the drug effects of nicotine. Organic acids have been used since the 1950s to improve “smoothness” of cigarettes. For example, Philip Morris found that lactic acid decreased subjective ratings of harshness and bitterness, and produced a sweeter flavour. Citric additives have been used not only for reduced harshness and flavour modification, but also to modify smoke pH, to neutralise nicotine “impact” (an industry term denoting the organoleptic sensation caused by nicotine; smokers often describe this as “throat catch” or “throat hit”). Tartaric and lactic acids likewise modify the pH of smoke. All of these organic acids increased smoothness and are associated with a decrease in nicotine “impact” (Philip Morris, 1989) However, it is unclear whether these

effects are due directly to pH modification. Unregulated botanical and chemical additives might have “multiple-use” purposes, such as enhancing flavour and producing “smoother” cigarette smoke, as well as potentially preventing or masking symptoms associated with smoking-related illnesses (Rabinoff *et al.*, 2007).

Facilitate the inhalation of tobacco smoke. Certain ingredients have local anaesthetic effects. As a result, coughing due to inhalation of irritating smoke is dampened and the smoker can inhale the smoke deeper (and more frequently). Examples are components of etheric oils, such as menthol and thymol (SCENIHR 2010).

Appearance, smell and irritation of tobacco smoke. In order to make the smoke more attractive not only to the smoker, but also to other people in the proximity of the smoker, it is important that the smoke is appealing and not annoying. This may be attained with additives that make the smoke whiter and more attractive to people seeing the smoke. The TPD prohibits additives having colouring properties for emissions.

Reduced visibility of side-stream is accomplished by the addition of magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate and calcium carbonate to the wrapper (cigarette paper). This has an effect on particle size; particles become smaller and therefore do not easily scatter light and become less visible. Reducing side-stream emissions is based on encapsulating the smoke in an impermeable cone using different types of additives such as potassium succinate, potassium citrate and magnesium carbonate. By combining the use of additives and the look of the tobacco product, greater acceptance of the smoke may be created. Less resistance may be encountered from persons who do not smoke, and at the same time greater pleasure for the smoker may be created. The same agents may also be used to target the individual product at certain target groups (Carpenter *et al.*, 2005a, Connolly, 2004).

The smell of the smoke may be also changed so that it is also more attractive and less irritating (Connolly *et al.*, 2000, Ling and Glantz, 2005). Connolly *et al.* (2000) examined tobacco industry patents covering the function of environmental tobacco smoke masking. These strategies include reducing smoke odour and reducing side-stream smoke visibility and emissions. Methods to neutralise or reduce lingering smoke odour include addition of acetylpyrazine, anethole and limonene to modify the side-stream odour. These compounds have rather low odour thresholds, and are subsequently easily picked up, while they elicit no trigeminal nerve response. Aroma precursors, e.g. polyanethole provided a noticeable fresher, cleaner and less irritating cigarette side-stream aroma, while others (e.g. cinnamic aldehyde, pinanediol acetal) produce slightly sweet, spicy, clean, fresh, and less cigarette-like aroma. In addition, more “classic” additives (e.g. vanillin, benzaldehyde, bergamot oil, cinnamon/cinnamon extract, coffee extract and nutmeg oil) modify sidestream odour.

Studying sensory effects

Neuro-imaging techniques are used to provide insight into brain processes related to sensory perception. The brain integrates sensory inputs such as taste, touch and smell, and the resulting neural activation can be studied by e.g. fMRI and PET (Small, 2012). Many brain areas are involved, such as brainstem, amygdala, and the orbitofrontal cortex. Odours, tastants, and trigeminal stimuli (intranasal irritants) are processed within the olfactory network, gustatory network, and trigeminal network, which are interacting networks (Lundstrom *et al.*, 2011). The widespread network involved in the processing

of odorants, tastants, and chemical irritants recruits several key cerebral areas, including those responsible for emotions, memories and reward. Reward consists of the psychological components learning, affect, and motivation (Berridge and Robinson, 2003). Physiological, emotional, cognitive and sensory responses caused by flavours and odours can be tested, also in relation to 'reward dose' in the brain (SCENIHR, 2010b). In research projects conducted by Philip Morris from 1982 to 1995, electroencephalography (EEG), pattern reversal evoked potential (PREP), and chemo-sensory event-related potential (CSERP) were used to measure physiological, sensory and cognitive changes related to nicotine and to cigarette additives (Rabinoff *et al.*, 2007).

3.4.3.7 Interaction of the additive with other additives/ingredient

Tobacco smoke is a complex mixture: 9582 chemical components have been identified so far in tobacco and tobacco smoke (Rodgman and Perfetti, 2013), its composition is qualitatively and quantitatively not fully known and may change, depending on the brand. In addition, tobacco being a natural product, its composition is variable over time from batch to batch even within the same brand. One of the major limitations of using *in vitro* and *in vivo* test systems for the toxicological study of inhalational exposures to compounds in tobacco mixtures is the very high number of components in tobacco smoke and the extreme variability of the mixture. The large number of additives (~ 1260 –SCENIHR Tobacco Opinion 1, 2016) present in the Industry's repertoire add further qualitative and quantitative variability to the mixture. The list is open ended, which means that at present there is no restriction on the number of additional additives as alternative chemicals, precursors etc. Moreover, several of these additives are botanical extracts, which, per se are composed of hundreds of components such as flavours, sugars, pH modifiers. These botanical and phytochemical additives are claimed to have a number of properties, including anaesthetic, antibacterial, anticancer, anti-inflammatory, antifungal, and antiviral properties (Rabinoff, 2007), but these 'apparently beneficial' activities cannot justify their use as additives in tobacco products. Indeed, in some cases, they provide for a "smoother" smoking experience by masking adverse symptoms caused by smoking (e.g., cough), preventing awareness in the consumer and reductions in cigarette consumption.

The specific purpose and the required concentration is well researched and optimised by the tobacco industry before any product is marketed; therefore additives included in the composition of each tobacco product represent a mixture itself. This 'optimal' mixture of additives is intentionally added to a known toxic, carcinogenic and addictive product in order to make the product more palatable by masking the bitter taste, improving the flavour and reducing the irritation of inhaled smoke, optimising nicotine uptake.

The possibility that chemical interactions can occur among different tobacco components and additives and among different additives cannot be excluded. These interactions can consist of direct chemical reactions, forming additional different compounds, or being translated in addition, potentiation or antagonism of the effects induced by additives and tobacco components (with the possibility that such an interaction occurs at the level of toxicokinetics and at toxicodynamic level). The number of possible interactions and the number of test combinations increase exponentially with increasing numbers of compounds in a mixture. Moreover, the number of experimental groups will also increase with the number of doses of each compound.

As for the general issue of mixture toxicity, in this specific case it can also not be solved by applying an experimental approach, since to test the thousands of possible mixtures is not feasible, beside the fact that, as previously discussed, their composition is variable. This is an additional reason for considering the approach of using comparative testing strategies insuitable, where differences in effect of the tobacco product with and without the additive are evaluated (see paragraph 3.4.1).

The frameworks for assessing chemical mixtures have been proposed at international and national levels and addressed in several guidance documents.

In most of these documents, the focus is on the mode of action of specific compounds: chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. The approach suggested by the non-food SC on mixture toxicity opinion (SCHER, 2012) as well as the one proposed by EFSA (EFSA, 2012 and EFSA, 2013 on pesticides) can be followed. A case-by-case approach could be useful to define specific testing.

Both the non-food SC and EFSA made use of the WHO/IPCS Framework for Risk Assessment of Combined Exposures to Multiple Chemicals. It is a tiered framework for organising risk assessment tools and data in order to conduct an assessment of combined exposure to multiple chemicals, starting with screening level assessment and proceeding to more complex approaches. The tools and data to be employed are decided on a case-by-case basis by the risk assessor, in order to address the problem at hand, e.g. contaminated site, chemicals in surface water, etc. (Meek *et al.*, 2011).

In the future, the EU project EuroMix (European Test and Risk Assessment Strategies for Mixtures) will provide a test strategy and test instruments using novel techniques for mixture testing based on new and already existing toxicological tests. The tests will result in data needed for refining future risk assessment of mixtures relevant to national food safety authorities, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders. <https://www.euromixproject.eu/>

The following examples show the interactions of compounds with similar function/activity on the one hand and the camouflaged effect of botanicals on the other hand, revealing the complexity of ascertaining interaction between additives.

Any additives able to interfere with nicotine bioavailability independent of the mechanism can be the cause of addition or synergism of effects. Using at the same time additives altering the pH of tobacco (e.g. alkalisng agents such as ammonium compounds facilitating nicotine passage through the cell membrane in the uncharged volatile form), together with substances such as menthol and thymol, facilitating the inhalation of tobacco smoke (due to local anaesthetic effects) or bronchodilators, such as theobromine (generated from cocoa, caffeine and glycyrrhizine) would, all together, have a non-negligible impact on nicotine bioavailability in the body. Although they present a low concentration singularly and their action can be deemed as non-relevant (SCENIHR 2010), taken together the impact is very different.

To these considerations should be added the use of additives that interfere with nicotine metabolism, additives such as the gamma-aliphatic lactones group (see SCENIHR opinion 1, 2016) are mild to weak inhibitors of CYP2A5 and CYP2A6. As CYP2A6 is

involved in the metabolism of nicotine, the presence of these chemicals could decrease smokers' metabolism of nicotine and maintain higher blood levels (thus increasing smokers' exposure to nicotine by slowing degradation of nicotine in the bloodstream). Furthermore, the inhibitory effect of these chemicals on CYP2A6, although relatively weak in isolation, might be greater when the chemicals act in combination.

