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)

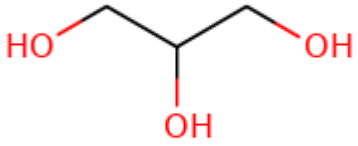
1.2. Synonyms

4-01-00-02751 (Beilstein Handbook Reference); 90 Technical glycerine; AI3-00091; BRN 0635685; CCRIS 2295; Caswell No. 469; Citifluor AF 2; Clyzerin, wasserfrei [German]; Dagralax; EINECS 200-289-5; EPA Pesticide Chemical Code 063507; FEMA No. 2525; Glicerina [DCIT]; Glicerol [INN – Spanish]; Glycerin; Glycerin, anhydrous; Glycerine; Glycerinum; Glyceritol; Glycerol; Glycerolum [INN – Latin]; Glycyl alcohol; Glyrol; Glysanin; Grocolene; HSDB 492; IFP; Incorporation factor; MOON; NSC 9230; Ophthalgan; Optim; Osmoglyn; Propanetriol; Star; Synthetic glycerin; Synthetic glycerine; Trihydroxypropane; UNII-PDC6A3C0OX; Vitrosupos; 1,2,3-Propanetriol; 1,2,3-Trihydroxypropane (ChemIDplus)

1.3. Molecular formula

$C_3H_8O_3$

1.4. Structural Formula



(ChemIDplus)

1.5. Molecular weight (g/mol)

92.09

1.6. CAS registration number

56-81-5

1.7. Properties

1.7.1. Melting point

17.8-20ºC (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)

1.7.3. Solubility

Miscible (ChemSpider; PubChem); 1e+006 mg/L at 25°C (EPISuite, 2017; PubChem); 5.296 x 10^6 mg/L at 25°C (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); 2.1–13% (GSBL, 2020)

1.7.7. (Auto)ignition temperature

739 deg F [393°C] or 698 ºF [370ºC] (PubChem); 400°C (IGS, 2019)

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).

1.7.10. Vapor pressure

0.000106 hPa at 25°C (calculated) and 0.0033 hPa at 50°C (measured); 0.01 Pa at 25°C and 0.003 mmHg at 50°C (PubChem); 0.000168 mmHg at 25ºC (EPISuite, 2017)

1.7.11. log Kow

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

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).

Reported levels from use as a flavouring (ppm): (FEMA, 1994)

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	0.49	0.92	Hard candy	21.71	22.49
Baked goods	17.84	34.04	Jams, Jellies	42.00	54.00
Chewing gum	13.63	18.55	Meat products	1.11	1.76
Confection, frosting	69.72	69.74	Milk products	83.59	83.59
Fats, oils	0.20	0.21	Nonalcoholic beverages	0.72	1.39
Frozen dairy	0.33	0.46	Snack foods	21.50	23.50
Fruit juice	0.02	0.07	Soft candy	1.78	3.75
Gelatins, puddings	25.64	42.75	Sweet sauce	74.02	74.02
Gravies	0.17	0.20			

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

As taken from Burdock, 2010.

“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 available at http://www.ncbi.nlm.nih.gov/pubmed/23496861

“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, diacetin, 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 available at http://www.ncbi.nlm.nih.gov/pubmed/22672560

"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 available at http://www.ncbi.nlm.nih.gov/pubmed/25460033

"….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 available at http://www.ncbi.nlm.nih.gov/pubmed/25455424

Glycerin is listed as an ingredient (at given concentrations, where specified) in hobby/craft (15-19%), auto (0-40%), home maintenance (1-60%), inside the home (0-60%), personal care (0-100%), commercial/institutional (1-100%), pesticides (4.77-10%) and pet care (0.0119-55%) 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 (Cosmetic substances and ingredients database). Accessed April 2020, available at https://ec.europa.eu/growth/tools-databases/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 proucts, 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.

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Glycerol (CAS RN 56-81-5) by Occupation*

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
008	PERSONNEL AND LABOR RELATIONS MANAGERS	542	
015	MANAGERS, MEDICINE AND HEALTH	7	7
018	FUNERAL DIRECTORS	11,025	
019	MANAGERS AND ADMINISTRATORS, N.E.C.	3,767	431
023	ACCOUNTANTS AND AUDITORS	225	113
027	PERSONNEL, TRAINING, AND LABOR RELATIONS SPECIALISTS	460	430
036	INSPECTORS AND COMPLIANCE OFFICERS, EXC. CONSTRUCTION	32	32
037	MANAGEMENT RELATED OCCUPATIONS, N.E.C.	303	303
044	AEROSPACE ENGINEERS	148	

045	METALLURGICAL AND MATERIALS ENGINEERS	29	
048	CHEMICAL ENGINEERS	81	6
055	ELECTRICAL AND ELECTRONIC ENGINEERS	976	
056	INDUSTRIAL ENGINEERS	383	331
057	MECHANICAL ENGINEERS	141	
059	ENGINEERS, N.E.C.	211	106
069	PHYSICISTS AND ASTRONOMERS	1,354	829
073	CHEMISTS, EXCEPT BIOCHEMISTS	11,519	4,548
074	ATMOSPHERIC AND SPACE SCIENTISTS	22	
075	GEOLOGISTS AND GEODESISTS	28	
078	BIOLOGICAL AND LIFE SCIENTISTS	1,984	1,173
083	MEDICAL SCIENTISTS	2,372	1,803
084	PHYSICIANS	26,371	13,976
085	DENTISTS	1,634	523
086	VETERINARIANS	7,323	633
095	REGISTERED NURSES	781,450	708,800
096	PHARMACISTS	13,900	7,719
097	DIETITIANS	886	844
098	INHALATION THERAPISTS	13,156	7,837

099	OCCUPATIONAL THERAPISTS	1,295	1,132
103	PHYSICAL THERAPISTS	18,815	14,388
104	SPEECH THERAPISTS	25	25
105	THERAPISTS, N.E.C.	798	606
106	PHYSICIANS' ASSISTANTS	4,929	2,464
163	COUNSELORS, EDUCATIONAL AND VOCATIONAL	181	127
185	DESIGNERS	945	475
188	PAINTERS, SCULPTORS, CRAFT-ARTISTS, AND ARTIST PRINTMAKERS	2,383	1,327
189	PHOTOGRAPHERS	846	183
194	ARTISTS, PERFORMERS, AND RELATED WORKERS, N.E.C.	291	143
195	EDITORS AND REPORTERS	285	23
203	CLINICAL LABORATORY TECHNOLOGISTS AND TECHNICIANS	59,714	43,599
204	DENTAL HYGIENISTS	18	18
206	RADIOLOGIC TECHNICIANS	31,627	21,652
207	LICENSED PRACTICAL NURSES	66,416	62,265
208	HEALTH TECHNOLOGISTS AND TECHNICIANS, N.E.C.	22,412	17,451
213	ELECTRICAL AND ELECTRONIC TECHNICIANS	4,142	727

215	MECHANICAL ENGINEERING TECHNICIANS	72	
216	ENGINEERING TECHNICIANS, N.E.C.	5,985	2,925
217	DRAFTING OCCUPATIONS	409	
223	BIOLOGICAL TECHNICIANS	7,251	3,681
224	CHEMICAL TECHNICIANS	8,310	2,916
225	SCIENCE TECHNICIANS, N.E.C.	1,665	780
228	BROADCAST EQUIPMENT OPERATORS	20	
235	TECHNICIANS, N.E.C.	12,000	3,281
274	SALES WORKERS, OTHER COMMODITIES	1,613	1,613
303	SUPERVISORS, GENERAL OFFICE	457	408
307	SUPERVISORS; DISTRIBUTION, SCHEDULING, AND ADJUSTING CLERKS	17	
313	SECRETARIES	5,705	5,705
319	RECEPTIONISTS	1,826	1,779
335	FILE CLERKS	180	60
336	RECORDS CLERKS	2,067	517
337	BOOKKEEPERS, ACCOUNTING, AND AUDITING CLERKS	513	513
345	DUPLICATING MACHINE OPERATORS	82	65
346	MAIL PREPARING AND PAPER HANDLING MACHINE OPERATORS	38	

356	MAIL CLERKS, EXC. POSTAL SERVICE	4,377	1,929
363	PRODUCTION COORDINATORS	177	
364	TRAFFIC, SHIPPING, AND RECEIVING CLERKS	7,856	2,829
365	STOCK AND INVENTORY CLERKS	5,986	2,900
366	METER READERS	1,495	
368	WEIGHERS, MEASURERS, AND CHECKERS	236	42
379	GENERAL OFFICE CLERKS	1,050	481
384	PROOFREADERS	303	303
385	DATA-ENTRY KEYERS	568	568
389	ADMINISTRATIVE SUPPORT OCCUPATIONS, N.E.C.	1,422	1,293
435	WAITERS AND WAITRESSES	1,438	1,233
436	COOKS, EXCEPT SHORT ORDER	251	188
444	MISCELLANEOUS FOOD PREPARATION OCCUPATIONS	2,573	1,850
445	DENTAL ASSISTANTS	107	57
446	HEALTH AIDES, EXCEPT NURSING	11,287	7,794
447	NURSING AIDES, ORDERLIES, AND ATTENDANTS	67,048	58,207
448	SUPERVISORS, CLEANING AND BUILDING SERVICE WORKERS	642	368
449	MAIDS AND HOUSEMEN	23,663	13,785

453	JANITORS AND CLEANERS	100,290	37,790
458	HAIRDRESSERS AND COSMETOLOGISTS	54,800	42,306
469	PERSONAL SERVICE OCCUPATIONS, N.E.C.	244	
487	ANIMAL CARETAKERS, EXCEPT FARM	5,428	3,948
496	TIMBER CUTTING AND LOGGING OCCUPATIONS	269	
503	SUPERVISORS, MECHANICS AND REPAIRERS	9	
505	AUTOMOBILE MECHANICS	22,410	
507	BUS, TRUCK, AND STATIONARY ENGINE MECHANICS	5,480	
508	AIRCRAFT ENGINE MECHANICS	9,695	256
514	AUTOMOBILE BODY AND RELATED REPAIRERS	3,005	
515	AIRCRAFT MECHANICS, EXC. ENGINE	2,405	42
516	HEAVY EQUIPMENT MECHANICS	9,921	75
518	INDUSTRIAL MACHINERY REPAIRERS	6,431	
519	MACHINERY MAINTENANCE OCCUPATIONS	1,447	6
523	ELECTRONIC REPAIRERS, COMMUNICATIONS AND INDUSTRIAL EQUIPMENT	1,981	3
526	HOUSEHOLD APPLIANCE AND POWER TOOL REPAIRERS	164	65
533	MISCELLANEOUS ELECTRICAL AND ELECTRONIC EQUIPMENT REPAIRERS	45	

534	HEATING, AIR CONDITIONING, AND REFRIGERATION MECHANICS	10,068	
535	CAMERA, WATCH, AND MUSICAL INSTRUMENT REPAIRERS	39	
538	OFFICE MACHINE REPAIRERS	431	
539	MECHANICAL CONTROLS AND VALVE REPAIRERS	2,515	
544	MILLWRIGHTS	3,964	105
547	SPECIFIED MECHANICS AND REPAIRERS, N.E.C.	5,947	1,582
549	NOT SPECIFIED MECHANICS AND REPAIRERS	17,643	1,069
557	SUPERVISORS; PLUMBERS, PIPEFITTERS, AND STEAMFITTERS	3,250	
558	SUPERVISORS, N.E.C.	374	
567	CARPENTERS	1,794	244
569	CARPENTER APPRENTICES	64	
575	ELECTRICIANS	3,457	
577	ELECTRICAL POWER INSTALLERS AND REPAIRERS	4,059	87
579	PAINTERS, CONSTRUCTION AND MAINTENANCE	2,619	297
585	PLUMBERS, PIPEFITTERS, AND STEAMFITTERS	5,762	
597	STRUCTURAL METAL WORKERS	1,319	10

599	CONSTRUCTION TRADES, N.E.C.	6,660	254
616	MINING MACHINE OPERATORS	8,072	
633	SUPERVISORS, PRODUCTION OCCUPATIONS	5,601	495
634	TOOL AND DIE MAKERS	5,058	58
635	TOOL AND DIE MAKER APPRENTICES	584	
636	PRECISION ASSEMBLERS, METAL	1,119	947
637	MACHINISTS	14,935	351
639	MACHINIST APPRENTICES	139	
644	PRECISION GRINDERS, FITTERS, AND TOOL SHARPENERS	65	43
646	LAY-OUT WORKERS	124	
647	PRECIOUS STONES AND METALS WORKERS (JEWELERS)	698	695
649	ENGRAVERS, METAL	313	7
653	SHEET METAL WORKERS	1,130	41
655	MISCELLANEOUS PRECISION METAL WORKERS	122	
657	CABINET MAKERS AND BENCH CARPENTERS	709	
658	FURNITURE AND WOOD FINISHERS	549	
667	TAILORS	32	
668	UPHOLSTERERS	282	34

669	SHOE REPAIRERS	120	120
675	HAND MOLDERS AND SHAPERS, EXCEPT JEWELERS	1,918	95
676	PATTERNMAKERS, LAY-OUT WORKERS, AND CUTTERS	255	
677	OPTICAL GOODS WORKERS	3,006	1,618
678	DENTAL LABORATORY AND MEDICAL APPLIANCE TECHNICIANS	1,616	21
679	BOOKBINDERS	8,465	5,494
683	ELECTRICAL AND ELECTRONIC EQUIPMENT ASSEMBLERS	13,360	10,685
684	MISCELLANEOUS PRECISION WORKERS, N.E.C.	3,106	2,707
686	BUTCHERS AND MEAT CUTTERS	3,501	645
687	BAKERS	4,856	1,214
688	FOOD BATCHMAKERS	7,346	5,242
689	INSPECTORS, TESTERS, AND GRADERS	469	253
695	POWER PLANT OPERATORS	666	172
696	STATIONARY ENGINEERS	1,161	217
699	MISCELLANEOUS PLANT AND SYSTEM OPERATORS	800	
703	LATHE AND TURNING MACHINE SET-UP OPERATORS	581	48
704	LATHE AND TURNING MACHINE OPERATORS	4,791	34