Several patents discussed direct "beneficial" physiological actions of botanical additives. In one US patent cited, it was noted that nicotine in cigarettes has a deleterious vasoconstrictive effect on the cardiovascular system, particularly the blood vessels within and surrounding the heart. It was also noted that vaporised niacin in cigarette smoke has a vasodilating action that helps counteract the vasoconstrictive effect of nicotine. Furthermore, additional "beneficial" effects may be obtained when niacin is combined with rutin (a chemical found in many botanicals), "which is considered effective in reducing and preventing capillary fragility." The patent listed 33 botanicals or vegetable materials, or compounds within them, which also appear on the tobacco industry cigarette additive list (Rabinoff, 2007).

Usage of fruit and vegetable extract concentrates/ botanicals can also give an impression of health benefit to the consumer, so could be considered under the TPD Article 7 2 a. This kind of information is very important as more than 100 of 599 documented cigarette additives have pharmacological actions that camouflage the odour of environmental tobacco smoke emitted from cigarettes, enhance or maintain nicotine delivery, could increase the addictiveness of cigarettes and mask symptoms and illnesses associated with smoking behaviours (Rabinoff, 2007).

3.4.4 Step 4: Reporting

In the fourth and last step, a report needs to be drafted on the activities carried out in Steps 1 to 3, to be sent to the relevant authorities. The report should include an overall evaluation of the results from Step 1 to 3. In Annex I a reporting template is provided for this purpose.

The formation of consortia and joint reports by industry is endorsed in order to limit the financial and administrative burden for both industry and authorities, and for the subsequent evaluation of the submitted reports by independent institutes.

3.5 Specific knowledge gaps for the priority list tobacco additives used in cigarettes and roll-your-own tobacco

In addition to the general strategy described in the previous paragraphs, the data gaps already identified in Tobacco Opinion 1 for the 15 additive included in the EU Commission priority list have been analysed. The analysis was based on the 'Rational for inclusion' taken from Opinion I.

Based on that, the activities to be performed upfront will be described, in order to identify the most appropriate steps (and end-points) to be carried out and to speed up the process, making possible testing feasible in the 18 month time-frame. In some cases (e.g. identification of CMR properties of the unburnt form) it would be possible to identify whether or not they should enter the evaluation procedure (e.g. having or not having properties that do not meet the criteria of the TPD, art. 7). Starting at the lowest step, for each of the 15 additives on the priority list, recommendations for experimental

activities to fill the data gaps recognised in Tobacco Opinion 1 are given. If the outcome is negative (i.e. no effect which does not meet the TPD criteria is demonstrated), they will enter the general strategy of testing and be considered as any other compound. It is recommended to address the extensive data search also for the 15 priority list chemicals and to apply the WoE approach, as described in step 1, since the SCENIHR in Opinion 1 was not asked to carry out a risk assessment but a prioritization (based on hazard) of a large number of additives to assist the Commission in the compilation of the priority list.

3.5.1 Carob bean

Synonyms: Locust bean extract, St. Johns bread extract

CAS number: 9000-40-2/84961-45-5

Rational for inclusion

Carob bean extract is a chemically undefined complex additive containing hundreds of chemicals, rich in carbohydrates/sugars. Information on the exact chemical composition of this complex tobacco additive is lacking (e.g. carbohydrate, proteins/amino acids and fats, pH modifiers. It pyrolyses extensively and the combustion of the high carbohydrate/sugars leads to formation of carcinogenic and toxic compounds (e.g. benzene, polycyclic aromatic hydrocarbons, and phenol), aldehydes (acetaldehyde, formaldehyde, furfural and acrolein), organic acids and caramel colour and flavours (Baker & Bishop, 2004, Coggins *et al.*, 2011).

Some of these pyrolysis compounds are reported to reduce nicotine delivery, leading to increased smoking frequency and deeper inhalation of smoke to enable higher absorption of nicotine in the airways. Compounds resulting from sugar combustion, such as acetaldehyde and more complex aldehydes, are MAO inhibitors, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT_{1A} autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive.

Moreover, it should be noted that pyrazines (reported as additives to cigarettes to impart flavour in low tar cigarettes; Alpert *et al.*, 2016) may be formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Carob bean extract has a sweet, fruity, chocolaty flavour and contributes to making smoking more attractive by improving flavour, thereby masking its bitter taste and reducing the harshness of smoking.

This information can facilitate the assessment of the influence on the carob bean extract on palatability (a key component of attractiveness), the pro-addictive effect and the interaction with other additives and tobacco chemicals.

Priority activities

Step 1: Data on the chemical composition of the carob bean extract should be provided by industry with emphasis on the concentrations of constituents of relevance;

Step 2: Some information on the effect of pyrolysis of carob bean extract is available, however, it is necessary to:

- further chemically define its pyrolysis products and

- evaluate the CMR properties of its pyrolysis products.

In case of concern for genotoxicity/carcinogenicity of its pyrolysis products, additional testing would be required to continue with **Step 3** in order to generate additional data for a WoE assessment,

If no concerns arise, the additive can enter the tiered procedure for evaluation. The assessment of its pyrolysis product on palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals should be presented (Step 4).

3.5.2 Cocoa and cocoa products (powder, extracts, shells of cocoa bean etc.)

Complex mixture from Theobroma cocoa beans

CAS Numbers: 95009-22-6 (cocoa powder), 84649-99-0, 84649-99-3 (cocoa extract)

Rational for inclusion

Many forms of cocoa additives such as extracts and powders are used frequently and in relatively high amounts. Added as flavour or casing to tobacco (cocoa extract is the most abundantly used, with 847 counts in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 0.105 (0.198). The maximum amount of cocoa as tobacco additive is around 1 % of the total tobacco weight (RIVM, 2012).

Regarding toxicity, the effects of cocoa inhalation through smoking have not been studied. The risk associated with the generation of combustion products produced upon cocoa pyrolysis has not been thoroughly studied and thus, conducting an adequate risk assessment for cocoa or its pyrolysis products is currently not possible.

Regarding addictiveness, several pharmacological effects of cocoa-derived ingredients were reported, including the bronchodilatory effect of theobromine and caffeine, which result in improved bioavailability of nicotine, although data available so far indicate that the content of theobromine per cigarette seems to be too low to have a bronchodilating effect on the lungs (SCENIHR, 2010). Furthermore, reaction products of tryptophan, phenylethylamine, tryptamine and tyramine, are thought to exert monoamine oxidase-inhibiting properties. In general, the pharmacologically active substances present in cocoa do not exclude a psychopharmacological effect in humans, owing to the low exposure concentrations and/or the inability of these substances to cross or reach the blood-brain barrier. Due to a lack of studies specifically on the psychoactive effects of cocoa added to tobacco, there is insufficient evidence that adding cocoa to tobacco makes cigarettes more addictive.

The addition of cocoa to tobacco is intended to enhance flavour. More data are needed on the amount of cocoa needed to impart a noticeable flavour.

Priority activities

Based on the available data, cocoa and cocoa products may result in a characterising flavour and increase inhalation and nicotine uptake. The reported percentage of cocoa

used in cigarettes ranges from 0.2% to 0.66%. Taking this information into account, the content of theobromine and caffeine per cigarette seems too low to have a bronchodilating effect on the lungs and thereby increase the absorption of nicotine. Therefore, there is uncertainty with regard to the direct effect of cocoa additives on the bioavailability of nicotine and more studies are required.

Step 1: Additional data should be sought and evaluated to address the above mentioned questions and all the other available information on the additive in the unburnt form.

Step 2: Pyrolysis of cocoa results in the generation of minor amounts of phenol, o-, m-, p-cresol, xylenols, catechol, palmitic acid and stearic acid (<0.001% (w/w) in tobacco) and nitrous gases, carbon monoxide and dioxide. Tryptophan combustion can generate 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole and 3-amino-1-methyl-5H-pyrido-(4,3-b)indole. Furthermore, tryptophan contains reactive groups and forms reaction products with other compounds during combustion, such as beta-carbolines, including harman (RIVM, 2002). The resulting anti-depressive effects of harman have been suggested to contribute to addiction caused by cigarette smoking. Reaction products of tryptophan, phenylethylamine, tryptamine and tyramine, which are formed during combustion, are thought to exert monoamine oxidase inhibiting properties. Nevertheless, the risk associated with the generation of combustion products produced upon cocoa pyrolysis has not been thoroughly studied and should be carefully evaluated.

Step 3: The exposure to cocoa and cocoa-derived ingredients transferred to cigarette smoke in their pure forms is negligible compared with the exposure to these compounds through food and drinks (RIVM, 2002). However, the consequences of the exposure through inhalation have not been studied. Exposure through smoking should not be neglected as it represents two different types of exposure through inhalation of (1) cocoa itself and (2) combustion products of cocoa and its ingredients (RIVM, 2002).

Several mechanisms of enhancing addictiveness of smoking have been proposed, however, it is unclear whether sufficient amounts of psychoactive compounds are produced to exert psychopharmacological effects that would increase addictiveness. Characterising flavour may make cigarettes more palatable to younger, first-time users and may indirectly facilitate dependence by providing enhanced flavour and mouth sensations, potentially serving as a cue for drug reward. Due to a lack of studies specifically directed to the psychoactive effects of cocoa compounds added to tobacco on addiction, there is insufficient evidence that the addition of cocoa to tobacco contributes to the addictive properties of cigarette smoking.

The addition of cocoa to tobacco is intended to enhance flavour and therefore smoking may result in a characterising flavour. However, although a considerable percentage of cigarette weight could be cocoa additives, it is not known to what degree this influences the flavour of inhaled mainstream or side stream smoke, and especially how this might influence smoking initiation in youths (Fowles, 2001).

3.5.3 Diacetyl

CAS-nr:431-03-8

Synonyms: butanedione, butane-2,3-dione

Rational for inclusion

Diacetyl exposure may lead to serious lung disease after inhalation. For a proper risk assessment, it is necessary to better characterise the concentrations in mainstream smoke. SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. There were no data on carcinogenicity. In addition, it can result in a characterising flavour, which can contribute to increasing attractiveness.

Priority activities

Based on the rationale for inclusion, the main open questions concerning diacetyl are:

- Uncertainty concerning the genotoxicity of diacetyl and no data on carcinogenicity.
- No sufficient data was found concerning the burned (pyrolysis) product.
- Exposure may lead to lung disease after inhalation and should be assessed in appropriate tests.
- Due to the typical flavour of diacetyl it is unclear whether the compound can add to the olfactory cue and attractiveness of the smoking product. The question whether this flavour is distinguishable (attractiveness) and/or has a "smoothing" effect on the smoke (attractiveness/addictiveness) remains unclear.

The first activity to be carried out is related to the need to rule out the genotoxicity of the compound. In case of identification of genotoxic potential, the additive will be out according to the TPD provisions; otherwise it should enter the step-wise procedure.

Step 1: Additional data should be sought and evaluated to address the above mentioned questions

Step 2: Pyrolysis products should be studied and evaluated using the available data.

Step 3: In order to fill the gaps identified in Step 1 and 2, toxicity testing for inhalation exposure and then for characterising flavour, inhalation facilitation and addictiveness (considering its potential characterising flavour as well as the "smoothing" effects) for the additive in its unburnt and burnt form.

3.5.4 Fenugreek extract

Synonyms: fenugreek (*trigonella foenum graecum* L.) extract, resin, & absolute
CAS number: 84625-40-1

Physical properties: Complex mixture, dark brown paste

Rationale for inclusion

Natural/botanical concentrates/extracts/resins (e.g. from several fruits - fig, plum, raisins, fenugreek, carob, cocoa, caramel, rum, etc.) form a large number of tobacco additives. They are poorly characterised complexes of several to hundreds of chemicals; the composition further depends upon variable factors influencing botanical source and preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of each of these additives is not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, many of the botanical extracts have a rich carbohydrate, polysaccharides and sugar content, together with varying amounts of proteins, amino acids and other flavour compounds. Fenugreek seeds contain mucilage (glycoproteins and polysaccharides) and some amount of sugar. The pyrolysis of this class of compounds has been well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, give rise to a complex mixture of toxic, carcinogenic and mutagenic compounds, as well as aroma/flavour compounds.

Compounds formed include smoothing agents (e.g. organic acids), flavours (e.g. caramel), compounds that facilitate nicotine delivery (e.g. aldehydes) and compounds with CMR properties (e.g. PAHs, formaldehyde). Moreover, pyrazines are important flavour impact compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also added as additives to cigarettes to impart flavour to low tar cigarette (Alpert *et al.*, 2016). The complex mixtures used as additives cause tremendous harm and contribute to CMR properties, addictiveness and attractiveness of tobacco smoke.

Priority activities

Step 1: Data on the chemical composition and specification of the Fenugreek extract (powder, concentrate) should be provided by industry with emphasis on the concentrations of constituents of relevance, production procedure, maximum levels for microorganisms and possible contaminants. According to available information, *Trigonella foenum-graecum* seeds contain mucilage, trigonelline, 4-hydroxyisoleucine, sotonol, diosgenin, phenolic acids, and protodioscin.

The use of fruit and vegetable extract concentrates are acknowledged to be beneficial to health. Fenugreek extract can thus give an impression of health benefit (*TPD Article 7 6a*) to the consumer. Moreover, as it is also used as a medicinal product, this could also give the impression of health protection.

Step 2: Fenugreek extract does not transfer intact to the mainstream smoke, but undergoes extensive pyrolysis. Based on the available studies (Baker and Bishop 2005), pyrolysis products from fenugreek extract include pyridine, benzene (carcinogen), toluene and furfural. The pyrolysis products, once characterised, should be evaluated according to the procedure.

If the evaluation shows that it is warranted to move on to **Step 3**, the effects which have been considered as matters of concern (e.g. neuropharmacological activities, CNS depressant and stimulant as well as allergic reaction and exacerbation of asthma) should be investigated first. Therefore, the industry should use the proposed step-wise system and the general strategy described to prove that the additive in the unburnt and burnt form does not contribute to toxicity, addictiveness, the characterising flavour or to facilitating inhalation of the tobacco product concerned.

3.5.5 Fig extract

Complex mixture (figus carica l. extract)

CAS number: 90028-74-3 (any other related one if used)

CoE number: 198

Rationale for inclusion

Natural/botanical concentrates/extracts/resins (e.g. from several fruits - fig, plum, raisins, fenugreek, carob, cocoa, caramel, rum, etc.) form a large number of tobacco additives. They are poorly characterised complexes of several to hundreds of chemicals; the composition further depends upon variable factors influencing botanical source and preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of each of these additives is not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, many of the botanical extracts have a rich carbohydrate/sugar content, together with varying amounts of proteins, amino acids and other flavour compounds. The pyrolysis of this class of compounds has been well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, give rise to a complex mixture of toxic, carcinogenic and mutagenic compounds, as well as aroma/flavour compounds.

Compounds formed include smoothing agents (e.g. organic acids), flavours (e.g. caramel), facilitating nicotine delivery (e.g. aldehydes) and with CMR properties (e.g. PAHs, formaldehyde). Moreover, pyrazines are important flavour impacting compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also added as additives to cigarettes to impart flavour to low tar cigarettes (Alpert *et al.*, 2016). The complex mixtures used as additives cause tremendous harm and contribute to CMR properties, addictiveness and palatability (a key component of attractiveness) of tobacco smoke.

Priority activities

Step 1: Data on the chemical composition of the fig extract should be provided by industry with emphasis on the concentrations of constituents of relevance, production procedure, maximum levels for microorganisms and possible contaminants. The use of fruit and vegetable extract concentrates are acknowledged to be beneficial to the health. Fig extract can thus give an impression of providing a health benefit (*TPD Article 7 6 a*) to the consumer.

Step 2: It does not transfer intact to the mainstream smoke, but undergoes extensive pyrolysis. Information available so far indicates that pyrolysis products include formation of benzene, toluene; in addition the combustion of the sugars lead to the formation of carcinogenic polyaromatic hydrocarbons, a variety of aldehydes, such as acetaldehyde (irritant and possible carcinogen), acrolein (irritant), 2-furfural and a mixture of organic acids. Converging data indicate that MAO (monoamine oxidase) inhibitors contained in tobacco and tobacco smoke act synergistically with nicotine to enhance addiction potential. Smokers have reduced levels of MAO in the brain. Among MAO inhibitors, compounds resulting from sugar combustion, such as acetaldehyde and more complex aldehydes, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT_{1A} autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive. As yet, data about the role of acetaldehyde are the only ones available. They are inconclusive and further investigation about other aldehydes is needed before the role of sugars as indirectly addictive compounds can be confirmed (SCENIHR 2010 report). Therefore, any information available on these compounds should also be reported.

Step 3: In order to fill the gaps identified in Step 1 and 2, toxicity testing for inhalation exposure and then for characterizing flavour, inhalation facilitation and addictiveness for the additive in its unburnt and burnt form. Overall, the industry should use the proposed step-wise system and the general strategy described, to prove that the additive does not contribute to the tobacco product toxicity and does not fall into the four categories listed in Art. 6 of the TPD.

3.5.6 Geraniol

CAS number 106-24-1

Rational for inclusion

Geraniol is a known flavouring agent for food and is added to tobacco products for flavouring (one of the factors potentially contributing to attractiveness). More data are needed on the amount of geraniol that imparts a noticeable flavour other than tobacco. No data are available regarding addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of geraniol through cigarette smoking. Therefore, research is needed to determine the amount of geraniol in mainstream cigarette smoke. However, considering that the toxicological properties of geraniol are mainly linked to a high potential for skin sensitisation (in addition to skin and eye irritation), no levels considered safe for the majority of consumers could be established from the available data. Geraniol oxidation products (e.g. geranial, epoxy-geraniol, epoxy-geranial) are also potent sensitisers in animals. It could be expected that geraniol would be a respiratory sensitiser (although no information is available on this issue).

It is unknown if geraniol combustion products (about 10-15% of the additive) formed upon smoking a cigarette are toxic or not. Additional pyrolysis experiments are recommended.

Priority activities

Step 1: The chemical characterization of the additive is of paramount importance: indeed, geraniol can contain the relevant impurity methyleugenol, which is a genotoxic carcinogen. Only in the absence of that impurity, proven by specific certificate of analysis, could geraniol be considered as a possible candidate as a tobacco additive.

If it could be demonstrated that geraniol does not contain methyleugenol, the additive can enter the procedure for evaluation.

Step 1: The collection of the available data could be useful anyway, although as already indicated in Tobacco Opinion I, this would lead to confirming that data are available to demonstrate that pure geraniol did not induce gene mutations in *Salmonella typhimurium* and mammalian cells and although equivocal response resulted in an *in vitro* clastogenicity test, its genotoxicity can be reasonably considered eligible. In addition, after long-term studies, no carcinogenicity potential was attributed to food grade geranyl acetate (29 % citronellyl acetate and 71% geranyl acetate). Therefore those end-points are already addressed. Since the oral absorption has been demonstrated to be >80%, the systemic toxicity after inhalation (also assuming a total absorption through the lung (100%)) the effects are not expected to be different. Since

the relevant NOAEL are relatively high (558 mg geraniol/kg bw per day for rats and 279 mg geraniol/kg bw/day for mice), systemic general toxicity is not considered relevant at the doses used as tobacco additive. Therefore data are available to address these end-points. In addition the ECHA REACH registration dossier updated on 18 March 2016 reported a general population inhalation DNEL (Derived No Effect Level) of 47.8 mg/m³ for geraniol.

Step 2: Additional standardised pyrolysis experiments are recommended to identify the products formed other than geraniol, to be then evaluated for their toxicological properties plus addictiveness, characterising flavour and facilitating inhalation.