705	MILLING AND PLANING MACHINE OPERATORS	891	
706	PUNCHING AND STAMPING PRESS MACHINE OPERATORS	774	177
708	DRILLING AND BORING MACHINE OPERATORS	49	32
709	GRINDING, ABRADING, BUFFING, AND POLISHING MACHINE OPERATORS	4,731	1,444
715	MISCELLANEOUS METAL, PLASTIC, STONE, AND GLASS WORKING MACHINE OPERATORS	29	
717	FABRICATING MACHINE OPERATORS, N.E.C.	750	178
719	MOLDING AND CASTING MACHINE OPERATORS	1,886	283
723	METAL PLATING MACHINE OPERATORS	8,770	2,276
724	HEAT TREATING EQUIPMENT OPERATORS	800	
725	MISCELLANEOUS METAL AND PLASTIC PROCESSING MACHINE OPERATORS	1,255	376
727	SAWING MACHINE OPERATORS	4,218	163
728	SHAPING AND JOINING MACHINE OPERATORS	595	595
733	MISCELLANEOUS WOODWORKING MACHINE OPERATORS	2,327	
734	PRINTING MACHINE OPERATORS	42,659	6,498
735	PHOTOENGRAVERS AND LITHOGRAPHERS	3,435	411
736	TYPESETTERS AND COMPOSITORS	629	224

737	MISCELLANEOUS PRINTING MACHINE OPERATORS	6,341	1,677
738	WINDING AND TWISTING MACHINE OPERATORS	3,256	2,259
739	KNITTING, LOOPING, TAPING, AND WEAVING MACHINE OPERATORS	390	
743	TEXTILE CUTTING MACHINE OPERATORS	1,046	
744	TEXTILE SEWING MACHINE OPERATORS	12,971	11,520
745	SHOE MACHINE OPERATORS	1,305	661
747	PRESSING MACHINE OPERATORS	3,006	2,968
748	LAUNDERING AND DRY CLEANING MACHINE OPERATORS	9,398	3,967
749	MISCELLANEOUS TEXTILE MACHINE OPERATORS	2,616	703
753	CEMENTING AND GLUING MACHINE OPERATORS	1,895	1,010
754	PACKAGING AND FILLING MACHINE OPERATORS	5,318	1,200
755	EXTRUDING AND FORMING MACHINE OPERATORS	997	132
756	MIXING AND BLENDING MACHINE OPERATORS	19,108	4,455
757	SEPARATING, FILTERING, AND CLARIFYING MACHINE OPERATORS	2,316	242
758	COMPRESSING AND COMPACTING MACHINE OPERATORS	91	

759	PAINTING AND PAINT SPRAYING MACHINE OPERATORS	5,078	717
764	WASHING, CLEANING, AND PICKLING MACHINE OPERATORS	384	16
765	FOLDING MACHINE OPERATORS	5,048	3,217
766	FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD	7,070	1,360
768	CRUSHING AND GRINDING MACHINE OPERATORS	142	
769	SLICING AND CUTTING MACHINE OPERATORS	6,790	2,711
774	PHOTOGRAPHIC PROCESS MACHINE OPERATORS	7,204	3,481
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	35,657	6,664
779	MACHINE OPERATORS, NOT SPECIFIED	27,500	7,181
783	WELDERS AND CUTTERS	8,774	254
784	SOLDERERS AND BRAZERS	6,970	4,571
785	ASSEMBLERS	145,244	99,877
789	HAND PAINTING, COATING, AND DECORATING OCCUPATIONS	202	56
793	HAND ENGRAVING AND PRINTING OCCUPATIONS	549	36
794	HAND GRINDING AND POLISHING OCCUPATIONS	51	

796	PRODUCTION INSPECTORS, CHECKERS, AND EXAMINERS	2,377	629
797	PRODUCTION TESTERS	2,665	1,136
798	PRODUCTION SAMPLERS AND WEIGHERS	111	
799	GRADERS AND SORTERS, EXCEPT AGRICULTURAL	12	
804	TRUCK DRIVERS, HEAVY	9,184	
825	RAILROAD BRAKE, SIGNAL, AND SWITCH OPERATORS	354	
844	OPERATING ENGINEERS	865	
855	GRADER, DOZER, AND SCRAPER OPERATORS	314	
856	INDUSTRIAL TRUCK AND TRACTOR EQUIPMENT OPERATORS	950	5
859	MISCELLANEOUS MATERIAL MOVING EQUIPMENT OPERATORS	859	32
869	CONSTRUCTION LABORERS	2,201	5
873	PRODUCTION HELPERS	3,882	
875	GARBAGE COLLECTORS	157	52
877	STOCK HANDLERS AND BAGGERS	32	
878	MACHINE FEEDERS AND OFFBEARERS	300	
883	FREIGHT, STOCK, AND MATERIAL MOVERS, HAND, N.E.C.	326	93
885	GARAGE AND SERVICE STATION RELATED OCCUPATIONS	12,357	1,098

887	VEHICLE WASHERS AND EQUIPMENT CLEANERS	2,606	261
888	HAND PACKERS AND PACKAGERS	20,112	13,127
889	LABORERS, EXCEPT CONSTRUCTION	11,694	2,107
TOTAL		2,135,546	1,346,631

*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH, available at https://web.archive.org/web/20111028103130/http://www.cdc.gov/noes/noes2/35085occ.html

“

Occupational limit values:

NPK-P (CZ) – 15 mg/m^3 (fog)

SUVA STEL – 100 mg/m^3 (einatembar)

“Average values per shift”: 5, 10, 15, 20, 50 and 200 mg/m^3

As taken from IGS, 2019

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 ≥215°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 ≥270°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

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 tryglycerides 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/m^3 (total dust); 5 mg/m^3 (respirable fraction)

DFG MAKs TWA: 200 mg/m^3 (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, 2020.

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.

US Army Military exposure guidelines (MEGs) for Short-Term exposures to chemicals in ambient air:

1 hour Critical Air MEG 5.0E+02 mg/m^3

1 hour Marginal Air MEG 5.0E+02 mg/m^3

1 hr Negligible air MEG 1.3E+02 mg/m^3

14 day Negligible air MEG 3.4E+00 mg/m^3

8 hour Negligible air MEG 1.0E+01 mg/m^3

Potential health effects head , irrit eyes , irrit resp sys , irrit skin , kidney inj , nau , vomit

US Army Military exposure guidelines (MEGs) for Long-Term exposures to chemicals in ambient air:

1 year Negligible air MEG 3.40E+00 mg/m^3

Potential health effects head , irrit eyes , irrit resp sys , irrit skin , kidney inj , nau , vomit

As taken from US EPA ACToR database, 2015.

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, 2020a,b.

"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 (2020) 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, 2020).

Workplace exposure limits for glycerol mist of 10 mg/m^3 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, 2020a).

Glycerol (CAS RN 56-81-5) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2020b).

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 (US EPA, 2019).

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

As taken from US EPA ACToR database, 2015.

Glycerol / 1,2,3-Propanetriol (CAS RN 56-81-5) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2012 CDR (Chemical Data Reporting Rule) and the 2016 CDR Partial Exempt lists. The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory, and 2012 CDR and 2016 CDR Partial Exempt lists are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Substance	Glycerol, mist	
CAS No.	56-81-5	
	Limit value - Eight hours	Limit value - Short term

	ppm	mg/m³	ppm	mg/m³
Australia		10 (1)		
Belgium		10		
Canada - Ontario		10		
Canada - Québec		10		
Finland		20		
France		10		
Germany (AGS)		200 (1)		400 (1)(2)
Germany (DFG)		200 (1)		400 (1)(2)
Ireland		10		
New Zealand		10 (1)		
Poland		10		
Singapore		10		
South Korea		10		
Spain		10		
Switzerland		50 inhalable aerosol		100 inhalable aerosol
USA - OSHA		15 inhalable dust		
		5 respirable dust		
United Kingdom		10		
	Remarks			
Australia	(1) This value is for inhalable dust containing no asbestos and < 1% crystalline silica.			
Germany (AGS)	(1) Inhalable fraction (2) 15 minutes average value			
Germany (DFG)	(1) Inhalable fraction (2) 15 minutes average value			
New Zealand	(1) The value for inhalable dust containing no asbestos and less than 1% free silica.			

As taken from GESTIS, 2019, available at https://limitvalue.ifa.dguv.de/.

1,2,3-Propanetriol (CAS RN 56-81-5) is listed in the New Zealand Inventory of Chemicals and may be used as a single component chemical under an appropriate group standard (NZ EPA, 2006).

“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.

The California Division of Occupational Safety and Health lists glycerine mist as a “particulate not otherwise regulated” and has set permissible exposure limits (PELs) of 10 mg/m^3 for the total dust and 5 mg/m^3 for the respirable fraction (Cal/OSHA, undated).

Glycerol has been designated as GRAS (generally recognized as safe) for use in food by FEMA (Hall RL and Oser BL, 1965).

1,2,3-Propanetriol (CAS RN 56-81-5) is “not considered to pose an unreasonable risk to the health of workers and public health on the basis of the Tier I IMAP assessment” and has been “identified as low concern to human health by application of expert validated rules”. As taken from NICNAS, 2018.

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:

Inactive Ingredient	**Route**	**Dosage Form**	**CAS Number**	**UNII**	**Maximum Potency per unit dose**
GLYCERIN	AURICULAR (OTIC)	SOLUTION	56815	PDC6A3C0OX	63.64%w/v
GLYCERIN	AURICULAR (OTIC)	SOLUTION/ DROPS	56815	PDC6A3C0OX	2.5%w/w
GLYCERIN	AURICULAR (OTIC)	SUSPENSION	56815	PDC6A3C0OX	0.05%w/w
GLYCERIN	BUCCAL	GUM, CHEWING	56815	PDC6A3C0OX	28.8mg
GLYCERIN	BUCCAL	MOUTHWASH	56815	PDC6A3C0OX	100mg/1ml
GLYCERIN	BUCCAL	PATCH, EXTENDED RELEASE	56815	PDC6A3C0OX	66mg
GLYCERIN	DENTAL	PASTE	56815	PDC6A3C0OX	20%w/w
GLYCERIN	DENTAL	PASTE, DENTIFRICE	56815	PDC6A3C0OX	18%w/w

GLYCERIN	DENTAL	SOLUTION	56815	PDC6A3C0OX	15%w/v
GLYCERIN	DENTAL	TABLET	56815	PDC6A3C0OX	0.53mg
GLYCERIN	INTRADERMAL	INJECTION	56815	PDC6A3C0OX	1.6%w/v
GLYCERIN	INTRAMUSCULAR	INJECTION	56815	PDC6A3C0OX	15.36%w/v
GLYCERIN	INTRAMUSCULAR	INJECTION, SOLUTION	56815	PDC6A3C0OX	15%w/v
GLYCERIN	INTRAVENOUS	EMULSION	56815	PDC6A3C0OX	2.2%w/v
GLYCERIN	INTRAVENOUS	INJECTION	56815	PDC6A3C0OX	15.36%w/v
GLYCERIN	INTRAVENOUS	INJECTION, EMULSION	56815	PDC6A3C0OX	225mg
GLYCERIN	INTRAVENOUS	INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION	56815	PDC6A3C0OX	225mg
GLYCERIN	INTRAVENOUS	INJECTION, SOLUTION	56815	PDC6A3C0OX	15%w/v
GLYCERIN	IONTOPHORESIS	PATCH, EXTENDED RELEASE	56815	PDC6A3C0OX	168.1mg
GLYCERIN	NASAL	DROPS	56815	PDC6A3C0OX	10mg/1ml
GLYCERIN	NASAL	SOLUTION	56815	PDC6A3C0OX	25mg/1ml
GLYCERIN	NASAL	SPRAY	56815	PDC6A3C0OX	23mg

GLYCERIN	NASAL	SPRAY, METERED	56815	PDC6A3C0OX	0.02mg/mg
GLYCERIN	OPHTHALMIC	EMULSION	56815	PDC6A3C0OX	2.2%w/w
GLYCERIN	OPHTHALMIC	GEL	56815	PDC6A3C0OX	8.8%w/w
GLYCERIN	OPHTHALMIC	SOLUTION	56815	PDC6A3C0OX	2%w/v
GLYCERIN	OPHTHALMIC	SOLUTION/ DROPS	56815	PDC6A3C0OX	2.25%w/v
GLYCERIN	OPHTHALMIC	SUSPENSION	56815	PDC6A3C0OX	2.5%w/v
GLYCERIN	OPHTHALMIC	SUSPENSION/ DROPS	56815	PDC6A3C0OX	2.5%w/v
GLYCERIN	ORAL	CAPSULE	56815	PDC6A3C0OX	249.1mg
GLYCERIN	ORAL	CAPSULE, COATED	56815	PDC6A3C0OX	14.21mg
GLYCERIN	ORAL	CAPSULE, EXTENDED RELEASE	56815	PDC6A3C0OX	132.32mg
GLYCERIN	ORAL	CAPSULE, GELATIN COATED	56815	PDC6A3C0OX	3.37mg
GLYCERIN	ORAL	CAPSULE, LIQUID FILLED	56815	PDC6A3C0OX	63.29mg
GLYCERIN	ORAL	CONCENTRATE	56815	PDC6A3C0OX	750mg/1ml
GLYCERIN	ORAL	DROPS	56815	PDC6A3C0OX	300mg/1ml

GLYCERIN	ORAL	ELIXIR	56815	PDC6A3C0OX	500mg/1ml
GLYCERIN	ORAL	LIQUID	56815	PDC6A3C0OX	885.5mg/1ml
GLYCERIN	ORAL	PASTE	56815	PDC6A3C0OX	29302mg/230ml
GLYCERIN	ORAL	SOLUTION	56815	PDC6A3C0OX	1004.8mg
GLYCERIN	ORAL	SOLUTION, CONCENTRATE	56815	PDC6A3C0OX	200mg/1ml
GLYCERIN	ORAL	SUSPENSION	56815	PDC6A3C0OX	2870.55mg/5ml
GLYCERIN	ORAL	SUSPENSION, EXTENDED RELEASE	56815	PDC6A3C0OX	500mg/5ml
GLYCERIN	ORAL	SUSPENSION/ DROPS	56815	PDC6A3C0OX	250mg/2.5ml
GLYCERIN	ORAL	SYRUP	56815	PDC6A3C0OX	1650mg/5ml
GLYCERIN	ORAL	TABLET	56815	PDC6A3C0OX	16mg
GLYCERIN	ORAL	TABLET, CHEWABLE	56815	PDC6A3C0OX	1mg
GLYCERIN	ORAL	TABLET, COATED	56815	PDC6A3C0OX	1mg
GLYCERIN	ORAL	TABLET, EXTENDED RELEASE	56815	PDC6A3C0OX	3.64mg
GLYCERIN	ORAL	TABLET, FILM COATED	56815	PDC6A3C0OX	1.55mg