Step 3: In the case of geraniol, the local toxicity is relevant, as it is a known skin and eye irritant. Furthermore, geraniol and many of its oxidation products (by air oxidation and by metabolic transformation) have already been proven to be skin sensitisers in predictive animal tests. Indeed, geraniol is included among the fragrance substances of clinical importance known to be a prehapten as well as a prohaptens. For skin sensitisation, the SCCS considered that 0.01% could be efficient in limiting elicitation. No data are available for irritation of mucosa in the airways as well as for respiratory sensitisation, but since there is a high potential for inducing that kind of effects, also considering that the few pyrolysis studies available indicate that geraniol is mainly (85-90%) transferred intact to smoke, these should be tested first. In case results would indicate a potential for respiratory irritation sensitisation, the use of geraniol as a tobacco additive should be carefully evaluated.

Characterising flavour should be addressed first, since geraniol is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of geraniol that imparts a noticeable flavour other than tobacco. Geraniol has been demonstrated to have the property to activate cold-menthol "transient receptor potential melastatin 8" (TRPM8). TRPM8 mediates a pleasant cooling sensation in the upper airways that can mask the harsh and irritation effects of tobacco smoke. TRPM8 activation is therefore a suitable physiological mechanism to promote inhalation, especially by unexperienced smokers during the initiation and adoption stages (Behrendt *et al.*, 2004; Lübbert *et al.*, 2013).

3.5.7 Glycerol

CAS number: 56-81-5

Rational for inclusion

Glycerol is added as a humectant to tobacco (to help keep it moist). Its addition is mostly during the "casing" of the tobacco. The amount of glycerol present in cigarettes depends on the cigarette brand. The levels of glycerol added to tobacco in the EU is reported to be on average 1.1 %, with a maximum level comprising 4.5 % of the total weight.

Regarding toxicity, it was reported by the tobacco industry that the transfer rate of glycerol to mainstream smoke is 12 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that there are risks of effects on the respiratory tract epithelium from glycerol. No thorough assessment on systemic effects was done.

Pyrolysis studies indicate almost 100 % intact transfer of glycerol (Baker & Bishop, 2004; Purkis *et al.*, 2011). However, it was found that less than 0.1 % of the blend glycerol is converted to acrolein in mainstream smoke for different cigarette designs and smoking regimes tested (Yip *et al.*, 2010). Acrolein is a toxic compound that is highly reactive and causes irritation in the respiratory tract. The relationship between added glycerol and acrolein formation is unclear and further research is needed.

Regarding addictiveness, no data were reported to suggest that glycerol plays a role in smokers' addictiveness to cigarettes.

Humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Glycerol is, therefore, considered to positively influence cigarette smoking, given that humidification improves the palatability of cigarettes. Glycerol does not have a strong flavour, and is, therefore, not expected to impart a noticeable flavour.

Priority activities

Step 1: Additional data should be sought and evaluated to address the issues raised above. This should include evaluating information available on the assessment of the systemic effects of glycerol.

Step 2: Pyrolysis studies have found that a small proportion of glycerol (<0.1%) is converted to acrolein in mainstream smoke and is also generated during the combustion of many other products in tobacco. Both glycerol and acrolein cause irritation to the respiratory tract and acrolein is highly reactive. The relationship between added glycerol and acrolein formation is unclear. If no other information can be retrieved from the available data search, it will be necessary to produce new data (Step 3).

Step 3: Further data is needed to study the additive effects of glycerol or its reactivity with other compounds.

3.5.8 Guaiacol

CAS number 90-05-1

Rational for inclusion

Guaiacol is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of guaiacol that imparts a noticeable flavour other than tobacco.

Its use as a local anaesthetic can enhance smoke inhalation, thus potentially contributing to addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of guaiacol through cigarette smoking. Therefore, research is needed to determine the amount of guaiacol in mainstream cigarette smoke.

Guaiacol is a severe eye irritant, a skin irritant and also reported to be a respiratory tract irritant. Other toxicological information on repeated exposure is scant. On the basis of results on structurally related compounds, effects are likely related to the irritation potential at the contact site, generating hyperplasia. Apart from the absence of

mutagenicity tested with the Ames test, the only genotoxicity test on mammalian cells gave positive results (SCE in human lymphocytes). More data are needed for a better evaluation.

Pyrolysis experiments performed with lignin found many guaiacol derivatives besides guaiacol itself and suggest that it transfers largely intact into the smoke.

Priority activities

Since the genotoxic potential of guaiacol is of concern, this is the first issue to be clarified. The SCHEER is aware that negative results have been already published by using the *Ames test*; however, positive results were obtained with human lymphocytes: these data have to be confirmed or denied by means of results coming from appropriate *in silico/in vitro* methods (**Step 3**).

In case of positive results, guaiacol would not meet the TPD requirement (see art.7), no additional testing would be necessary, therefore the procedure can go directly to **Step 4**.

If guaiacol could be proved not to have genotoxic properties, the additive can enter the step-wise procedure for evaluation, starting with:

Step 1: evaluation of available data on the additive in unburnt form

Step 2: Pyrolysis products should be considered

If there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: Guaiacol is reported to be a respiratory tract irritant: this property should be specifically addressed as a priority. In addition, other toxicological end-point and properties as local anaesthetic, potentially contributing to addictiveness, should be also investigated, together with characterising flavour and inhalation facilitation.

3.5.9 Guar gum

Synonyms: Guaran, Guar Flour, Jaguar

CAS number: 900-30-0 (Guar depolymerised **CAS number:** 68411-94-9) and others

Rational for inclusion

Guar gum is an extract of the seeds of the guar bean plant. Guar gum consists of high molecular weight polysaccharides and some amount of protein. Reconstituted tobacco is made up of mashed tobacco stems and other parts of the tobacco leaf that would otherwise be discarded. Guar gum (and its derivatives) is added to reconstituted tobacco in cigarettes. Guar gum is also used to prepare the cigarette paper that wraps the tobacco.

The amount of guar gum added to bind the tobacco can make up between 0.6-1.8 % of the total weight of the tobacco used in one cigarette. Guar gum is generally regarded as safe for use in food and cosmetics. However, guar gum does not transfer intact to the mainstream smoke, but undergoes pyrolysis, giving rise to toxic/carcinogenic

(e.g. formaldehyde, benzo(a)pyrene and benzene) compounds. Irritating and toxic fumes, gases and acrid smoke can be formed when the additive is heated to decomposition.

Regarding flavours, it is well known that the thermal degradation of sugars and carbohydrates at lower temperatures as in foods contribute to complex aromas. Several flavour compounds were reported due to pyrolysis reactions of guar gum. These flavour compounds singly or in combination with the thousands of other smoke constituents can act synergistically and contribute to the attractiveness of smoking by improving smoke flavour, thereby masking its bitter taste, reducing the harshness of smoking, creating sensory cues, which all could contribute to the optimisation of nicotine dosing and enhance abuse potential.

Guar gum is hazardous when heated to decomposition, emitting acrid smoke and irritating fumes. Although some information on the effect of pyrolysis is available from the internal industry documents, further chemically defining this additive from the point of view as a tobacco additive and its pyrolysis products would help confirm/facilitate the assessment of the influence on the carob bean extract on toxicity/carcinogenicity, palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals.

Priority activities

Step 1: Data on the chemical composition of the guar gum should be provided by industry with emphasis on the concentrations of constituents of relevance.

Step 2: Some information on the effect of pyrolysis of guar gum is available, however, it is necessary to:

- further chemically define its pyrolysis products and
- evaluate the CMR and other toxicological properties of its pyrolysis products.

In case of unequivocally positive results for genotoxicity/carcinogenicity of its pyrolysis products the use of guar gum as a tobacco additive should be carefully evaluated. Additional testing would be required for a WoE assessment in case of uncertainties.

Step 3: If CMR properties are not proven by available data, the additive can enter the step-wise procedure for evaluation, analysing data other than those related to CMR properties. The assessment of its pyrolysis product on palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals should be analysed.

3.5.10 Liquorice

CAS numbers: 1405-86-3 (α -D-Glucopyranosiduronic acid), 103000-77-7 (β -D-Glucopyranosiduronic acid)

Rational for inclusion

Liquorice is a natural extract of the root of the liquorice (*Glycyrrhiza glabra*) plant – logically an incompletely defined complex mixture of compounds. More than 400 compounds were isolated from *Glycyrrhiza* species. Liquorice extracts are used to

improve the organoleptic properties of tobacco smoke, making the harsh cigarette smoke palatable, thereby facilitating inhalation and resulting in characterising flavour. The taste and flavour of tobacco with added liquorice/licuorice root are described as sweet, woody and round. The major active principle of liquorice is the sweet tasting triterpene glycoside glycyrrhizin.

Glycyrrhizin has been hypothesised as a bronchodilator, although not confirmed. It is not clear whether the levels present are sufficient for this effect, although a synergistic effect with other compounds in cigarette smoke maybe expected.

It is expected to pyrolyse extensively, but there is a lack of information on the pyrolysis products formed, which would help facilitate the assessment of the influence on toxicity/carcinogenicity. Additionally, the effect of liquorice on bronchodilation, alone or in combination with other additives and/or tobacco constituents, needs to be ascertained to better understand its effect on the ease of inhalation of nicotine and other alkaloids, thereby potentiating addictiveness.

Priority activities

The potential genotoxic effects of liquorice extract have been postulated but this is controversial: glycyrrhizinic acid and its derivatives have previously been reported to give both positive and negative results in mutagenicity tests.

Starting with **Step 1** and if necessary continuing to **Step 3**, this end-point should be addressed first to identify alert for the genotoxicity and carcinogenicity of the additive.

If results are negative, the additive could enter the procedure.

Step 1: Evaluation of available data on properties other than genotoxicity/carcinogenicity for the additive in its unburnt form

Step 2: Additional standardised pyrolysis experiments are recommended to identify the products formed during the combustion process of liquorice, to be then evaluated for their CMR properties

Step 3: All the testing regarding general systemic toxicity in chronic inhalation exposure and the one regarding characterising flavour should be addressed; since liquorice can mask the undesirable characteristics of tobacco smoke and acts as a bronchodilator, the possibility for facilitating nicotine uptake should be analysed. More data are needed on the amount of liquorice and glycyrrhizic acid that imparts a noticeable flavour other than tobacco. No data are available regarding addictiveness.

The evaluation of the effects of long-term inhalation exposure to liquorice with different content of glycyrrhizic acid should consider that both renal and hepatic 11-beta-hydroxysteroid dehydrogenase (converts cortisol to cortisone) as well as hepatic delta-4-5-beta-steroid-reductase (inactivates glucocorticoids and mineralocorticoids) are inhibited by glycyrrhetic acid, which can lead to pseudohyperaldosteronism and elevated blood pressure. The flavonoids licochalcone A and B inhibit the elevation of calcium ions induced by thrombin, in a dose-dependent manner. They also inhibit thrombin-induced platelet aggregation *in vitro*. Licochalcone A and B were tested with human neutrophils and were found to inhibit the formation of leukotrienes B1 and C4,

cyto B-induced lysosomal enzyme, platelet activating factor, n-formyl-methionyl-leucyl-phenylalanine and calcium ionophore A.

Hypokalemia, hypernatremia, and water retention are primary problems associated with chronic liquorice ingestion. Changes in the sodium/potassium ratios may result in pH changes. Cardiomyopathy, pulmonary edema, myoglobinuria, ptosis, myopathy, tetany, cramping, seizures, and rhabdomyolysis have also been reported in patients following chronic, excessive liquorice ingestion. Only if it could be demonstrated that chronic liquorice inhalation with tobacco smoke has no systemic effects could the additive enter the step-wise procedure for evaluation. The safety evaluation of glycyrrhizic acid should be based on the data from humans. Since the oral absorption has been demonstrated to be high, the systemic toxicity after inhalation (also assuming a total absorption through the lung (100%)) the effects are not expected to be different. Since the relevant NOAEL is relatively high (2 mg glycyrrhizic acid / kg bw per day for healthy volunteers) and the blood serum half-life is 5 hours, the risk of systemic general toxicity may not be high at the doses used as tobacco additive.

3.5.11 Maltol

CAS Number: 118-71-8

Synonyms: 3-Hydroxy-2-methyl-4-pyrone, 3-Hydroxy-2-methyl-4H-pyran-4-one, Palatone, Larixinic acid, Talmon.

Rational for inclusion

Following the EFSA report on maltol (FGE19 and FGE213 and FGE213 rev 1 - EFSA, 2008, 2009 and 2014), the concern for genotoxicity could not be excluded. Therefore, maltol will be on the priority list until data on its genotoxicity are clarified. In addition, possible effects on the CNS must be clarified.

Priority activities

The main open questions concerning maltol are therefore uncertainty concerning the genotoxicity.

If the existing information (**Step 1**) does not clarify the uncertainties, some additional testing should be undertaken based on the OECD TG (**Step 3**).

If maltol is proven not to be genotoxic, the additive can enter the step-wise procedure for evaluation.

Step1: Evaluation of available data on the additive in unburnt form

Step 2: Pyrolysis products should be considered

In case there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: Since an inhibition of the response of the GABAA receptors in the presence of maltol has been reported which may contribute to CNS stimulation/addictiveness, these are the priority effects that should be investigated in Step 3, where all the other end-points should be considered.

In addition to the above, the following issues were identified:

Maltol and other hydroxyl cyclohexanone derivatives (such as ethyl maltol) are used to augment or enhance the taste of consumable materials, with a typical odour of cotton candy and caramel. No information on minimum levels of odour awareness was found. Reports on the health effect of maltol also underline its potential anti-apoptotic effect. It is unclear whether the anti-apoptotic is specific directed to healthy cells, neoplastic cells and/or cells undergoing mutations.

3.5.12 Menthol

CAS numbers: l-Menthol: 2216-51-5; D-Menthol: 15356-70-4; D/L Menthol: 89-78-1; Menthol: 1490-04-6

Rational for inclusion

Menthol is one of the most commonly used tobacco additives worldwide. It is a monocyclic terpene alcohol that is used primarily for its chemosensory effects of creating perceptions of a cooling minty taste and smell. Menthol is added at a continuum of concentrations, from imperceptible amounts to levels imparting different levels of a characterising flavour.

In addition, several additives and formulations are used to simulate menthol effects. Menthol induces anaesthetic and sensory effects, facilitates deeper inhalation and adds to the impact of nicotine.

Menthol is a multifunctional additive. It is an effective anaesthetic, antitussive agent that may increase the sensation of airflow and inhibit respiratory rate, thereby allowing increased lung exposure to nicotine, tar and toxic constituents, while masking reactions like coughing or other early warning signs of respiratory disease. It may increase the absorption and lung permeability of smoke constituents, thereby increasing nicotine and carcinogen uptake. It may also decrease nicotine/cotinine metabolism leading to higher doses of nicotine. It is one of the additives that was originally added to create the impression that a tobacco product has health benefits and/or reduced health risks. It affects multiple sensations including taste, aroma and tactile smoothness, and enhances abuse liability. Its pharmacological actions reduce the harshness of smoke and the irritation from nicotine, and may increase the likelihood of nicotine addiction in adolescents and young adults who experiment with smoking and it may make it more difficult to quit.

In 2011, the FDA Tobacco Products Scientific Advisory Committee (TPSAC, 2011) concluded that menthol 1) impacts youth initiation, 2) contributes to adults continuing to smoke, and 3) has an adverse impact on public health by increasing the numbers of smokers with resulting premature death and avoidable morbidity. Finally, they concluded that the "removal of menthol cigarettes from the marketplace would benefit public health in the United States" (TPSAC, 2011; FDA, 2011).

Independently, the US Food and Drug Administration undertook a thorough review and concluded that the data suggested that menthol use is likely associated with increased smoking initiation by youth and young adults, greater addiction, greater signs of nicotine dependence and less likelihood of successfully quitting smoking. These findings, combined with the evidence indicating that menthol's cooling and anaesthetic properties

may reduce the harshness of cigarette smoke and the evidence indicating that menthol cigarettes are marketed as a smoother alternative to non-menthol cigarettes, make it likely that menthol cigarettes pose a public health risk above that seen with cigarettes without menthol (FDA, 2013). The review concluded that although there is little evidence that menthol cigarettes *per se* are more toxic than menthol-free cigarettes, adequate data indicate that menthol presence is associated with increased smoking initiation and greater addiction, especially among young people, as confirmed later by the studies of Nonnemaker *et al.* (2013) and Brennan *et al.* (2015).

Indeed, smokers usually using menthol cigarettes develop greater nicotine dependence, which is likely associated to the anaesthetic properties that reduce the harshness of smoke. In addition, menthol cigarette smokers are less successful quitting smoking (Smith *et al.*, 2014). Recent perception studies confirm earlier work showing that smokers, especially young adults, perceive menthol cigarettes as less harmful (Brennan *et al.*, 2015; Wackowski and Delnevo, 2016).

With regard to toxicity, Noriyasu *et al.* (2013) exposed cell cultures to menthol and non-menthol smoke and found that cell death was significantly enhanced by mentholated smoke, whereas menthol alone was inert. This suggests a synergistic effect with other smoke-compounds and requires further study. A recent study conducted in mice showed that menthol at low concentration strongly suppressed respiratory irritation due to acrolein and cyclohexane, which are smoke irritants in naïve mice. Additionally, menthol suppressed irritation by tobacco smoke in mice. Menthol increased blood cotinine levels, which is a biomarker of nicotine uptake. Thus, menthol appears to suppress smoke-induced irritation, making it easier to inhale smoke and increasing the dosage of nicotine. Due to the similarities in menthol's pharmacology in humans, experiments in animal models suggest that beginning smokers likely prefer menthol-containing cigarettes because their respiratory tract is less irritated. At the same time, these smokers are exposed to higher levels of nicotine and become addicted faster and are less likely to quit smoking (Ha *et al.*, 2015).

At lower application levels, menthol can be used to increase smoothness and reduce harshness in cigarette smoke. This is likely the main reason for use of menthol as an additive, also in "non" menthol brands. Therefore, research to ascertain the physiological and pharmacological impact of low menthol and its interaction with other chemicals, interaction with nicotine, on palatability and inhalation of smoke/nicotine, etc. is recommended.

Baker and Bishop (2004) and Jenkins (1970) reported that after pyrolysis of menthol, it is transferred intact into smoke (99%). Smoking studies resulted in intact transfer of around 98-99%. There was some formation of menthone, menthene and menthane. In contrast, in earlier pyrolysis experiments (Schmeltz and Schlotzhauer, 1968) 84% of the menthol was pyrolysed and phenol and benzo[a]pyrene were found in the pyrolysate.

Priority activities

Step 1: Additional data should be sought and evaluated to address the above mentioned questions.

Step 2: Based on the available studies, pyrolysis of menthol may result in carcinogenic substances (concern category d). There is uncertainty with regard to the nature of the

pyrolysis products. Pyrolysis studies should be carried out to identify these products and the products should be evaluated.

Step 3: Based on the available data, menthol is concluded to impart a characterising flavour if added in sufficient amounts, facilitate inhalation and addictiveness of tobacco products and increase inhalation and nicotine uptake (concern categories A and C). Uncertainty is low. Further testing to show whether menthol is or is not addictive or does or does not increase attractiveness is not recommended in view of the strength of the data.

Further studies are needed into the suggested synergistic effect of menthol with other smoke compounds. Research to ascertain the physiological and pharmacological impact of low menthol and its interaction with other chemicals, interaction with nicotine, on palatability and inhalation of smoke/nicotine, etc. is recommended.