GLYCERIN	RECTAL	SUPPOSITORY	56815	PDC6A3C0OX	128mg
GLYCERIN	RESPIRATORY (INHALATION)	SOLUTION	56815	PDC6A3C0OX	5%w/w
GLYCERIN	SUBCUTANEOUS	INJECTION	56815	PDC6A3C0OX	32.5%w/v
GLYCERIN	SUBCUTANEOUS	INJECTION, SOLUTION	56815	PDC6A3C0OX	15%w/v
GLYCERIN	SUBCUTANEOUS	INJECTION, SUSPENSION	56815	PDC6A3C0OX	1.6%w/v
GLYCERIN	TOPICAL	AEROSOL	56815	PDC6A3C0OX	3%w/w
GLYCERIN	TOPICAL	AEROSOL, FOAM	56815	PDC6A3C0OX	2.11%w/w
GLYCERIN	TOPICAL	CLOTH	56815	PDC6A3C0OX	0.05mg/mg
GLYCERIN	TOPICAL	CREAM	56815	PDC6A3C0OX	20%w/w
GLYCERIN	TOPICAL	CREAM, AUGMENTED	56815	PDC6A3C0OX	4%w/w
GLYCERIN	TOPICAL	GEL	56815	PDC6A3C0OX	20%w/w
GLYCERIN	TOPICAL	JELLY	56815	PDC6A3C0OX	NA
GLYCERIN	TOPICAL	LIQUID	56815	PDC6A3C0OX	2.5%w/w
GLYCERIN	TOPICAL	LOTION	56815	PDC6A3C0OX	11.2%w/w

GLYCERIN	TOPICAL	OINTMENT	56815	PDC6A3C0OX	NA
GLYCERIN	TOPICAL	PATCH	56815	PDC6A3C0OX	0.18mg/mg
GLYCERIN	TOPICAL	PATCH, EXTENDED RELEASE	56815	PDC6A3C0OX	168.1mg
GLYCERIN	TOPICAL	SHAMPOO	56815	PDC6A3C0OX	5%w/w
GLYCERIN	TOPICAL	SOAP	56815	PDC6A3C0OX	0.76%w/w
GLYCERIN	TOPICAL	SOLUTION	56815	PDC6A3C0OX	11.5%w/w
GLYCERIN	TOPICAL	SPONGE	56815	PDC6A3C0OX	2%w/w
GLYCERIN	TOPICAL	SUSPENSION	56815	PDC6A3C0OX	0.6%w/v
GLYCERIN	TRANSDERMAL	FILM, EXTENDED RELEASE	56815	PDC6A3C0OX	NA
GLYCERIN	TRANSDERMAL	GEL	56815	PDC6A3C0OX	5%w/w
GLYCERIN	VAGINAL	CREAM	56815	PDC6A3C0OX	5%w/w
GLYCERIN	VAGINAL	DOUCHE	56815	PDC6A3C0OX	1%w/w
GLYCERIN	VAGINAL	JELLY	56815	PDC6A3C0OX	NA
GLYCERIN	VAGINAL	SUPPOSITORY	56815	PDC6A3C0OX	227.9mg

As taken from FDA, 2020c.

Glycerol is listed by the US EPA Office of Pesticide Programs (2020) and was first registered as a conventional chemical pesticide on 9 December 2003.

Protective Action Criteria:

Value	PAC-1	PAC-2	PAC-3
Glycerine (mist)	45 mg/m^3	170 mg/m^3	1100 mg/m^3

As taken from IGS, 2019

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 glyceral-dehyde-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**

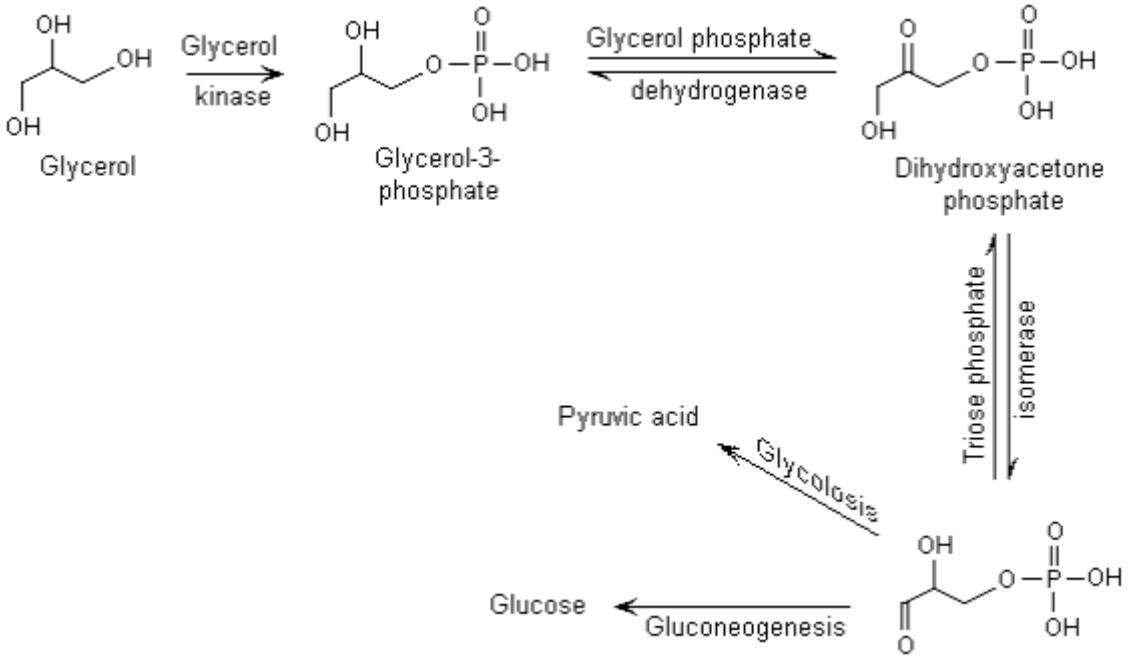
Glycerol, pyruvic acid, and lactic acid are endogenous in humans. Glycerol and pyruvic acid are metabolized completely and are not excreted. ... Glycerol is metabolized via the glycolytic pathway after it has been converted in the liver to glycerol-3-phosphate. [WHO/FAO: Expert Committee on Food Additives. 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 14CO2. At lower concentrations, the proportion converted to CO2 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/

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/OECDSIDS/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 absorbption 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 adminstration 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 ± 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% 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

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 14C-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 14CO2 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 14CO2. Peak levels of 14CO2 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 presentedby 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 14Clabelled 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 _ 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 lmol/kg bw per minute (mean 1.7 lmol/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 lmol/minute was reported in adult volunteers. At plasma concentrations up to 0.45 mM (41.4 mg/L), the turnover rate increased to 700 lmol/minute in obese subjects, and to a maximum of 400 lmol/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 lmol/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 CO2, 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% 13C3-glycerol. Skin distribution of 13C3-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

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: Glycerolin 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 ofglycerolon 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 addedglycerol1.45% (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 containingglycerol(P < 0.01). With ethanol, this was also true for the immediate effect. CONCLUSION:Glycerol1.45% (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 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

"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 Gastrointestinal - 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	FEPRA7 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 percent kill	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, 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	JIHTAB 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 decreased Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - catalases	PHMCAA Pharmacologist. (American Soc. for Pharmacology and Experimental Therapeutics, 9650 Rockville Pike, Bethesda, MD 20014) V.1- 1959- 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	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982-

				mediation of inflammation	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 pylores 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.

LC50 (rat) > 570 mg/m3/1h (Haz-Map, 2020).

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 (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.

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

Oral LD50 rat – 20 ml/kg

Intravenous rat LD50 – 4.4 ml/kg

As taken from IGS, 2019

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 abcesses, 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 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	At 20%:	-	Loeser 1954, Bornmann 1955

			water (= 667-13340 mg/kg bw)	Mortality 2/12 rats at top dose; initial growth and development ↓ (with recovery)		
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, 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	↓= decrease
BWG = body weight gain	RBC=red blood cells
CHO = cholesterol	UV= urinary volume
CL = chloride	WC = water consumption
n.i.= not indicated	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 lowdensity 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%, 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.

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 mg/kg bw/day

POD Owner - COSMOS TTC (NON-CANCER)

Critical study: RAT (Chronic Toxicity) Oral - Dietary exposure for 730 day

NOEL/LOEL Owner - Munro

Original NOEL - 3442.0 mg/kg bw/day

Original LOEL - 6883.0 mg/kg bw/day

Critical Sites - UNSPECIFIED ORGANS

Critical Effects - UNSPECIFIED ORGANS - WEIGHT CHANGES

As taken from the COSMOS database available at https://cosmosdb.eu/cosmosdb.v2/dbsearch/full

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 Administration	Species/Test System	Dose Data	Sex/Duration	Toxic Effects	Reference

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-l 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, 2020.

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 (Nfκb1), 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 death), unspecified assay	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, 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 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 - 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	In vitro	Human - ocular	454605 mg/L/1H	In Vitro Toxicity Studies - cell	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V.,

Concentration 50				viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 212,198,2012
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

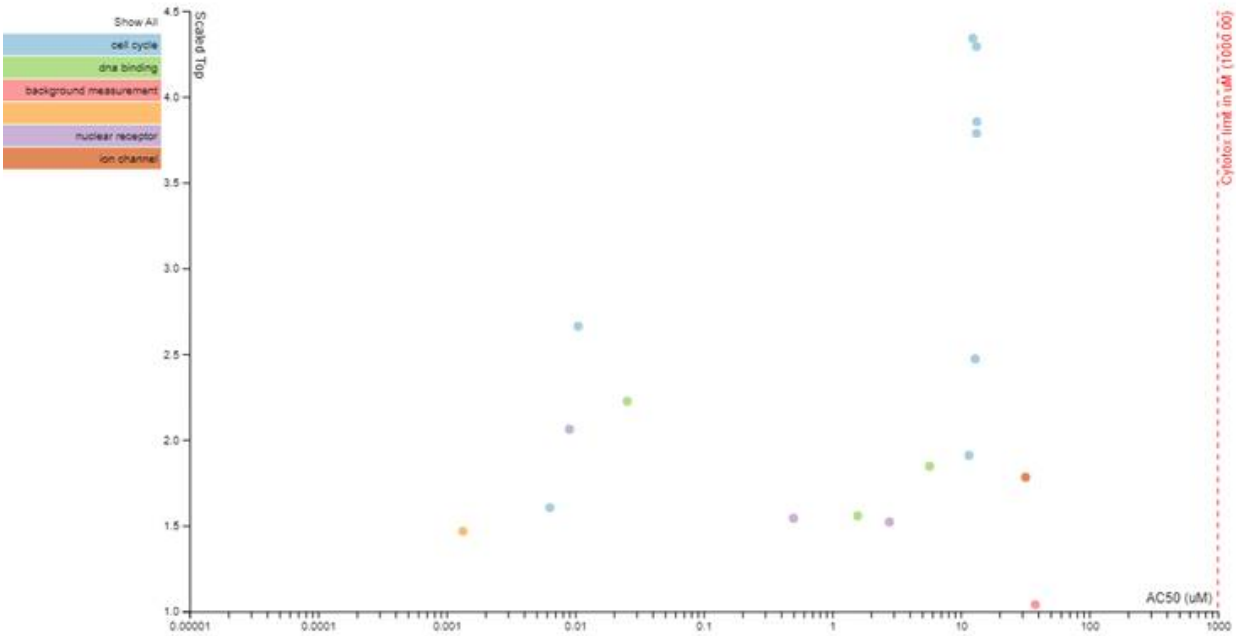
High-throughput Assay Data

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

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 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 PASMC. 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-1α and MIP-1β 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. 2019. Respirology. Epub ahead of print. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31542893/

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	CALEDQ Cancer Letters (Shannon, Ireland). (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1975- Volume(issue)/page/year: 229,181,2005

				- tumors Tumorigenic - facilitates action of known carcinogen	

As taken from RTECS, 2019.

“BACKGROUND: We investigated the effect of corn-derived biodiesel glycerolon 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 purifiedglycerolfrom 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 (Nfκb1), 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 biodieselglycerolthat compared to control mice; these alterations reached significant levels only in few cases. CONCLUSION: Biodieselglycerolpresents no higher risk forcarcinogenicityortoxicity.” 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 treatmentrelated 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).

Conclusion

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 sensitiser. 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 sensitiser 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; Organicke 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; Organicke 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 ToxicityCriteria (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 µmol/l, p-phenylenediamine (PPD) 15.6-125 µmol/l, 2-mercaptobenzothiazole (MBT) 50-1000 µmol/l, coumarin, and resorcinol (both: 250-1500 µmol/l)] and four non-sensitizers (monochlorobenzene, caprylic acid, glycerol, and salicylic acid (all: 125-1000 µmol/l)]. DNCB and MBT increased a subset of IL-23 receptor+/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% 13C3-glycerol. Skin distribution of 13C3-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

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 [electonic 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&pident_articulo=90337413&pident_usuario=0&pcontactid=&pident_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 andglycerolproduce mouth and throatirritationand 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/24 H	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 - other assays	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 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/44 H	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	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20-

				morphology: overgrowth of cell appendixes etc.	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 30	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
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. The infantile form is the most severe and is associated with vomiting, lethargy, severe developmental delay, and adrenal insufficiency. The mechanisms of glycerol toxicity in infants are not known, but it appears to shift metabolism towards chronic acidosis. Acidosis typically occurs when arterial pH falls below 7.35. In infants with acidosis, the initial symptoms include poor feeding, vomiting, loss of appetite, weak muscle tone (hypotonia), and lack of energy (lethargy). These can progress to heart, liver, and kidney abnormalities, seizures, coma, and possibly death. These are also the characteristic symptoms of untreated GKD. Many affected children with organic acidemias experience intellectual disability or delayed development. 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.