3.5.13 Propylene glycol

CAS number: 57-55-6

Rational for inclusion

Propylene glycol (PG) is added as humectant to tobacco, rather frequently and in relatively high amounts (1599 counts in NL ingredient lists, 23 in NTM, total number of brand 4265), average (weight %) 1.579 (1.636).

Regarding inhalation facilitation, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Internal tobacco industry documents reported that adding 3-7 weight percent of PG increased the mildness and reduced irritation (although this is higher than amounts typically present in tobacco cigarettes). Propylene glycol is, therefore, considered to positively influence the attractiveness of cigarette smoking given that humidification improves palatability of expected to impart a noticeable flavour.

Regarding addictiveness, no data were reported to suggest that propylene glycol plays a role in smokers' addictiveness to cigarettes.

Regarding toxicity, it was reported by tobacco industry that the transfer rate of propylene glycol to mainstream smoke is 10 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that risks of effects on the respiratory tract epithelium from propylene glycol exist. No thorough assessment on systemic effects was made.

Propylene oxide is regarded as possibly carcinogenic to humans and trace amounts are present in propylene glycol. Additionally, pyrolysis of propylene glycol results in formation of small amounts (<10 %) of 1,3-propylene glycol, acetol or acetic anhydride, and pyruvaldehyde.

Finally and importantly, propylene glycol and/or its combustion products is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

Priority activities

Step 1: Since propylene oxide, which is regarded as possibly carcinogenic to humans (IARC Group 2B carcinogen), is found in trace amounts in industrially-produced propylene glycol, the specification should be provided.

Data available should be collected to prove or disprove whether propylene glycol increases the risks of effects on the respiratory tract epithelium.

Step 2: Pyrolysis products should be better characterised also considering that Propylene oxide has been reported to be generated during cigarette smoking.

Step 3: The effect of propylene glycol on inhalation facilitation to cigarettes at levels found in European cigarettes (range of 0.2 to 2.4%) warrants investigation. The additive effects of propylene glycol and/or its reactivity with other compounds should be further investigated.

No thorough assessment of the systemic effects of propylene glycol has been done, so this should be looked at further.

3.5.14 Sorbitol

CAS number: 50-70-4

Rational for inclusion

Sorbitol is added as a humectant to tobacco (210 times in NL ingredient lists, 30 in NTM, total no of brands 4265), average (weight %) 0.232 (0.458).

Regarding inhalation facilitation, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Sorbitol is, therefore, considered to positively influence attractiveness of cigarette smoking given that humidification improves palatability of cigarettes. Sorbitol gives tobacco smoke a slightly bitter taste and a vague odour of cellulose and is, therefore, not expected to impart a noticeable attractive flavour when used in higher amounts.

Regarding addictiveness, no data were reported to suggest that sorbitol plays a role in smokers' addictiveness to cigarettes. However, its combustion products, such as acetaldehyde and formaldehyde, were proposed to increase the addictive effect of nicotine, although data on acetaldehyde produced by pyrolysis entering the brain through the smoke inhaled are inconclusive (SCENIHR 2010).

Regarding toxicity, sorbitol was reported to pyrolyse at 900°C to compounds, such as 2-furfural (31.4 %, see section on furfural), acetaldehyde (irritant and possible human carcinogen), formaldehyde (irritant, carcinogen). Other pyrolysis products of sorbitol include furan, 2-methyltetrahydrofuran, propionaldehyde, acetone, methanol, and carbon monoxide (Baker and Bishop, 2004). Further research is needed to confirm these effects, especially if sorbitol pyrolysis results in carcinogenic compounds.

Finally, it must be borne in mind that sorbitol (and/or its combustion products) is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

Priority activities

Step 2: The main concern for sorbitol to be addressed, before it can enter the evaluation procedure, is the formation of toxic pyrolysis and CMR products. Pyrolysis experiments must be carried out using conditions relevant for cigarette smoking (see section 3.4.1). In case of positive results, sorbitol as a tobacco additive would fall in one of the four categories listed in art. 6 of the TPD and its use as an additive in tobacco product should be carefully evaluated.

If it can be demonstrated that no toxic or carcinogenic pyrolysis products are formed, the additive can enter the step-wise procedure for evaluation.

Step1: Evaluation of available data on the additive in unburnt form

If there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: In particular, it needs to be assessed whether it increases inhalation facilitation of cigarette smoking. It is not expected that sorbitol will give a characterising flavour but data regarding addictiveness should be produced.

3.5.15 Titanium Dioxide

CAS numbers: 13463-67-7 (mixture of mainly rutile and anatase); 1317-80-2 (rutile); 1317-70-0 (anatase)

Rational for inclusion

The SCCS evaluated its use as cosmetic ingredient (sunscreen). With regard to inhalation toxicity, it was concluded that in subacute repeated dose inhalation toxicity studies, nano-size TiO₂ induce an acute inflammation in the lungs, that may be reversible depending on the dose and the time after exposure. In view of this, acute inflammation (spray) applications, which may result in inhalation exposure, were not recommended by the SCCS. Titanium dioxide was classified by IARC as a Group 2B carcinogen (i.e. possibly carcinogenic to humans) (IARC, 2010).

To perform a risk evaluation, it is necessary to know the exposure level of titanium dioxide through cigarette smoking. Therefore, research is needed to determine the amount of titanium dioxide in mainstream cigarette smoke. Because inhalation toxicity is also related to the size of the particles, a distinction needs to be made between nano and non-nano size.

Priority activities

Step1: Evaluation of available data on the additive in unburnt form

Step 2: Not applicable because titanium is already in its highest oxidised state

Step 3: In subacute repeated dose inhalation toxicity studies, nano-size TiO₂ induces an acute inflammation in the lungs. Based on the available data, titanium dioxide is classified as a IARC Group 2B carcinogen (concern category d).

Within the scope of the EU CLP Regulation, a proposal for harmonised classification of TiO₂ was submitted (ECHA, CLH report, Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2,

Substance Name: Titanium dioxide, Version 2, May 2016). It is proposed to reclassify TiO₂ (all forms) specifically by inhalation as Carcinogen Cat 1B. It was concluded that no carcinogenic concern was reported by both oral and dermal routes but that there is sufficient evidence of carcinogenicity in experimental animals after inhalation. Indeed, a causal relationship has been established between TiO₂ and the increase of malignant lung tumours in female rats and benign lung tumours in males and female rats in 2 inhalation and 2 instillation studies. Human data do not suggest an association between occupational exposure to TiO₂ and risk for cancer. However, all these studies have methodological limitations and the level of exposure reported is debatable. Although the full mode of action is still unclear, an inflammatory process and indirect genotoxic effect through ROS production seems to be the major mechanism to explain the effects induced by TiO₂. It is considered that this mode of action is principally due to the biopersistence and poor solubility of the TiO₂ particles.

Evidence may be provided to address the uncertainties with regard to the genotoxicity and carcinogenicity of TiO₂ by inhalation for humans.

4 OPINION

As requested by Article 6 of the Tobacco Products Directive 2014/40/EU (TPD), the Commission has established a priority list of 15 additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations that is to be updated on a regular basis. SCENIHR Tobacco Opinion 1, provided the scientific basis for the establishment of such priority list.

The TPD prescribes that Member States shall require manufacturers and importers of tobacco products to carry out comprehensive studies on these additives. The SCHEER was requested to provide guidance on the type and criteria for the comprehensive studies, and the most suitable methodologies to be used for the first list of 15 priority additives, as well as for additives on future updated lists.

In the first part of the current Opinion, the SCHEER proposed a step-wise strategy (Section 3.4), as the most pragmatic and efficient way to proceed in the assessment of the toxic and addictive effects as well as characterising flavour properties, and inhalation facilitation as factors potentially contributing to attractiveness, of additives used in cigarettes and roll-your-own tobacco. The tiered approach proposed by DKFZ (DKFZ, 2010) was used as a starting point, but the SCHEER developed its own approach, also including the evaluation of addictive effects, characterising flavour properties and the inhalation facilitation of additives. The proposed strategy ensures that testing is minimised. For this reason the application of grouping different additives with similar chemical structures and of the read-across principle are endorsed.

First, the chemical specification of the additive has to be available (Step 1). Then collection/evaluation of the available open and grey literature needs to be carried out by applying a WoE approach, for the additive in its unburnt form (Step 1) and its pyrolysis products (Step 2). If no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions (Step 2). Here, it needs to be noted that no validated methods are available for the pyrolysis of tobacco additives.

In case data retrieved in Step 1 and 2 are not sufficient or robust enough to make the evaluation possible, non-testing methods such as QSAR and read across are proposed, followed by *in vitro* approaches addressing the different endpoints to be considered (Step 3). Regarding types of effects, unless the previous step highlighted some concern for a specific end-point, CMR properties and toxicity are assessed first, as accepted methods and evaluation frameworks are available, followed by characterising flavour, because procedures are available for the assessment of these end-points. Next, addictiveness is assessed, an effect for which no validated tests are currently available, although some mechanisms underlying addictiveness are known. It is strongly advised that *in silico* and *in vitro* test to assess additive-induced addictiveness are developed and validated by independent organisations in the near future.

In line with the provisions of other regulatory contexts, the SCHEER recommends the use of a Quality system (e.g. Good Laboratory Practice or ISO17025) for carrying out the pyrolysis or other physico-chemical studies as well as pre-clinical studies. Human studies should follow the appropriate Quality System.

The issue related to interaction of the additive with other additives/ingredients is also considered. The industry is asked to provide all known information on the interaction of

additives and their pyrolysis products leading to the intended formation of flavours / pH modifiers/ smoothing agents and other important compounds.

In addition to proposing specific steps and tests to be considered by industry, some general criteria were also identified. A pre-ambule here is that additives in tobacco products have no health benefits for the consumer, but rather may promote the use of and addiction to an extremely toxic product. Therefore, a risk-benefit analysis is not the appropriate paradigm for assessing additives.