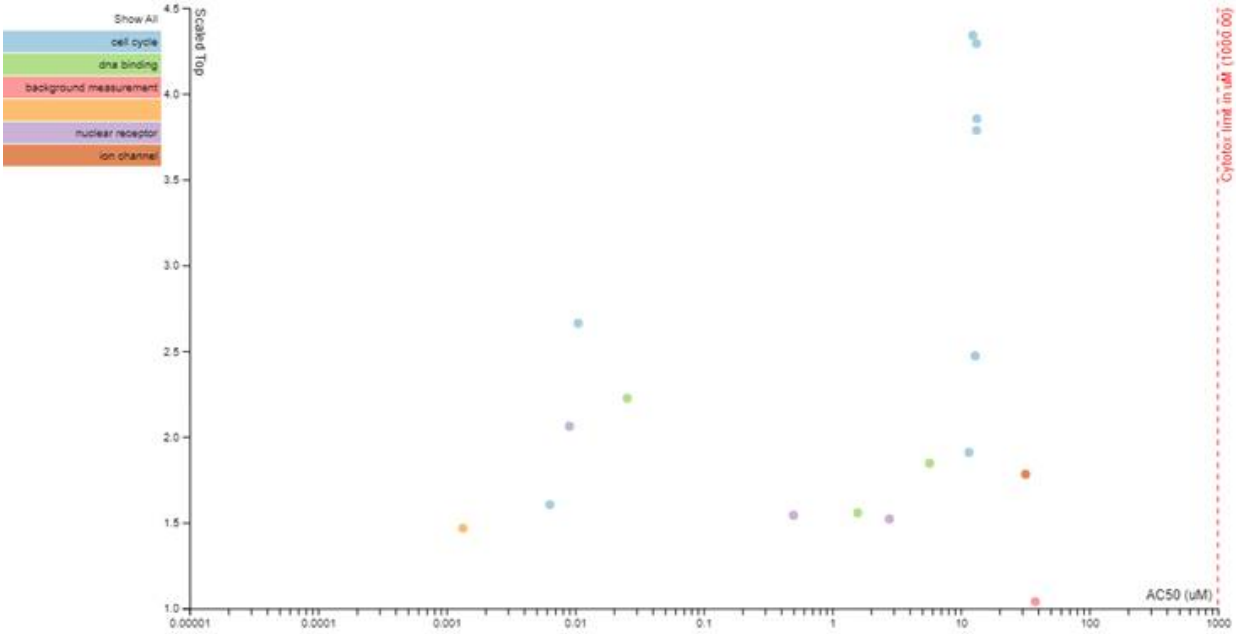
High-throughput Assay Data

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

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 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/

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 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**

Severe dehydration, cardiac arrhythmias, and hyperosmolar nonketoic 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.

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 overdosage 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 apperance 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-1β (IL-1β) mRNA, transforming growth factor-β(1) (TGF-β(1)), phospho-p65 of nuclear factor-κB (NF-κB), 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, hemeoxygenase-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-1β and NF-κB) 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. Opthalmol. 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 ofglycerol/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
	70,000	Baker et al., 2004c
Skin painting	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).

In a water pipe transfer study, 17% of Glycerol was transfer intact to the smoke (JTI/Oekolab study).

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 l (-1) for the EHC and 40-170 microg l (-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 mutagenicitytest (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 mutagenicitycould 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% glyceroland 50% propylene glycol, with nonicotineor 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

withnicotineconcentration 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)

A prototype electronic cigaret 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-κB 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, dualuse 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. 2020. Chem. Res. Toxicol. Epub ahead of print. PubMed, 2020 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 μA/cm2 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 ± 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/

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"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 exits 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 eliquids. In other words, glycidol can form and appear in the aerosol when a glycerol-containing solvent such as an eliquid is heated and aerosol is produced (Refs. 1-2)."

As taken from FDA, 2019

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
Reference: 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.		
Test Substance (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
Method		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Not applicable	
Test design / conditions		
Study type (photodegradation, hydrolysis, biodegradation, other –specify, do not assess): Biodegradation		
Test type (aerobic or anaerobic - specify, do not assess):		
Test medium (air, water, soil, sediment - specify, do not assess):		
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 ºC	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. (do not assess this item): % biodegradation by BOD or TOC (see file on N drive)		
Information on breakdown products (do not assess this item)		X
Overall score: 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 1.68X10-4 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 1.73X10-8 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 pharmaceutic 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 1.73X10-8 atm-cu m/mole(3). Glycerin is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 1.68X10-4 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 1.73X10-8 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/episuitedl.htm (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 1.68X10-4 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 1.9X10-11 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 1.9X10-11 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 5X10+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/episuitedl.htm (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 1.73X10-8 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 1.68X10-4 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 AI 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 (PM2.5) 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.

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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)

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BFA

Rate : 36; 63

Units : %BODT

Test Method : BOD5

Oxygen Condition : AEROBIC

Incub Time (days): 5; 5

Inoculum : SEWAGE

Temp (deg C) : 20

Remarks : SECOND SAMPLE USED ADAPTED SEED

Reference : BRIDIE,AL ET AL.(1979)

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 68

Units : %BODT

Test Method : WARBURG

Oxygen Condition : AEROBIC

Analysis Method : MANO

Incub Time (days): 5

Chem Conc (ppm) : 50-250

Microbial Pop : 10 (%)

Inoculum : SEWAGE

Temp (deg C) : 20

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

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 64

Units : %BODT

Test Method : BOD

Oxygen Condition : AEROBIC

Analysis Method : BOD

Incub Time (days): 5

Chem Conc (ppm) : 150-500

Microbial Pop : 10 (%)

Inoculum : SEWAGE

Temp (deg C) : 20

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

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 55.75

Units : %BODT

Test Method : BOD5

Oxygen Condition : AEROBIC

Analysis Method : BOD

Incub Time (days): 5

Inoculum : SEWAGE

Temp (deg C) : 20

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

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 28.19

Units : %BODT UTILIZATION IN 22-24 HRS

Test Method : BOD

Oxygen Condition : AEROBIC

Analysis Method : BOD

Incub Time (days): 1

Chem Conc (ppm) : 720

Microbial Pop : 2316 (MG/L)

Inoculum : ACTIVATED SLUDGE

Temp (deg C) : 20

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

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 52.57

Units : %BODT

Test Method : BOD5

Oxygen Condition : AEROBIC

Analysis Method : BOD

Incub Time (days): 5

Temp (deg C) : 20

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

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: BF

Rate : 75.3

Units : %BODT

Half Life (days) : .89

Test Method : SEWAGE DIE-AWAY

Oxygen Condition : AEROBIC

Analysis Method : BOD

Incub Time (days): 8-10

Chem Conc (ppm) : 6000

Inoculum : SEWAGE

Reference : WAGNER,R (1976)

****

CAS #: 56-81-5 GLYCEROL

Parameter Type : Screening Test

Study Biodeg Eval: NE

Oxygen Condition : ANAEROBIC-WATER

Inoculum : SEWAGE

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)

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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)

****

OXID GLYCEROL CAS# 56-81-5

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)

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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)

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MICD GLYCEROL CAS# 56-81-5

Species Name :

1 : ALCALIGENES SP., MC11

2 : ALCALIGENES SP., TE8

3 : ALCALIGENES SP., PE18

4 : CORYNEBACTERIUM SP., OEH8

Rate :

1 : GREW WELL

2 : GREW WELL

3 : GREW WELL

4 : GREW WELL

Analyt. Meth. : GROWTH

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)

****

MICD GLYCEROL CAS# 56-81-5

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)

****

MICD GLYCEROL CAS# 56-81-5

Summary : DEGR

Species Name :

1 : MYCOBACTERIUM SMEGMATIS 422

2 : MYCOBACTERIUM RHODOCHROUS 382

Rate :

1 : DEGR

2 : 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)

****

MICD GLYCEROL CAS# 56-81-5

Species Name :

1 : ARTHROBACTER- 2 STRAINS

2 : BACILLUS- 3 STRAINS

3 : ENTEROBACTERIACEAE- 4 STRAINS

4 : PSEUDOMONAS- 5 STRAINS

Rate :

1 : GROWTH

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

3 : GROWTH

4 : GROWTH

Analyt. Meth. : 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)

****

MICD GLYCEROL CAS# 56-81-5

Species Name : ARTHROBACTER GLOBIFORMIS- 10 STRAINS

Rate : GOOD TO EXCELLENT DEGR

Analyt. Meth. : GROWTH

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

Abbrev. Ref. : JENSEN,HL (1964)

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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): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	1.1799 0.9937 3.4756 (days-weeks) 4.1032 (days) 0.9697 0.9744 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)): Mackay model: Octanol/air (Koa) model:	 0.000134 6.03E-009

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 cm3/molecule-sec
Half-Life =	0.571 Days (12-hr day; 1.5E6 OH/cm3)
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)	*Carassius auratus*
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.		
Test Substance (CAS # and name): 56815 Glycerol		
Item	Yes	No
Test Substance		
*Chemical composition of the substance (including purity, by-products)	X	
Persistence/stability of test substance in aquatic solution		
Method		
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	
Test organisms (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
Test design / conditions		
Test type – acute or chronic (specify, but do not assess this item): 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	
Results		
Toxicity values (LC_{50}, EC_{50}, or IC_{50} - specify, do not assess this item): 24-hr TLm >5000 mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, do not assess this item):		
*Was toxicity value below the chemical's water solubility?	X	
Other adverse effects (carcinogenicity, mutagenicity, etc. Do not assess this item):		
Score: 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 Lglass 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: 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 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 assignable19.12.2001 (10)

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 turbity 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 treshold 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 Pseudomonas putida with Microcystis aeruginosa and in Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) the toxicity of Microcystis aeruginosa was compared to the toxicity of Scenedesmus quadricauda.
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 turbity 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: 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 turbity 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 turbity 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. 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. 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

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 paramaecium (Protozoa)
Exposure period:	48 hour(s)
Unit:	mg/l
EC5:	> 10000
Year:	1980
Test substance:	other TS
Method:	TEST ORGANISMS - Species: Chilomonas paramaecium 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 uM) 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 C. pasteurianum 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 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 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:	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 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 l2(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 Pseudomonas putida. 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:	Pseudomonas putida (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 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)
Remark:	A validated method for biological monitoring of glycerol is not available.
Source:	Unichema Chemie B.V. Gouda EUROPEAN COMMISION - 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	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	

Dinoflagellate								
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 Coccolithophorid	S U	SW LAB	 28			POP/ GPOP	F 46000000* ug/L	
Dunaliella tertiolecta Green Algae	S U	SW LAB	 28			POP/ GPOP	F 46000000* ug/L	
Entosiphon sulcatum Flagellate Euglenoid		FW LAB	 NR		CHG	POP PGRT/	F 3200000 ug/L	
Entosiphon sulcatum Flagellate Euglenoid		FW LAB	 NR		CHG	POP PGRT/	F 3200000 ug/L	

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

Scenedesmus quadricauda Green Algae	AQUA - NR U	FW LAB	10		DEC	POP BMAS/	F > 10000000 ug/L	
Scenedesmus quadricauda Green Algae		FW LAB	NR		CHG	POP PGRT/	F > 10000000 ug/L	
Scenedesmus quadricauda Green Algae	S U	LAB	8			POP/ GPOP	F > 10000000 ug/L	
Tetraselmis maculata Prasinophyte	S U	SW LAB	28			POP/ GPOP	F 4600000* ug/L	
Thalassiosira pseudonana Diatom	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	
Skeletonema costatum Diatom	S U	SW LAB	28			POP/ GPOP	F 46000000* ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 10210000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 10960000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 11100000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 11350000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 11350000 ug/L	

Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 12130000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 5750000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 6750000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	EC50	INC	DVP DFRM	F 7500000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 10000000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 10000000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 10320000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 17030000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 17160000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 17990000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	4	LC50	INC	MOR MORT	F 18640000 ug/L	

Xenopus laevis African Clawed Frog	S U	FW LAB	 4	LC50	INC	MOR MORT	F 20680000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	 4	LC50	INC	MOR MORT	F 8250000 ug/L	
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 10000000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 11250000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 11250000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 15000000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 15000000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 3630000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 3630000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 3630000 ug/L	SIG <0.05
Xenopus laevis African Clawed Frog	S U	FW LAB	WO 4	LOEC	DEC	GRO LGTH/	F 8000000 ug/L	SIG <0.05

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

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

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

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 PNECsediment of 479 mg/kg wwt and a tentative PNECsoil of 92.1 mg/kg wwt”.

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 PNECsediment of 479 mg/kg wwt and a tentative PNECsoil of 92.1 mg/kg wwt”.

Record for 1,2,3-propanetriol:

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

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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13. Last audited

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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 III[1]

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.

[2] Panel members: Diane Benford, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Eugenia Dogliotti, Lutz Edler, Peter Farmer, Peter Fürst, Laurentius (Ron) Hoogenboom, Helle Katrine Knutsen, Anne-Katrine Lundebye Haldorsen, Manfred Metzler, Carlo Stefano Nebbia, Michael O'Keeffe, Ivonne Rietjens, Dieter Schrenk, Vittorio Silano, Hendrik van Loveren, Christiane Vleminckx, and Pieter Wester. Correspondence: contam@efsa.europa.eu

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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 worse 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 852/2004[4] (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 2009[10] 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 852/2004[11] 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/EC[5] 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/EC[5] (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/EC[5] 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 854/2004 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/EC[5] 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/EC[5] 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: laureate, 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/EC[15]), 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

<u>Absorption, distribution, metabolism and excretion</u>

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 SiO_2:Na_2O. 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 posses 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

<u>Absorption, distribution, metabolism and excretion</u>

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).

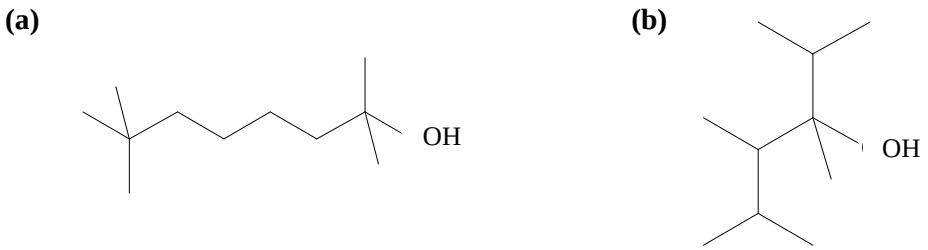

Figure 1: Two examples of oxo-alcohols representing the potential for most branching amongst the compounds likely to be present in the technical mixtures being assessed as previous cargoes.

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 LD_{50}s ranging from > 2 000 to 5 400 mg/kg b.w. Acute oral LD_{50}s 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 LD_{50}s 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 LD_{50}s 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 LD_{50} was 14.9 mL/kg b.w., corresponding to 15.8 g/kg b.w. In another experiment with rats administered by gavage the LD_{50} 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 LD_{50} 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/m^3) 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/m^3 (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/m^3 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/m^3 (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/EC[5] 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 H_2SO_4 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=Agree&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, not 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/m^3). 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 ≥15 000 mg/m^3. There was no increase in the incidence of malformations. The NOAEC for developmental toxicity was 11 000 mg/m^3 (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

<u>Absorption, distribution, metabolism and excretion</u>

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 LD_{50} 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 is 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/m^3 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 ≥ 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 substances[24] 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 Sata 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 minutes [25] (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 LD_{50} 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/m^3). 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_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 (accesed 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/m^3 (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 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 Crl: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 ≥ 1 000 ppm, with reduced food consumption, and at ≥ 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/m^3) 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/m^3, 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 being 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 LD_{50} of individual alkenes and mixtures of alkenes is > 5 g/kg b.w. in rats and, when tested, in mice. In most cases, the LD_{50} 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 affects 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

<u>Absorption, distribution, metabolism and excretion</u>

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).

<u>Acute toxicity</u>

Montan wax can be anticipated to be of low acute oral toxicity. An LD_{50} 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]

<u>Subacute, subchronic and chronic toxicity studies</u>

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 10/2011[24] 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 LD_{50} of greater than 5 000 mg/kg b.w. has been reported[29] (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-Goosens 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/EC[30] 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 LD_{50} 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 permited 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 LD_{50} 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 ECHA[31] 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 mm^2/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 mm^2/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 mm^2/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 mm^2/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 ≥ 11 mm^2/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 ≥ 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, AOEL[32] or ARfD[33] 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

<u>Absorption, distribution, metabolism and excretion</u>

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 LD_{50} 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/histocytosis in MLN[34] 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] Mesentheric 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 observed [31]. 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 at 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 LD_{50}s > 20 000 mg/kg b.w. in rats, mice and rabbits. At doses approaching the LD_{50}, 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/EC[5] 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/EC[5] 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 Sata Set
TDI	Tolerable daily intake
TTC	Threshold of toxicological concern
WHO	World Health Organization

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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), Alicja Mortensen, Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Pasquale Mosesso, Dominique Parent-Massin, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright, Maged Younes, Polly Boon, Dimitrios Chrysafidis, Rainer Gürtler, Paul Tobback, Ana Maria Rincon, Alexandra Tard and Claude Lambré

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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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 (LD_{50}) 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 1333/2008[1] 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 257/2010[2]. 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 EU[3] 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]

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[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-CosIng[6]).

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 Database[10]) 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.

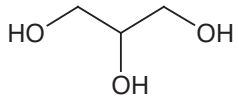

Figure 1: Structural formula of glycerol (E 422)

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 231/2 012). 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, glycyl alcohol, glycerin or glycerine (SciFinder[11], 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 ± 25°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 *Matching Fluid H*

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 1272/2008[12] (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 1333/2008[14] 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 refreshening microsweets	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 *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	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 1234/2007[17] (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/EC[18]' (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 GNPD[20] 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 recently[21] 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 refreshening 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 refreshening 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 refreshening 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 $^{14}CO_2$ 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 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 $^{14}CO_2$. 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 $^{14}CO_2$, 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 ± 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 μmol/kg bw per minute (mean 1.7 μmol/kg bw per minute). The half-life (at equilibration; t_o = 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 μmol/minute was reported in adult volunteers. At plasma concentrations up to 0.45 mM (41.4 mg/L), the turnover rate increased to 700 μmol/minute in obese subjects, and to a maximum of 400 μmol/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 μmol/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 LD_{50} 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	*Salmonella* Typhimurium TA100, TA1535, TA1537, TA98, TA1538; *Escherichia coli* 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	*S.* Typhimurium TA100, TA1535, TA1537, TA98	0.01–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Haworth et al. (1983)
Ames test	*S.* Typhimurium 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	*S.* Typhimurium TA100, TA1535, TA1537, TA98, TA1538	0.2–1 mg/plate n.d.	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
Ames test	*S.* Typhimurium TA102 and TA97	0.1–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Fujita et al. (1994)
Ames test	*S.* Typhimurium 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	*S.* Typhimurium TA100	Up to 92 mg/plate n.d.	No data	Negative	Negative	Stolzenberg and Hine (1979)
Ames test	*S.* Typhimurium 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	*S.* Typhimurium 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	*Bacillus subtilis* 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 ± 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 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-l 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 (bloatedness) 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 CO_2, 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 LD_{50} 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 the 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	physiololical saline
QS	*quantum satis*
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 microsweets	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	*Without taking into account NP*
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	
			–	1.1	1.9	Cutisin[(a)]	(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)
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 refreshening 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 refreshening 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 refreshening microsweets	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.

FOREWORD INTRODUCTION

GLYCEROL

CAS N°: 56-81-5

SIDS Initial Assessment Report

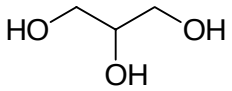
For

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	

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 LD_{50} 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 *in vitro*. Results of a limited gene mutation test in mammalian cells 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 the absence of a positive control, prevent any reliable conclusions to be drawn from the *in vivo* 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 LC_{50} 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 EC_{50} 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 calculated tentative PNECs for soil and sediment based on the $PNEC_{aquatic}$ 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 25ºC), 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

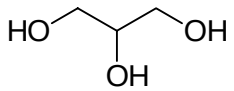
No further work is indicated, because of the low hazard potential of this substance.

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; glycyl 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 Kow = -1.76, water solubility = $1x10^5$ 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 BOD_5, COD and ThOD was determined. The standard dilution method for a period of 5 days (BOD_5) and the standard potassium dichromate method (COD) were used for the determinations. Both tests were performed according to APHA and ASTM guidelines, respectively. BOD_5 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] LogKoc = 0.39 logKow + 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 micro-organisms 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/m^3 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 LD_{50} value of 27,200 mg/kg bw was reported (Hine 1953). In other studies, with limited reporting, LD_{50}s 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 pylores and small intestine, pale spleen, lung congestion). The LD_{50}s for mice and guinea pigs were reported to be 23, 000 and 10, 000 mg/kg, respectively.

A number of acute oral toxicity LD_{50} 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 LD_{50} values were not available. The LD_{50} values reported are consistent with the range of values found in the available literature except in one case, where an oral LD_{50} 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 LD_{50} values are generally similar to those obtained by Hine (1953)

Other Routes of Exposure

In rats and mice, LD_{50} values for glycerol were between 4,420 and 10,100 mg/kg bw after intraperitoneal administration and 4250-6700 (rabbit LD_{50} = 53000) mg/kg bw after intravenous administration. Glycerol is much more toxic after subcutaneous administration (LD_{50} 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 LD_{50} 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 LD_{50} 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 sensitiser. 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/m^3. The mass median aerodynamic diameter (MMAD) was reported to be < 1.5 micrometres. Two males at 1000 mg/m^3 and 1 male and 1 female at 2000 mg/m^3 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/m^3 led to decreased triglyceride levels in males at 33 (34%) and 165 mg/m^3 (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/m^3 and 662 mg/m^3 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 abcesses, 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, 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

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/m^3. No other target organ involvement was identified. The NOAEL for local effects on the respiratory tract following exposure by inhalation is 165 mg/m^3.

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 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).

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 LD_{50} 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/m^3. The NOAEL is 167 mg/m^3.

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 of 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
Leuciscus idus melanotus (Golden Orfe)	n.i.	LC_{50} >10,000	Juhnke 1978
Carassius auratus (Goldfish)	24	LC_{50} >5,000	Bridie 1979
Leuciscus idus (Golden Orfe)	48	LC_0 >250	Wierich 1968
Oncorhynchus mykiss (Rainbow trout)	96	LC_{100} = 51,000-57000	Johnson 1980 [n.r]
Not specified	96	LC_{50} = 184,000	ECOSAR - QSAR
Daphnia magna	24	EC_{50} >10,000	Bringmann 1977, 1982
Daphnia magna	24	EC_0 > 500	Henkel [n.r.]
Daphnia	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 LC_{50} 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 EC_{50} 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 LC_{50}/EC_{50} values being in excess of 5000 mg/L.

A QSAR prediction for the 96-hour LC_{50} 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 10^4 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/EC_{50} 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 EC_{50} 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 (EC_0 >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 EC_{50} 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 x 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**
Chlimonas paramaecium	48	>10,000	Bringmann 1980, 1981
Clostridium sp.	n.i.	170,000	Dabrock 1992
Entosiphon sulcatum	72	3200	Bringmann 1978, 1980
Pseudomonas putida	16	>10,000	Bringmann 1976, 1977, 1980
Uronema parduzci	20	>10,000	Bringmann 1980, 1981

n.i.= not indicated

Conclusions

Glycerol is of low toxicity to bacteria with an EC_0 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 EC_{50} 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 EC_{50} 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 25ºC), 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.

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 LC_{50} 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 EC_{50} 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 calculated 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/EC_{50} 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 derm?); (kinet? or metabol? or endocrin?); (PBPK or PB-PK); (irritat? or sensitis? 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
Revision date	:	
Date of last update	:	29.01.2002
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
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 :

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; glycyl alcohol; trihydroxypropane; 1,2,3-trihydroxypropane; Citifluor AF 2; Glycerin mist; Glyceritol; Clyzerin, 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

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

08.01.2002

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

1.8.3 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 manchinery 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 Corrotion 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)

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 COMMISION - 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)

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 : 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 : < 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 COMMISION - 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 : CAS 56-81-5 (glycerine), purity 100%.
Reliability : (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

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/cm³ 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/cm³ 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/cm³ 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/cm³ 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/cm³ 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

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)logMW-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.

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 COMMISION - 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 :

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 COMMISION - 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

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

flash point.

03.01.2002 (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

EUROPEAN COMMISION - European Chemicals Bureau Ispra (VA)
19.12.2001 (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
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
13.05.1994 (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
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
21.05.1998

Value : ca. 180 °C
Type : open cup

Remark : Literature could not be retrieved.
Source : Wolff Walsrode AG Walsrode
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
14.05.1998

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
Handbook data are considered to be from a trusted source.
19.12.2001 (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

Handbook data are considered to be from a trusted source.
19.12.2001 (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
Handbook data are considered to be from a trusted source.
03.01.2002 (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
Handbook data are considered to be from a trusted source.
19.12.2001 (5) (4)

Value : = 422 °C at 1013.25 hPa
Method : other
Year : 1981
GLP :
Test substance :

Remark : Literature could not be retrieved.
Value given refers to autoignition temperature (739 Deg F)
Source : 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 COMMISION - 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

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%.

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

hydrolysis.

Reliability : (4) not assignable
11.12.2001

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, intracellulary 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

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

based on ThOD(NH3).

Test substance : CAS 56-81-5 (Glycerine), purity not indicated.

Conclusion :

Readily biodegradable.
Only study available, which was conducted to an OECD guideline.

Reliability : (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 $KMnO_4$-H_2SO_4 at 100 C for 30 minutes (catalyst: Ag_2SO_4)

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 : CAS 56-81-5 (glycerine), purity not indicated

Conclusion : 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

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

continously with photographic processing effluent.

19.12.2001 (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 CO2 & N2
- 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 NH4Cl, 400 mg/L KCl, 400 mg/L MgSO4.6H2O, 40 mg/L FeCl2.6H2O, 4 mg/L CoCl3, 80 mg/L (NH4)2HPO4, 10 mg/L cysteine, 10 mg/L KI, 10 mg/L Na hexameta phosphate, 0.5 mg/L MnCl2, 0.5 mg/L NH4V2O3, 0.5 mg/L ZnCl2, 0.5 mg/L Na2MoO4.2H2O, 0.5 mg/L H3BO3, NaHCO3 (to maintain an alkalinity of 3000 mg CaCO3/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)

Result : other: anaerobic biodegradation test. Degradation rate based on disappearance of test substance form 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), decreaese 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

Conclusion : Glycerol is degraded very easily to other reaction products, but ultimate biodegradation (formation CO2) 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 CO2). 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-14C] 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 14CO2 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 14CO2. 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

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 COMMISION - European Chemicals Bureau Ispra (VA)
Test condition : Concentration of activated sludge: 30 mg/l.
19.12.2001 (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 : CAS 56-81-5 (Glycerine)
25.01.2002 (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

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

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: 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).
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 :

Test substance :

Method : Calculation with Epiwin model.
Input logKow -1.65

Result : log BCF = 0.5

19.12.2001 (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/CO3 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 : CAS 56-81-5 (glycerine), purity not indicated.

15.11.2001 (36)

Memo : Microbial degradation

Remark : This study was performed to test the growth of Aspergillus versicolor and Pseudomonas aeruginosa on esters and alcohols. In this test glycerol was used as the control substrate. It can be concluded that both micro-organisms (A. versicolor and P. aeruginosa) are capable of growth using glycerol as the carbon source.

Test substance : CAS 56-81-5 (glycerine), chemically pure (probably >99%)

15.11.2001 (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

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

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 Lglass 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.

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
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

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

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)

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 turbity 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:
EC3 2900 mg/L

Test substance : CAS 56-81-5 (glycerine), purity not indicated

Conclusion : Most sensitive toxicity treshold 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 Pseudomonas putida with Microcystis aeruginosa and in Bringmann & Kuehn (Mitt. Internat. Verein. Limnol. 21: 275-284, 1978) the toxicity of Microcystis aeruginosa was compared to the toxicity of Scenedesmus quadricauda.

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
Limit test :
Analytical monitoring : no data
Method :
Year : 1978
GLP : no data
Test substance : other TS

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 turbity 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 : RESULTS:
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 turbity 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.

19.12.2001 (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 uM. 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 mu (test 1 and 2) & haemacytometrically (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 Agmenellum quadruplicatum and Anacystis marina.

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 Lemna gibba. The effects described for ethylene glycol are a relatively high EC50 with regard to frond reproduction, no metabolisation 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 : Pseudomonas putida (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 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 paramaecium (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 paramaecium 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 D-Biotin), 149 ug/L nicotinic acid, 74 ug/L thiamin.HCl, 74 ug/L p-aminobenzoic acid, 37 ug/L panthotenic acid (as D-Panthotenic acid, Na-salt), 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)
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 uM) 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 C. pasteurianum 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
Analytical monitoring : no
Method :
Year : 1980
GLP : no data
Test substance : other TS

Method : TEST ORGANISMS
- 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 $Ca(NO_3)_2.4H_2O$, 70 mg/L $Mg(NO_3)_2.6H_2O$, 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

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 $NaNO_3$, 0.12 g/L K_2HPO_4, 60 mg/L KH_2PO_4, 10 mg/L $FeSO_4.7H_2O$, 0.2 g/L $MgSO_4.7H_2O$, 0.083 mg/L $Al_2(SO_4)_3.18H_2O$, 0.042 mg/L KI, 0.042 mg/L KBr, 0.083 mg/L TiO_2, 0.042 mg/L $SnCl_2.2H_2O$, 0.042 mg/L LiCl, 0.58 mg/L $MnCl_2.4H_2O$, 0.92 mg/L H_3BO_3, 0.083 mg/L $ZnSO_4.7H_2O$, 0.083 mg/L $CuSO_4.5H_2O$, 0.089 mg/L $NiSO_4.6H_2O$, 0.083 mg/L $Co(NO_3)_2.6H_2O$

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 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 Pseudomonas putida.
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 : Pseudomonas putida (Bacteria)
Exposure period : 75 minute(s)
Unit : mg/l
Method :
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:
IC50 760000 mg/L
Test substance : CAS 56-81-5 (glycerine), purity not indicated.