When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies performed in Step 3, it is a SCHEER recommendation that the assessors consider the worst-case evaluation.

In order to provide a relevant outcome to the question whether an additive contributes to the toxicity, characterising flavour, facilitating inhalation or addictiveness of the tobacco product, the study design must adhere to some methodological criteria. Most importantly, the test outcomes should be relevant for tobacco smoking. This implies that they should be related to actual human exposure and tobacco-induced diseases, and be relevant not only for acute or subchronic, but also for chronic exposure in intermittent use sessions (Johnson *et al.*, 2009).

Furthermore, comparative toxicity testing strategies, where differences in effect of the tobacco product with and without the additive are evaluated, are not considered suitable to address the properties outlined in the Terms of Reference with the currently available methodology. Indeed, due to the high intrinsic toxicity of tobacco products, it is challenging to demonstrate any differences, whether they are increases or decreases, induced by an additive with the currently available tests and methodologies (Kienhuis *et al.*, 2016). Very sensitive tests would be required, with a clear dose-response relationship, in order to show any differences from these high background effects, which can associate assay outcomes to human risk and exposure. In this respect, *in vitro* tests combined with toxicogenomics, using biomarkers of exposure and disease, are the most promising (Kienhuis *et al.*, 2016). However, for the time being, no standardised methods have been validated for this purpose.

As adequately sensitive tests are not currently available, no new comparative studies (tobacco product with and without additives) using the available methodologies of toxicity testing will be considered for the moment, since these studies generally lack discriminative power.

Here, the effects of the pure additive, and its pyrolysis products, are considered in a relevant testing strategy, such as the tiered approach proposed by the SCHEER (Section 3.4) in order to evaluate their contribution to tobacco product toxicity. Studies that are already available could be presented, but the relevance of their results has to be evaluated in a WoE approach based on these considerations.

Comparative studies are also not endorsed for studying the effect of additives on addictiveness, for the same reasons. In human studies, there are two exceptions to this general rule: characterising flavour testing, and facilitating inhalation or nicotine uptake. The former is, by definition, a comparative testing paradigm, as clearly noticeable

flavour is a property of a tobacco product, caused by the addition of flavour additives, and not a property of an additive itself. The latter is related to additives that facilitate inhalation or nicotine uptake, and when large groups of subjects are enrolled, effects on e.g. sensory perception, smoking topography and biomarkers of exposure (e.g. nicotine in blood or cotinine in urine) can be discriminated.

Another problem with comparative testing is that the outcomes would only apply to that specific tobacco test product. The results (e.g. related to toxicity or addictiveness) cannot be generalised to all products and brands, having a different composition with respect to tobacco type, blend and additives. Therefore the obtained results may not lead to general prohibition/acceptance of specific additives but rather to prohibition/acceptance on a product-by-product basis (DKFZ, 2010).

It is ethically questionable to carry out any new animal studies to evaluate the contribution of an additive to the tobacco product toxicity, addictiveness, characterising flavour, and properties that facilitate inhalation or nicotine uptake. Similarly to most cosmetics, it is not necessary to use tobacco products. Moreover, apart from being unnecessary, tobacco products are highly harmful with no benefits to individual or public health. As additives are used for product improvement, often contributing to detrimental effects for the consumers (e.g. addictiveness), there is no health benefit in using tobacco additives. For the hazard assessment of tobacco additives, *in silico* and *in vitro* methods exist. QSAR methodology has been successfully used for decades for predicting toxicological and pharmacological properties of chemicals. The same applies to *in vitro* methods, which were validated, adopted as official protocols (e.g. OECD Test Guidelines) and accepted, also for regulatory purposes. Additionally, many promising *in vitro* methods are currently being developed to assess different adverse outcomes.

Therefore, as a principle, only the use of *in silico*, and *in vitro* methods will be considered suitable for new studies, following the EU policy to ban animal studies for chemicals to be used in voluntary products such as cosmetics (EU Regulation no. 1223/2009). Only already available data obtained with animal models will be evaluated. Human studies are generally discouraged. These may be used (e.g. in case of flavour assessment), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products.

The data gaps already identified in Tobacco Opinion 1 for the 15 additive included in the EU Commission priority list have been analysed (Section 3.5). Based on the data gaps described in the 'Rationale for inclusion' taken from Opinion 1, the activities to be performed upfront have been described, indicating the most appropriate steps (and end-points) to be carried out and then used for the evaluation, in order to speed up the process making possible testing feasible in the 18-month timeframe.

In general, data gaps for the 15 priority additives are information on addictiveness and characterising flavour or inhalation facilitation, as well as on the identity of the pyrolysis products. In the past, major emphasis was put on toxicity, whereas limited research was carried out on addictiveness (and even less on attractiveness). Regarding toxicity, data were often taken from the food sector, where pyrolysis and inhalation are not an issue.

In conclusion, this Opinion provides general guidance to tobacco industry to conduct studies and prepare reports on the evaluation of Tobacco additives to be sent to the competent authorities. To this purpose, a reporting template is provided as well (see

Annex I). In addition, specific advice is given for priority testing activities to fill the data gaps recognised in Tobacco Additives Opinion 1. In order to limit the testing and administrative burden, the formation of consortia and joint reports by industry is endorsed.

It needs to be noted that there is a lack of validated methods for the pyrolysis of tobacco additives. For the assessment of many toxicological end-points animal-free methodologies are still lacking, especially when the inhalation route is concerned. Similarly, no addictiveness and attractiveness tests (apart from a procedure for characterising flavours) are available, a knowledge gap already noted by the SCHENIHR in 2010 in its report 'Addictiveness and attractiveness of tobacco additives', and the situation has not significantly improved since then. It is advised that independent bodies or organisations conduct relevant research in these fields.

5 MINORITY OPINION

None.

6 CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the Scientific Committees from 22 July to 22 September 2016. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

Twenty-two organisations and individuals participated in the public consultation, providing input to different parts of the Opinion, resulting in 214 contributions and nearly 1000 comments.

Most comments, by far, were from the tobacco industry, which disagreed with some aspects they considered too demanding to implement; on the contrary, other organisations and individual researchers expressed their appreciation to the SCHEER, recognising the difficulties in fulfilling such a complex mandate and positively commenting on the rationale followed by the SCHEER and on the indication that no animal testing should be performed *ex novo*.

The most frequent comments were related to comparative testing, which was not endorsed by the SCHEER in the preliminary Opinion. The claim was that this is the only way to answer to TPD Art 6 (6), according to which TI should assess whether a given additive results in a significant or measureable increase in toxicity, addictiveness or CMR. Similar criticisms were directed toward the indication in the preliminary Opinion to carry out a pyrolysis study instead of smoke chemistry.

Tobacco industry also repeatedly criticised the preliminary Opinion for supposedly going beyond the Terms of References by examining properties such as 'attractiveness' as well as asking for the application of the precautionary principle.

The SCHEER provided an individual reply to each contributor. Each submission was carefully considered by the SCHEER and the preliminary Opinion has been revised in response to relevant comments.

More precisely, in the Final Opinion SCHEER clarified its position about comparative testing, specifying when it can be considered appropriate (e.g. in some human studies), but stating that at present, methodologies are not yet sensitive enough to discriminate between the very high background toxicity associated with tobacco with and without the additive. The SCHEER concludes that testing the effects of inhaling the pure additive and its pyrolysis products is the only meaningful way to comply with art. 6(2) of the TPD, i.e. to assess whether additives contribute to the toxicity or addictiveness of the products concerned.

For consistency with the terms of reference, the wording in the Final Opinion has been aligned with the TPD, avoiding the use of the term 'attractiveness' and replacing it with properties such as characterising flavour, facilitating inhalation and nicotine uptake, which fall under the TPD.

The SCHEER agreed that the reader might associate the precautionary principle with a risk measurement measure, although that was not the SCHEER's intention. For that reason, any reference to the precautionary principle has been deleted and indications

were given for the assessor on the evaluation of the collected available and new data on the additive and its pyrolysis products in case uncertainties could not be clarified by new testing.

The many papers provided by the tobacco industry were checked and the literature has been accordingly updated with relevant publications. However, in most cases they were not considered to have provided any additional information or any information relevant enough to require amending the Opinion.

In the final Opinion, some changes were included to address specific comments and editorial changes were made to address comments pointing out possible misunderstandings.

The SCHEER would like to thank all contributors for their comments and for the references provided during the public consultation.

The text of the comments received and the response provided by the SCHEER are available here:

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scheer_consultation_01_en

7 ABBREVIATIONS AND GLOSSARY OF TERMS

AOP	Adverse outcome pathway
ASL	Arterial Spin Labelling
CAS	Chemical Abstracts Service
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, mutagenic or toxic for reproduction
CNS	Central nervous system
COC	Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
COM	Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CVD	Cardiovascular disease
CYP	Cytochrome P450 monooxygenase
DA	Dopamine
DKFZ	Deutsches Krebsforschungszentrum (German Cancer Research Center)
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	(US) Food and Drug Administration
FEMA	(US) Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
fMRI	functional magnetic resonance imaging
FTND	Fagerström Test for Nicotine Dependence
GABA	Gamma (γ)-Aminobutyric acid
GABA	γ -aminobutyric acid
GLP	good laboratory practice
IARC	International Agency for Research on Cancer
IC50	The half-maximal inhibitory concentration
IPCS	(WHO) International Programme on Chemical Safety

JECFA	Joint FAO/WHO Expert Committee on Food Additives
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	(EU) Joint Research Centre
MoE	Mode of Exposure
MRI	Magnetic resonance imaging
nACh	nicotinic acetylcholine
NOAEL	No observed adverse effect level
NRC	(US) National Research Council
NTM	Non-tobacco material
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PAH	Polycyclic aromatic hydrocarbon
PBPK	Physiologically based pharmacokinetic
PET	Positron emission tomography
PG	Propylene glycol
pH	Measure of acidity or basicity of a solution
PITOC	EU project "Public Information Tobacco Control"
ppm	Parts per million
QSAR	Quantitative structure--activity relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The Netherlands National Institute for Public Health and the Environment)
RYO	Roll-your-own (cigarettes)
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental and Emerging Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCOEL	Scientific Committee on Occupational Exposure Limits
SPECT	Single-photon emission computed tomography
TG	(OECD) Test Guidelines
TPD	Tobacco products directive
UK	United Kingdom

US(A)	United States (of America)
WHO	World Health Organization
WoE	Weight of evidence

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9 Annex I

GUIDANCE AND TEMPLATE

A. GENERAL ISSUES

This brief guidance intends to support the understanding of “what was done”. Authors should take responsibility to be clear of the definitions and provide proper citations for any terms/data they use.