19.12.2001 (12)

Type : aquatic
Species : Uronema parduzci (Protozoa)
Exposure period : 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 $CaCl_2.2H_2O$, 86 mg/L $MgSO_4.7H_2O$, 29 mg/L KH_2PO_4, 57 mg/L K_2HPO_4
- medium used for pre-and test culture: 290 $Ca(NO_3)_2.4H_2O$, 70 mg/L $Mg(NO_3)_2.6H_2O$, 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)

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 COMMISION - 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

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)

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 glyerine, 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) (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 (64)

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

- 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 :

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.

17.12.2001 (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

NECROPSY FINDINGS: not reported

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.
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

EUROPEAN COMMISION - 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
The information was confined to the above.
16.11.2001 (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
The information was confined to the above.
16.11.2001 (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
The information was confined to the above.
16.11.2001 (65)

Type : LD50
Value : = 28000 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 : = 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

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 COMMISION - European Chemicals Bureau Ispra (VA)

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 COMMISION - 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 : Critical study for SIDS endpoint
17.12.2001 (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. Heamoglobinuria 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 distroyed.
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 COMMISION - 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)

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 COMMISION - 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 COMMISION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
25.01.2002 (83)

Type : LD50

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)

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 COMMISION - 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 :

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

The same method was used in 20 different laboratoria.
Result : 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

REVERSIBILITY: yes, within 24 h

Test substance : CAS 56-81-5 (glycerine), purity not indicated (Glycerol/Water mixture of unknown composition).
Conclusion : Not irritating.
Reliability : (4) not assignable
Secondary literature; the information in the report is confined to the above.

25.01.2002 (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
The information in the report is confined to the above.

25.01.2002 (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.

17.12.2001 (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

EXAMINATIONS
- Grading system: diameter, height and colour of the reaction compared to the reaction at the first sensitizing injection

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.

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

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 COMMISION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable

25.01.2002 (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 : (4) not assignable

25.01.2002 (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/m3
Control group : yes, concurrent no treatment
LOAEL : = 1000 mg/m³
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 um (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)

STATISTICAL METHODS: ANOVA, least significant difference

Result : 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/m3 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 : Critical study for SIDS endpoint

25.01.2002 (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/m3
Control group : yes, concurrent no treatment
NOAEL : = 167 mg/m³
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/m3 (calculated to be equiavlent 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 um (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

- 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

Result : 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 : CAS 56-81-5 (glycerine), purity >99.8%

Conclusion : NOAEL 167 mg/m3 based on local irritant effects on 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 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 : Critical study for SIDS endpoint

25.01.2002 (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-sqare 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 abcesses, taenia infestation of the liver, hydronephosis 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 : Critical study for SIDS endpoint

25.01.2002 (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

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 excercise, water intake and urinary output. All animals that died and a selection of the survivors were necropsied.
Another selection was sacrified 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 (calulated 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 euivalent 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

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 : (4) not assignable

25.01.2002 (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 : (4) not assignable

25.01.2002 (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/cm3, 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.

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/cm3, 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/cm3, 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/cm3, water intake of 22.5 ml/d and average bodyweight of 0.425 kg)

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 atrophia 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/cm3, 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 :

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 abormal 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 :

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

STATISTICAL METHODS: not indicated

Result : 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 histopathalogical 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
- O2-consumption: decreased on day 3, 9, 18 and 21

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)

OBSERVATIONS: gastro-intestinal irritation, macroscopic and microscopic investigation of stomach and intestinal mucosa

Result : 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

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 COMMISION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
25.01.2002 (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 severly hyperaemic with petechial haemorrhages
At 3800 mg/kg bw: the stomach mucosa was (slightly to) severly 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/cm3)
Control group :

Method : One animal/sex/treatment received glycerine during 4 months (after 1 months there was a two-week interruption).

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 avaerage 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 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

21.01.2002 (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: 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 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
21.01.2002 (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
- Deficiences: 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

CYTOTOXIC CONCENTRATION: no cytotoxicity up to 1000 ug/plate

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 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 : Critical study for SIDS endpoint
17.12.2001 (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
- Proficiences: 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 : Critical study for SIDS endpoint
17.12.2001 (106)

Type : Sister chromatid exchange assay
System of testing : CHO cells

Test concentration : 200-1000 ug/ml
Cycotoxic concentr. : no cytoxicity 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 (106)

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

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 : Critical study for SIDS endpoint
25.01.2002 (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)

CRITERIA FOR EVALUATING RESULTS:
A statistically significant, reproducible and dose-dependent increase in frequency of cells with aberrations compared to solvent control.

Result : 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 : Critical study for SIDS endpoint
25.01.2002 (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 <= 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 stimulatated 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.

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

STATISTICAL METHODS: Student's t-test, chi-square test

Result : 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:
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
- 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.

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

STATISTICAL METHODS: not specified

Result : 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 idetified 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
treament (4 weeks glycerine) 8/10
treament (25 weeks glycerine) 8/9
treament (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
treament (4 weeks glycerine) 3.5
treament (25 weeks glycerine) 2.3
treament (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 (118)

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)

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 COMMISION - 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-sqare 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 im 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 glycerin, purity 99.5% (rest mainly water with very small amounts of glycerin polymers and glyceraldehyde).

Conclusion : No increased incidence in tumour incidence follwing treatment with glycerol.

Reliability : (2) valid with restrictions
The report was confined to the above.

Flag : Critical study for SIDS endpoint

29.01.2002 (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 : Critical study for SIDS endpoint

18.12.2001 (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
HISTOPATHOLOLOGY:
Day 7: nucleated bodies in seminiferous tubules (almost no spermatogenic cells), normal germ cells and Leidig cells
Day 15 and 73: few nucleated bodies in seminiferous tubules (almost no spermatogenic cells), normal Leidig 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

Remark : Literature could not be retrieved.
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
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
25.01.2002 (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: TDL0
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 COMMISION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
25.01.2002 (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 female

PARAMETERS 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 foetuses
- Examination of foetuses: body weight, sex, external abnormalities, visceral (1/3 of foetuses) and skeletal (2/3 of foetuses) examination

ORGANS EXAMINED AT NECROPSY: urogenital tract

STATISTICAL METHODS: not indicated

Result : 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.

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 foetuses
- Examination of foetuses: body weight, sex, external abnormalities, visceral (1/3 of foetuses) and skeletal (2/3 of foetuses) examination

ORGANS EXAMINED AT NECROPSY: urogenital tract

STATISTICAL METHODS: not indicated

Result : 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 foetuses
- Examination of foetuses: body weight, sex, external

abnormalities on day 29, neonatal survival, visceral and skeletal examination on day 30

ORGANS EXAMINED AT NECROPSY: urogenital tract

STATISTICAL METHODS: not indicated

Result : 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 : Critical study for SIDS endpoint
25.01.2002 (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 wheteher 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 refereces 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 in 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 COMMISION - 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 COMMISION - 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 : No overt signs of toxicity or effect on food consumption
24.09.2001 (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 : During treatment, mortality of the group with cerebral infarction was 11%, howerver 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.
14.11.2001 (127) (129)

Method : Skin patch test in 15 workers with glycerol diluted 500 to 1000 times with water.
Result : Negative
24.09.2001 (90)

Result : 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.
24.09.2001 (130)

Remark : 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.
24.09.2001 (131) (132)

Result : A case of acute colonic ischemia following a glycerin enema in preparation for coronary artery bypass surgery was reported.
24.09.2001 (133)

Result : 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
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
16.05.1994 (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 COMMISION - 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 COMMISION - 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 COMMISION - 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 COMMISION - 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

pulmonary tumorgenesis, 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.

24.09.2001 (140)

Type : Biochemical or cellular interactions

Remark : Glycerol-related lung tumorgenesis enhancing effects found in tests with mice were inhibited by vitamine 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 COMMISION - 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

25.01.2002 (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 CO2;
In lean persons (long fasting) 76% of plasma glycerol (0.142 umol.ml) was turned into glucose and 5.4% into CO2;
In obese persons (short fasting) 56% of plasma glycerol (0.105 umol/ml) was turned into glucose and 3.1% into CO2;
In obese persons (long fasting) 96% of plasma glycerol (0.180) was turned into glucose and 4.4% into CO2;

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

levels were reduced; it cannot be stated unequivocally however, that glycerol is
hypocholesteremic.

24.09.2001 (148)

Remark : Glycerol has a hemolytical potency. With 0.002 ml Glycerol/ml blood hemolysis in a 10% extent was observed for bovine erytrocytes.
Pharmacodynamics: glycerol acts as a contact laxative.
Literature could not be retrieved.

Source : Unichema Chemie B.V. Gouda

Reliability : (4) not assignable

25.01.2002 (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 intraperitonealy 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

days), both men and women showed increased serum glyceride concentration. The increase was significantly greater in men, however.

11.12.2001 (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
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
25.01.2002

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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 (*1*) 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 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/pcs

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 assessment[1], 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 adulturated 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 *116*), 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 mm^2/s; P70 oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s; P70(H) oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation); N70(H) oil, crude: naphthenic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation; P15(H) oil, crude: paraffinic, viscosity (40 °C): 15 mm^2/s, hydrotreated (catalytic hydrogenation; N15(H) oil, crude: naphthenic, viscosity (40 °C): 15 mm^2/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 [^{14}C]1-eicosanylcyclohexane 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 immunoglobin 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.

Toxicological and epidemiological data

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*-nitrosopiperidine.

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 nitate 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 **s**hort- **a**nd **l**ong-chain **a**cyl**tri**glyceride **m**olecules. 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 *131*), 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 ≤ 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	Brillant Black BN	0151		-	2	-	-
Colour	Brillant 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 *Blakeslea trispora*	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 *Bacillus subtilis*	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.

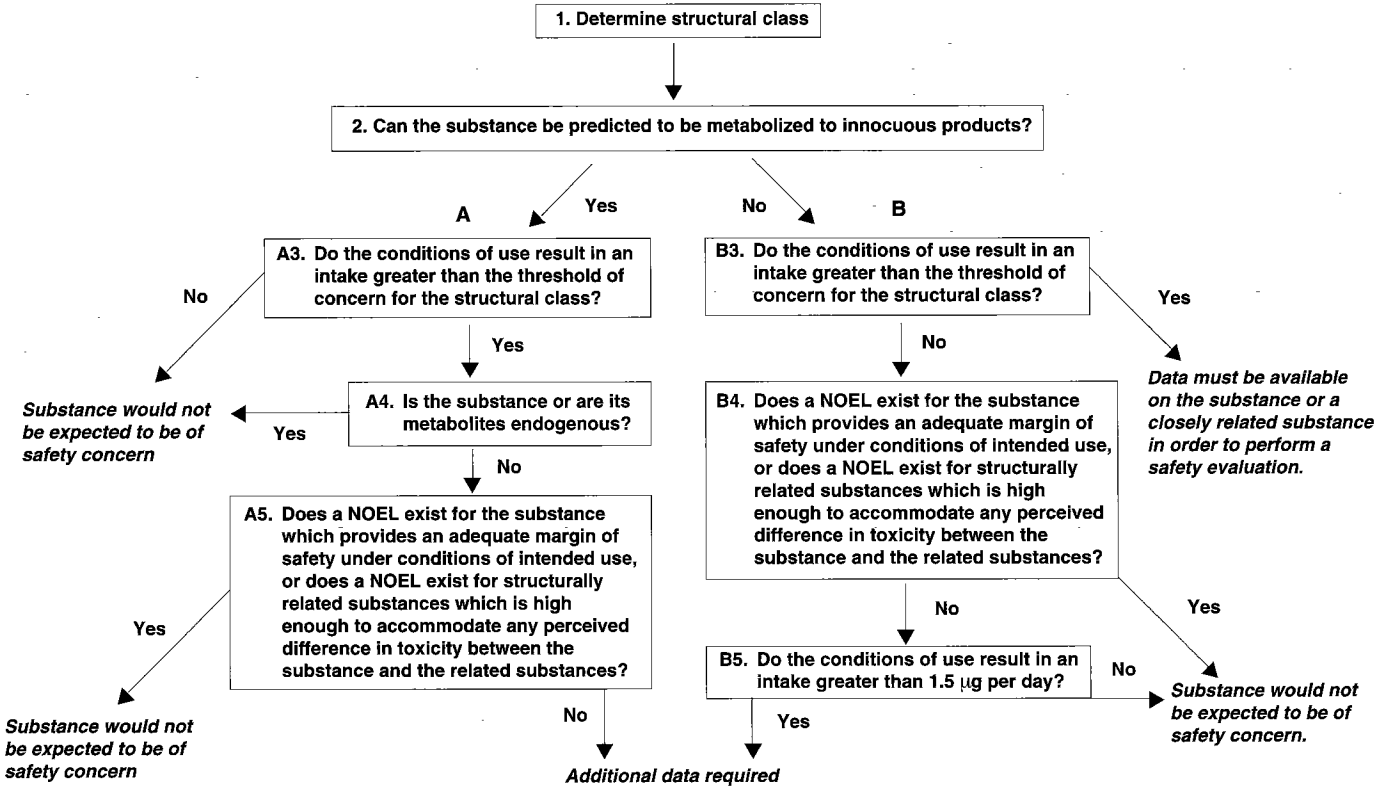


Figure 1
Procedure for the safety evaluation of flavouring agents

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.

$$\text{Intake (}\mu\text{g per person per day)} = \frac{\text{annual volume of production (kg)} \times 10^9\ (\mu\text{g/kg})}{\text{population of consumers} \times 0.6 \text{ or } 0.8 \times 365 \text{ days}}$$

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

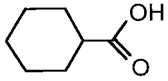
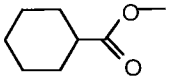
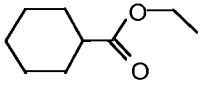
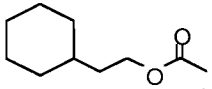
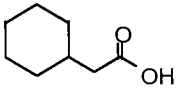
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Structural class I						
Cyclohexanecarboxylic acid	961	98-89-5 	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
cis-5-Isopropenyl-*cis*-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)

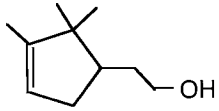
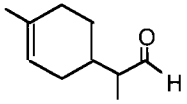
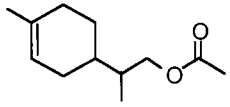
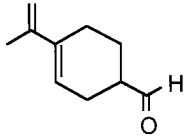
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
α-Campholenic alcohol	970	1901-38-8 	A3 – No Europe: 0.01 USA: ND	N/R	See note 4	No safety concern
p-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-*p*-Menthen-9-yl acetate	972	17916-91-5 	A3 – No Europe: 1 USA: ND	N/R	See notes 2,4	No safety concern
p-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)

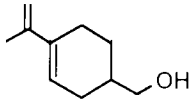
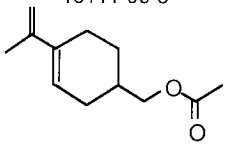
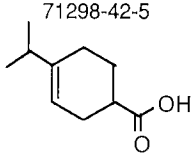
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
p-Mentha-1,8-dien-7-ol	974	536-59-4 	A3 – No Europe: 2 USA: 1	N/R	See note 4	No safety concern
p-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)

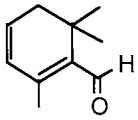
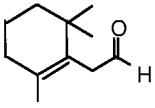
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	977	116-26-7 	B3 – 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)

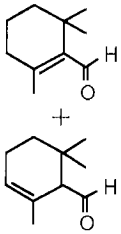
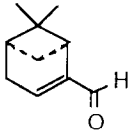
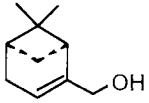
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethyl-1-cyclohexen-1-carboxaldehyde	979	432-25-7 	A3 – 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[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
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)

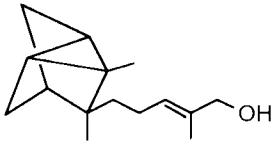
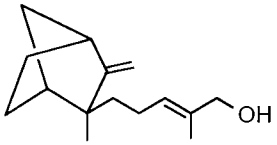
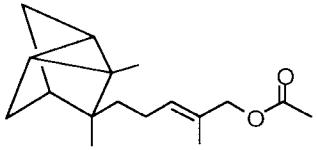
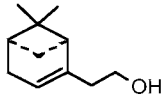
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Santalol (α and β)	984	115-71-9  α 77-42-9  β	A3 – No Europe: 0.04 USA: ND	N/R	See note 4	No safety concern
Santalyl 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-dimethyl-bicyclo[3.1.1]hept-2-ene (No. 980; myrtenal). The estimated daily *per capita* intake of 11 of the substances in this group is ≤ 10 μg.