1. TITLE PAGE

The title page should contain the following information:

Identification of the additive

Abstract and keywords, if applicable

Name of sponsor (and bodies that fund or commission the analysis)

Name and affiliation of person or persons responsible for producing and signing off the report

Date and version of report.

2. SUMMARY

The summary is intended to provide a concise description of the key elements.

3. REPORTING SOURCES OF INFORMATION

This section should describe any data source or sources that were used (e.g. existing data and/or databases, *in silico* techniques/models used, experimental studies).

This section addresses the key features of the design.

The rationale for the overall study design should be documented

If needed also ethical approval (approval number – approved by ...- date) for *in vivo* experiments (animals or humans) should be given.

4. REPORTING DATA QUALITY / DATA COLLECTION QUALITY ASSURANCE

This section addresses the reporting of the elements of data collection and pre-processing that could influence data quality.

- How was the literature search conducted and which quality controls were in place and used
- In own experimental studies, were quality controls in place:

In silico

In vitro

In vivo

5. PUBLIC SUMMARY OF THE DOSSIER

The target group of a public summary is the non-professional public. The structure and content of the public summary shall be elaborated accordingly. The document should be less extensive compared to the *summary*. A scientific/professional terminology shall be avoided if possible.

6. CD/DVD

The applicant shall submit a dossier with the full information on standard electronic media such as CD ROMs or DVDs. Two or three CD ROMs or DVDs shall be submitted.

Common electronic formats should be used (e.g. MS Office, Adobe Acrobat Reader) allowing content copying and printing (no content copy protection). The text of the files should be searchable using the search facilities of standard software packages. The CD or DVD shall be structured in folders that reflect the structure of the submission.

Also a full paper copy of the dossier is requested, it has to be declared by the applicant on a separate sheet or in the accompanying letter that the electronic and the paper versions are identical.

7. LIST OF PARTS OF THE DOSSIER REQUESTED TO BE TREATED AS CONFIDENTIAL

Applicants have the right to request a confidential treatment of certain information. They shall indicate which sections and data they wish to be treated as confidential (and give verifiable justification for each part for which a confidential treatment is required).

Furthermore, the applicant shall provide the Commission with two electronic versions of the dossier, namely the complete dossier and a second version of the complete dossier without confidential information.

B. SPECIFIC ISSUES – GENERAL LAYOUT

1. CHEMICAL AND PHYSICAL SPECIFICATIONS OF ADDITIVE

A. Chemical identity

Primary name and/or INCI name

Chemical names

Trade names and abbreviations

CAS / EC number

Structural formula

Empirical formula

B. Physical form

Molecular weight

Purity, composition and substance codes

Impurities / accompanying contaminants

Solubility

Partition coefficient (Log Pow)

Additional physical and chemical specifications

Where relevant:

- organoleptic properties (colour, odour, taste if relevant)

- vapour pressure (include also temperature used for the assessment)

- pKa

- ...

C. Function and use of the additive

2. (Each identified) PYROLYSIS PRODUCTS

Chemical identity

Molecular weight

% formed (at specific temperature)

Solubility

Partition coefficient (Log Pow)

Additional physical and chemical specifications

Where relevant:

- organoleptic properties (colour, odour, taste if relevant)

- vapour pressure (include also temperature used for the assessment)

- density

-- pKa

- ...

3. TOXICOLOGICAL EVALUATION

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original (own) study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report a study summary should be submitted, including, if applicable, the following (the summary reports should usually only be 1 page long):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one toxicological endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the Toxicology section, a brief general conclusion should be formulated.

4. ADDICTIVENESS ASSESSMENT

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original (own) study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report a study summary should be submitted, including, if applicable, the following (the summary reports should not usually exceed 1 page):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one addictiveness endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section addictiveness assessments, a brief general conclusion should be formulated.

5. CHARACTERISING FLAVOUR AND INHALATION FACILITATION PROPERTIES ASSESSMENT

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report, a study summary should be submitted, including the following (the summary reports should usually only be 1 page in length):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one attractiveness endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section attractiveness assessments a brief general conclusion should be formulated.

6. ASSESSMENT OF INTERACTION OF ADDITIVES WITH OTHER ADDITIVES/INGREDIENTS

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report, a study summary should be submitted, including the following (the summary reports should usually only be 1 page):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section, a brief general conclusion should be formulated on the Interactions with other additives/ingredients.

SUMMARY / OVERALL CONCLUSIONS

In the final section, a summary and an overall conclusion shall be formulated covering all issues discussed above (chemical and physical specifications, use, toxicity, addictiveness, attractiveness and interactions with other ingredients).

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.6

Revision Date 02.11.2023

Print Date 24.11.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking**1.1 Product identifiers**

Product name : Glycerol

Product Number : G5516

Brand : Sigma

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 56-81-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification**2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.



2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Ecological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Toxicological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	: 1,2,3-Propanetriol Glycerin
Formula	: C ₃ H ₈ O ₃
Molecular weight	: 92,09 g/mol
CAS-No.	: 56-81-5
EC-No.	: 200-289-5

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled

After inhalation: fresh air.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed

After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available



SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Water Foam Carbon dioxide (CO₂) Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Vapors are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

In the event of fire, wear self-contained breathing apparatus.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Evacuate the danger area, observe emergency procedures, consult an expert.

For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemisorb®).

Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed.

hygroscopic

Storage class

Storage class (TRGS 510): 10: Combustible liquids



7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN 16523-1 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN 16523-1 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

Respiratory protection

Recommended Filter type: Filter A-(P2)

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.



SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	viscous
b) Color	clear
c) Odor	odorless
d) Melting point/freezing point	Melting point/range: 20 °C
e) Initial boiling point and boiling range	182 °C at 27 hPa
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	Upper explosion limit: 19 %(V) at 1013 hPa Lower explosion limit: 2,7 %(V) at 1013 hPa
h) Flash point	199 °C at ca.1013,0 hPa - Pensky-Martens closed cup
i) Autoignition temperature	370 °C
j) Decomposition temperature	No data available
k) pH	5,5 - 8
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: 1.412 mPa.s at 20 °C 612 mPa.s at 30 °C 14,8 mPa.s at 100 °C
m) Water solubility	at 25 °C miscible
n) Partition coefficient: n-octanol/water	log Pow: -1,75 at 25 °C
o) Vapor pressure	0,26 hPa at 100 °C 5,7 hPa at 150 °C
p) Density	1,25 g/mL
Relative density	No data available
q) Relative vapor density	No data available
r) Particle characteristics	No data available
s) Explosive properties	No data available
t) Oxidizing properties	none

9.2 Other safety information

Surface tension ca.63,4 mN/m at 20 °C



Relative vapor density 3,18 - (Air = 1.0)

SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Rat - female - 27.200 mg/kg

Remarks: (ECHA)

LC50 Inhalation - Rat - male and female - 4 h - > 5.850 mg/l - aerosol

Remarks: (ECHA)

LD50 Dermal - Guinea pig - male and female - 56.750 mg/kg

Remarks: (ECHA)

Skin corrosion/irritation

Skin - Rabbit

Result: No skin irritation - 24 h

Remarks: (ECHA)

Serious eye damage/eye irritation

Eyes - Rabbit

Result: No eye irritation - 7 Days

Remarks: (ECHA)

Respiratory or skin sensitization

Local lymph node assay (LLNA) - Mouse

Result: negative

(OECD Test Guideline 429)

Germ cell mutagenicity

Test Type: Ames test

Test system: S. typhimurium



Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 471
Result: negative
Remarks: (IUCLID)
Test Type: In vitro mammalian cell gene mutation test
Test system: Chinese hamster ovary cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 476
Result: negative
Test Type: sister chromatid exchange assay
Test system: Chinese hamster ovary cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 476
Result: negative
Test Type: unscheduled DNA synthesis assay
Test system: rat hepatocytes
Method: OECD Test Guideline 482
Result: negative
Test Type: Chromosome aberration test in vitro
Test system: Chinese hamster ovary cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 473
Result: negative

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Repeated dose toxicity - Rat - male - Oral - 28 Days - NOAEL (No observed adverse effect level) - > 1.600 mg/kg

Remarks: (ECHA)

RTECS: MA8050000



To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Oncorhynchus mykiss (rainbow trout) - 54.000 mg/l - 96 h Remarks: (ECHA)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 1 d Result: 94 % - Readily biodegradable. Remarks: (ECHA)

Biochemical Oxygen Demand (BOD)	870 mg/g Remarks: (External MSDS)

Chemical Oxygen Demand (COD)	1.160 mg/g Remarks: (External MSDS)

Theoretical oxygen demand	1.217 mg/g Remarks: (Lit.)

Ratio BOD/ThBOD	71 % Remarks: (Lit.)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment	: The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects



13.1 Waste treatment methods

See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

14.1 UN number

ADR/RID: - IMDG: - IATA: -

ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

ADR/RID: - IMDG: - IATA: -

ADR/RID: - IMDG: - IATA: -

ADR/RID: no IMDG Marine pollutant: no IATA: no

No data available

Not classified as dangerous in the meaning of transport regulations.

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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