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 dehydro-dihydroionol (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[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
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[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
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)

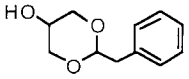
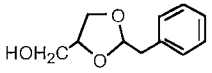
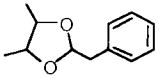
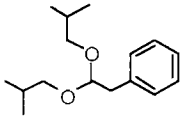
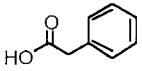
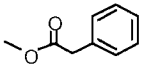
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenylacetaldehyde 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

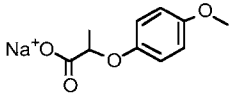
Table 4 (contd)

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 α β	1323-75-7	No Europe: ND USA: 1	N/R	N/R	See note 9.	No safety concern
p-Tolyacetaldehyde	1023	104-09-6	No Europe: 6 USA: 3	N/R	N/R	See note 5.	No safety concern
p-Isopropylphenyl-acetaldehyde	1024	4395-92-0	No Europe: 0.1 USA: 0.01	N/R	N/R	See note 5.	No safety concern
Methyl *para-tert*-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 (*p*-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 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-methoxy-phenoxy)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 phenyacetaldehyde (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*-tolyloxy 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 phenyacetaldehyde 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*-tolyloxy)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*-tolyloxy)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*-tolyloxy)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]

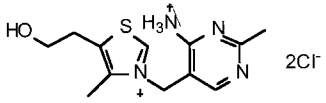
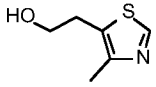
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
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-methyl-propyl)-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-dimethyl-thiazole 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-trimethyl-thiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

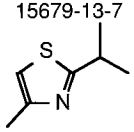
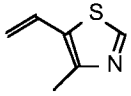
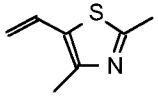
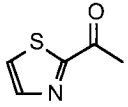
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-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	1759-28-0 	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)*

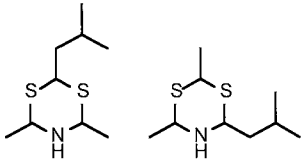
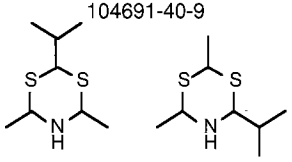
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyl-dihydro-1,3,5-dithiazine (mixture)	1046	101517-87-7 101517-86-6  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-dimethyl-dihydro-1,3,5-dithiazine (mixture)	1047	104691-41-0 104691-40-9  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 *H*-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 *H*-1,3,5-dithiazine when used as a flavouring agent.	No safety concern
2,4,6-Trimethyl-dihydro-4 *H*-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 *H*-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 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
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-dimethyl-thiophene when used as a flavouring agent.	No safety concern
4-Methyl-5-thiazoleethanol acetate	1054	656-53-1	Yes[e] 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)*

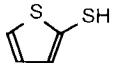
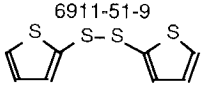
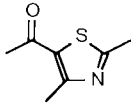
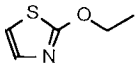
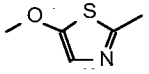
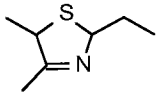
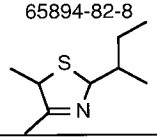
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring 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 aromatic[1]. 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 pyrole 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 thesulfur-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-dimethyldihdro-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*H*-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*H*-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-carboxyaldehyde	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-carboxyaldehyde	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
[a]90-day study in rats
[b]14-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]

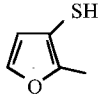
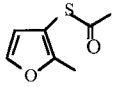
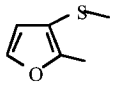
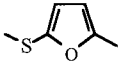
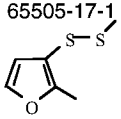
Flavouring agent	No.	CAS No. and structure	*Step B3*[b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Structural class II					
2-Methyl-3-furanthiol	1060	28588-74-1 	No Europe: 0.6 USA: 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-thiofuroyl 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-thiofuroyl 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 estimqted intake of 4-[(2-methyl-3-furyl)thio]-5-nonanone when used as a flavouring agent.	No safety concern
Ethyl 3-(furfuryl-thio)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 at when used as a flavouring agent.	No safety concern
cis- and *trans*-2,5-Dimethyl-3-tetrahydro-furanthiol	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
cis- and *trans*-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 *cis*- and *trans*-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 age 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			5[a]	0.015	> 100 000
		Ethanoic acid, *S*-(2-methyl-3-furanyl) ester	1069		0.001	> 1 000 000
		2,5-Dimethyl-3-furanthiol	1063		0.01	> 100 000
Methyl 2-methyl-3-furyl disulfide	1064			1.2[a]	0.014	> 10 000
		Propyl 2-methyl-3-furyl disulfide	1065		0.01	> 100 000
		Methyl furfuryl disulfide	1078		0.017	> 10 000
		Furfuryl propyl disulfide	1079		0.04	> 10 000
Bis(2-methyl-3-furyl) disulfide	1066			0.45[a]	0.01	> 10 000
		Bis(2,5-dimethyl-3-furyl) disulfide	1067		0.01	> 10 000
Furfuryl mercaptan	1072			3[a]	0.58	> 1 000
		Furfuryl thioformate	1073		0.026	> 100 000
		Furfuryl thiopropionate	1075		0.0002	> 1 000 000
		2,2′-(Dithiodimethylene) difuran	1081		0.064	> 10 000
Furfuryl thioacetate	1074			0.83[a]	0.008	> 100 000
		Furfuryl thioformate	1073		0.026	> 10 000
		Furfuryl thiopropionate	1075		0.0002	> 1 000 000
4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084			54[b,c]	0.01	> 1 000 000
		2-Methyl-3-tetrahydrofuranthiol	1090	12[b,c]	0.07	> 100 000
2,2′-(Thiodimethylene) difuran	1080			10[b]	0.014	> 100 000

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
		Methyl thiofuroate	1083		0.002	> 100 000
Furfuryl isopropyl sulfide	1077			1.3[a]	0.002	> 100 000
		2-Methyl-3-(methylthio)furan	1061		0.024	> 10 000
		2-Methyl-5-(methylthio)furan	1062		0.021	> 10 000
		Furfuryl methyl sulfide	1076		0.02	> 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)thiol]-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-thioacetoxy-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

[a]90-day study
[b]14-day study
[c]This study was not used in the evaluation, as a 90-day study on a structurally related flavouring agent was available.
[d]1-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 μg 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-Trimethyl cyclohexanol	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					
cis- and *trans-para*-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

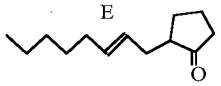
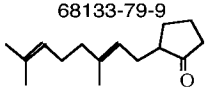
Table 9 *(continued)*

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-Trimethylcyclo-hexanone	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-cyclo-hexenone	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
Isojasmone	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[a,b] 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[a,b] 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	110-93-0	No Europe: 120 USA: 44	See notes 1 and 4.	No safety concern
3,4,5,6-Tetrahydro-pseudoionone	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

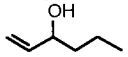
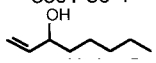
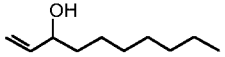
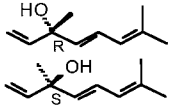
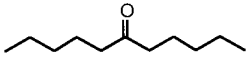
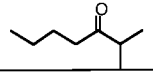
Table 10 *(continued)*

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[a,b] 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 decison 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.

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[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 adulturated 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 *tributyrin (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
850	Benzoic acid[a]	≤ 100	Aromatic acid with a sweet–sour, acrid 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-*para*-hydroxybenzoate	20 (baked goods) 1000 (beverages)	Flavour depends on hydrolysis to *butyl alcohol* and *para-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'
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 *oleic acid* 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 *glycerol* and *acetic acid*	No safety concern[b]
921	Glyceryl tripropionate (Tripropionin)	≤ 50	Flavour depends on hydrolysis in heated or otherwise processed foods to *glycerol* and *propionic acid*	No safety concern[b]
922	Tributyrin	≤ 100	Flavour depends on hydrolysis in heated or otherwise processed foods to *glycerol* and *butyric acid*	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-*decalactone* (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-*dodecalactone* (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 *stearic acid* 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 *para*-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-(*para*-Tolyl)-2-butanone	R
847	Benzyl 2,3-dimethylcrotonate	R
866	Tolualdehydes (mixed *ortho*, *meta* and *para*)	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 *cis*-3-hexenyl acetal	R
954	Ethyl vanillin propylene glycol acetal	R

[a]R, 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

[a]R, 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

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158. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 49, 2002.
159. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/14, 2002.

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 teolerance 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 ar ising 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 hat this criterion must be used with in the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be usedat 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 Annex3.

Food additive considered for specifications only

Food additive	Specifications[a]
Amyloglucosidase from *Aspergillus niger*, var.	R

[a]R, 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.	Specifications[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
para-Menth-1-en-9-al	971	N	No safety concern
1-*para*-Menthen-9-yl acetate	972	N	No safety concern
para-Mentha-1,8-dien-7-al	973	N	No safety concern
para-Mentha-1,8-dien-7-ol	974	N	No safety concern
para-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

[a]N, new specifications prepared

B. Phenethyl alcohol, aldehyde, acid and related acetals and esters

Flavouring agent	No.	Specifications[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.	Specifications[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
para-Tolylacetaldehyde	1023	N	No safety concern
para-Isopropylphenylacetaldehyde	1024	N	No safety concern
Methyl *para-tert*-butylphenylacetate	1025	N	No safety concern
Phenoxyacetic acid	1026	N	No safety concern
Ethyl (*para*-tolyloxy)acetate	1027	N	No safety concern
2-Phenoxyethyl isobutyrate	1028	N	No safety concern
Sodium 2-(4-methoxyphenoxy)propanoate	1029	N	No safety concern

[a]N, new specifications prepared

C. Sulfur-containing heterocyclic compounds

Flavouring agent	No.	Specifications[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*H*-1,3,5-dithiazine	1048	N	No safety concern
2,4,6-Trimethyldihydro-4*H*-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.	Specifications[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

[a]N, new specifications prepared

D. Sulfur-substituted furan derivatives

Flavouring agent	No.	Specifications[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-thioacetoxy-4,5-dihydrofuran	1089	N	No safety concern
2-Methyl-3-tetrahydrofuranthiol	1090	N	No safety concern
2,5-Dimethyl-3-tetrahydrofuranthiol, *cis* and *trans* isomers	1091	N	No safety concern
2,5-Dimethyl-3-thioacetoxy-tetrahydrofuran, *cis* and *trans* isomers	1092	N	No safety concern

[a]N, new specifications prepared

E. Alicyclic ketones, secondary alcohols and related esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Cyclohexyl acetate	1093	N	No safety concern
Cyclohexyl butyrate	1094	N	No safety concern
Cyclohexyl formate	1095	N	No safety concern
Cyclohexyl isovalerate	1096	N	No safety concern
Cyclohexyl propionate	1097	N	No safety concern
cis and *trans-para*-1(7)8-Menthadien-2-yl acetate	1098	N	No safety concern

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
Isojasmone	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

[a]N, 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.	Specifications[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

[a]N, 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-(*para*-Tolyl)-2-butanone	R
847	Benzyl 2 3-dimethylcrotonate	R
861	Glyceryl tribenzoate	R
862	Propylene glycol dibenzoate	S
866	Tolualdehydes (mixed *ortho* *meta* and *para*)	R
870	Butyl *para*-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

[a]N, 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-*para*-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)	Secondary components do not raise safety concern.
55	Neryl formate	85	4–6% geraniol; 1–3% nerol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
56	Rhodinyl 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-$^{\delta}$-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	*cis*-5-Octenal	85	10–15% *trans*-5-octenal	*trans*-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	*cis*-6-Nonenal	90	6–9% *trans*-6-nonenal	*trans*-6-Nonenal is oxidized to 6-nonenoic 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	Linalyl propionate	92	2–5% linalool	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.
384	β-Damascone	90 (sum of *cis* and *trans* 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 *cis* and *trans* isomers)	92–96% *trans*, 4–8% *cis* isomers	The NOEL in a 90-day study with α-damascone containing a *trans*- and *cis*-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 *cis* and *trans* 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 *cis* and *trans* 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-diethylcyclo-hexadien-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 cyclo-pentenolone	90	5–10% 3-ethylcyclo-pentan-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	*para*-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% citronellol	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 citronellol, see No. 53 above.	Secondary components do not raise safety concern.
604	3-(Hydroxymethyl)-2-heptanone	90	5–8% 1-hydroxy-3-ocatanone	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	Linalyl 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-homocyclogeranate; 0.6% ethyl β-homocyclogeranate	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-homocyclogeranate and ethyl β-homocyclogeranate 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 hydroysed 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-isopropy-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-dimethyldi-hydro-1,3,5-dithiazine; 27% 4-isopropyl-2,6-dimethyldi-hydro-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-triisopropyl-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% *cis*- and *trans*-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, *cis* and *trans* 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*-Butylcyclo-hexanone	94	2–2.5% 2-isobutyl-cyclohexanone	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).	Secondary components do not raise safety concern.
1128	3-Octen-2-one	94	4–6% 4-octen-2-one	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.
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	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.
1137	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadien-2-one	94	3–4% 4,8-dimethyl-3,7-nonadien-2-ol	As the secondary component is an alcohol which is interconvertible in vivo with the ketone, the safety evaluation appplies to both.	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	For data on linalool, see No. 358 above.	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
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EINECS Name	glycerol
EINECS No.	200–289–5
Molecular Formula	C3H8O3

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Street: rue de l'Industrie 52
Town: 10400 Brussels
Country: Belgium
Phone: 0032–2–288.91.11
Telefax: 0032–2–288.33.88

Name: Henkel KGaA
Street: Henkelstr. 67
Town: 40589 Duesseldorf
Country: Germany

Name: Huels AG
Street: Postfach
Town: D–45764 Marl
Country: Germany

Name: IFF
Street: Duddery Hill
Town: CB9 8LG Haverhill, Suffolk
Country: United Kingdom
Phone: +44 1440 715000
Telefax: +44 1440 762199

Name: KAO Corporation S.A.
Street: Puig dels Tudons 10
Town: 08210 Barbera del Valles
Country: Spain

Name: Karlshamns Tefac AB
Town: 374 82 Karlshamn
Country: Sweden
Phone: +46 454 82300
Telefax: +46 454 752005

Name: Kays (Ramsbottom) Ltd
Street: Kenyon Street
Town: BL0 0AE Ramsbottom, Bury, Lancs.
Country: United Kingdom
Phone: 01706 822216
Telefax: 01706 828615

Name: Lever Brother Ltd.
Street: Lever House 3, St James Road
Town: KT1 2BA Kingston Upon Thames, Surrey
Country: United Kingdom
Phone: 44 (0)81 541 8200
Telefax: 44 (0)51 641 4308

Name: Lever GmbH
Street: P.O. Box 104180
Town: 20028 Hamburg
Country: Germany

Name: NEUBER GES.M.B.H.
Street: BRÜCKENGASSE 1
Town: 1060 WIEN
Country: Austria
Phone: 0222/599950
Telefax: 0222/5970200

Name: Novartis Crop Protection UK Ltd. (formerly Ciba–Geigy Crop Protection UK Ltd.)
Street: Hill Farm Road
Town: CB2 4QT Whittlesford
Country: United Kingdom
Phone: 44 1 223 83 3621
Telefax: 44 1 223 83 5211

Name: PROCTER & GAMBLE (CHEMICALS EUROPE) LTD.
Street: P.O. BOX 9, HAYES GATE HOUSE, 27 UXBRIDGE ROAD
Town: UB4 OJD HAYES MIDDLESEX
Country: United Kingdom
Phone: 44812422200
Telefax: 44812422333
Telex: 936310

Name: Pronova Oleochemicals a.s.
Street: Framnesveien 54
Town: 3202 Sandefjord
Country: Norway
Phone: +4733448800
Telefax: 33448899
Telex: 70410

Name: Pulcra S.A.
Street: Pasaje Mariner, 9
Town: 08025 Barcelona
Country: Spain

Name: Quest International
Street: Lindtsedijk 8
Town: 3336 LE Zwijndrecht
Country: Netherlands
Phone: (78)109920
Telefax: (78)195279

Name: Simel S.p.A.
Street: Via Bergamo 66, Casella Postalc 204
Town: 26100 Cremona
Country: Italy

Name: Simel S.p.A. Industria Chimica
Street: Via Bergamo 66
Town: 26100 Cremona
Country: Italy
Phone: +39 (0)372 4871
Telefax: +39 (0)372 412123
Telex: 412123

Name: Solvay Alkali GmbH
Street: Postfach 110270
Town: 42662 Solingen
Country: Germany

Name: SPIGA nord S.p.A.
Street: Pontevecchio,55
Town: 16042 CARASCO (GE)
Country: Italy
Phone: ++39–185–350241
Telefax: ++39–185–350971
Telex: ++283058 SPIGA

Name: Unichema Chemicals Ltd
Street: Wirral, Merseyside L62 4UF
Town: Bebington
Country: United Kingdom
Phone: 44 (0)51 645 2020
Telefax: 44 (0)51 645 9197
Telex: 629408

Name: Unichema Chemie B.V.
Street: PO Box 2
Town: 2800 AA Gouda
Country: Netherlands

Name: Unichema Chemie GmbH
Street: Steintor 9
Town: D–46446 Emmerich
Country: Germany
Phone: +49–2822–720
Telefax: +49–2822–72276

Name: Unichema International B.V.
Town: 2800 AA Gouda
Country: Netherlands

Name: UNION DERIVAN S.A.
Street: Avda. Generalitat 175–179
Town: 08840 VILADECANS
Country: Spain
Phone: (93)6373537
Telefax: (93)6591902

Name: VOS B.V.
Street: Ondernemingsweg 1A
Town: 2404 HM Alphen aan den Rijn
Country: Netherlands
Phone: 31–172–431601
Telefax: 31–172–432494

Name: Witco Espana S.L.
Street: Poligono Industrial Congost – Avda. Sant Julia,
Town: 08400 Granollers
Country: Spain
Phone: E–93.864.21.00
Telefax: E–93.864.21.11

Name: WITCO Surfactants GmbH
Street: Industriegebiet West
Town: 36396 Steinau a. d. Straße
Country: Germany

Name: Wolff Walsrode AG
Street: Postfach 1515
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;Trihydroypropane
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

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 all (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 paises 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 limites de exposición profesional, sugirió el mismo valor de 10 mg/m3.
Un estudio reciente de inhalación subcrónica de Renne et all (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 limite 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 all (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 all (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 contries, 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 Exoposure 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 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.
Source: 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.
A recent subchronic inhalation study from Renne et all (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 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.
A recent subchronic inhalation study from Renne et all (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 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

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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

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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 gassess 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 gassess 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 gassess 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 gassess 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 x 10exp9 l/mol.sec.
Source: Simel S.p.A. Industria Chimica Cremona
Test condition: Sensitizer (OH radical) concentration in water: 10exp–17 mol/l.

(30)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance:
Result: Rate constant: 0.95 x 10exp9 l/mol.sec.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
Test condition: Sensitizer (OH radical) concentration in water: 10exp–17 mol/l.

(31)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance:
Result: Rate constant: 0.95 x 10exp9 l/mol.sec.
Source: Unichema Chemie GmbH Emmerich
Test condition: Sensitizer (OH radical) concentration in water: 10exp–17 mol/l.

(31)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance:
Result: Rate constant: 0.95 x 10exp9 l/mol.sec.
Source: Lever GmbH Hamburg
Test condition: Sensitizer (OH radical) concentration in water: 10exp–17 mol/l.

(31)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance:
Result: Rate constant: 0.19 x 10exp10 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: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

(34)

3.1.3 Stability in Soil

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Remark: no disponible
Source: UNION DERIVAN S.A. VILADECANS

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Remark: No data.
Source: Croda Universal Ltd Goole, North Humberside

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Source: Wolff Walsrode AG Walsrode

(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, intracellulary as lipids.
– It is an important intermediair 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 moleculesof 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, intracellulary as lipids.
– It is an important intermediair 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, intracellulary as lipids.
– It is an important intermediair 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, intracellulary as lipids.
– It is an important intermediair 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 x 10exp.–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 x 10exp.–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 x 10exp.–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 x 10exp.–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 continously 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: t1/2 – 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

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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: – Rainbouw 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;
– Unmeasered 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–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.
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
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 >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

Other species were tested and gave similar results.
Source: 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)

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)

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)

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)

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)

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)

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)

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)

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)

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)

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)

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)

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)

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 unknowen 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)

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 onhuman skin in a closed patch test. Further the investigatorsobserved a maximum score for irritation of 4 on a scale of 9at 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)

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)

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)

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)

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, andcalorie 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

(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 thegroups 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)

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)

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)

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)

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)

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: 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)

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)

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: 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)

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)

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 kindneys, livers, and bladders of the rats disclosed no untoward effects.
Source: Simel S.p.A. Industria Chimica Cremona

(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 invervals 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)

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 kindneys, 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 invervals 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 invervals of 6 weeks, remained without abnormal findings.
Source: Unichema Chemie GmbH Emmerich (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: 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 invervals of 6 weeks, remained without abnormal findings.
Source: Lever GmbH Hamburg

(128)

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
Year: **GLP:** no data
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 autopsiedat 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: 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
Year: **GLP:** no data
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 Brother Ltd. Kingston Upon Thames, Surrey
(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
Year: **GLP:** no data
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
Year: **GLP:** no data
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)

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 fromeither 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)

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)

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)

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: 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
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: Unichema Chemie GmbH Emmerich

(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
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: 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
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: Simel S.p.A. Industria Chimica Cremona

(131)

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)

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 wth cerebral infarction was 11%, howerver 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)

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 wth cerebral infarction was 11%, howerver 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)

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 wth cerebral infarction was 11%, howerver 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
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 wth cerebral infarction was 11%, howerver 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)

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

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–
Year: 1977 **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: 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 aberations in rat bone marrow cells. This resultis 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 structurallysimilar 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 aberations 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 aberations 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 aberations 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)

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 themean 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)

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)

Species: mouse **Sex:**
Strain: other
Route of admin.: drinking water
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses: 5% solution
Result:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: IUCLID DATA SHEET:
Remark:
The promoting effect of glycerol on the pulmanory 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 weeksafter 4NQO treatment, compared with mice given 4NQO alone.
Source: Wolff Walsrode AG Walsrode

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: Simel S.p.A. Industria Chimica Cremona

(153)

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)

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)

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)

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)

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 oftoxic or endocrine effects.
Source: Wolff Walsrode AG Walsrode

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)

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)

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 performace of the untreated F1 generation, and no histological changes occured 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 performace of the untreated F1 generation, and no histological changes occured in the tissues of both the F1 and F2 generation.
Source: Lever Brother Ltd. Kingston Upon Thames, Surrey
(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 performace of the untreated F1 generation, and no histological changes occured 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 performace of the untreated F1 generation, and no histological changes occured in the tissues of both the F1 and F2 generation.
Source: Lever GmbH Hamburg
(164)

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 ot 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 descernible effect on nidation or on maternal or fetal suvival.
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 descernible effect on nidation or on maternal or fetal suvival.
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 descernible effect on nidation or on maternal or fetal suvival.
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 descernible effect on nidation or on maternal or fetal suvival.
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 ratsand dogs.
Phosphorylation of glycerine to alpha–glycerophosphate takesprimarily 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 erytrozytes.
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 abilityto 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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(33) IUCLID DATA SHEET:
Photodegradation
Type: air
Sensitizer: OH
Rate constant: 0.95 x 10exp9 l/mol.sec

(34) IUCLID DATA SHEET:
keine Daten (no data)

(35) Criteria document for an occupational exposure limt value of: GLYCEROL. SEG/CDO/30 (1992).

(36) IUCLID DATA SHEET:
Glycerol occurs naturally in all animals and vegetables, in combined form as glycerides in fats and oils, or, intracellulary as lipids.

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(39) IUCLID DATA SHEET:
Type: other: soil permeability
Media: water – soil
Method: other: soil column transport

Result:
– Soil movement: 0.9 x 10exp.–9 cm/sec.

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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.

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(58) Juhnke I. & Lüdemann D. (1978). Z. Wasser–u. Abwasser–Forsch. 11, 161.

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Ambiguous. The authors state that glycerol induces chromosomal aberations in frat bone marrow cells. This result is in contrast to all in–vitro mammalian mutagenicitydata. Because the purity of the test substance is not mentioned, it is feasible that some undiluted contamination is responsible for the observed effect. ...

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7.1 Risk Assessment

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Safety Data Sheet

For Compliance with OSHA 29 CFR 1910.1200 and ANSI Z400.1-1998

Section 1: Identification

Product Name Glycerin USP (Natural)
Commercial Name Not available.
Product Use Intermediate for making glycerol derivatives.
Restrictions On Use Not available.

Product Code 30-2865

Company
PCCA
9901 South Wilcrest
Houston, TX 77099
Phone: 1-800-331-2498
Fax: 1-800-874-5760

In case of emergency contact:
CHEMTREC (24hr) 1-800-424-9300

Section 2: Hazard(s) Identification

OSHA Haz Com: CFR 1910.1200 Not available

Signal Word NON-HAZARDOUS

Hazard Statement(s) Not available

Pictogram(s) or Symbol(s)

Precautionary Statement(s):

Prevention Wear protective gloves, protective clothing, eye protection, face protection.
Response If skin irritation occurs: Get medical advice and attention. Wash hands thoroughly after handling. Take off contaminated clothing and wash before reuse
Storage Not available.
Disposal Not available.

Section 3: Composition/Information on Ingredients

Substance/Mixture Substance
Components 1,2,3-propanetriol
% By Weight 100
CAS# 56-81-5
Molecular Weight 92.09 g/mole
Chemical Formula $C_3H_5(OH)_3$
Synonym(s) 1,2,3-Propanetriol; Glycerol

Mixtures

Name	CAS#	% by Weight	TLV/PEL	LC50/LD50
1,2,3-propanetriol	56-81-5	99-100	N/A	N/A

Section 4: First-Aid Measures

Inhalation Remove to fresh air.

Skin Contact Wash with soap and water.

Eye Contact Immediately flush eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so.

Ingestion Rinse mouth thoroughly. Drink plenty of water. If ingestion of a large amount does occur, call a poison control center immediately.

Symptoms/Effects

Acute Not available.

Delayed Not available.

Immediate Medical Attention

Not available.

Section 5: Fire-Fighting Measures

Suitable Extinguishing Media

Water. Water spray. Foam. Dry powder. Carbon dioxide. Fire fighting equipment and instructions: Move containers from fire area if you can do so without risk. In the event of fire, cool tanks with water spray. Use water spray to cool unopened containers. Cool containers exposed to flames with water until well after the fire is out. In the event of fire, cool tanks with water spray. Use water spray to cool unopened containers.

Unsuitable Extinguishing Media

Not available.

Products of Combustion

These products are carbon oxides (CO, CO2), irritating and toxic fumes. Auto Ignition Temps:370°C (698°F) (NFPA Fire Protection Guide to Hazardous Materials, 13th ed., 2002; NIOSH ICSC, 2001; CHRIS, 2001) 392 C (739 F) (Lewis, 1997)

Firefighters Special Equipment and Precautions

Move containers from fire area if you can do so without risk. In the event of fire, cool tanks with water spray. Use water spray to cool unopened containers. Cool containers exposed to flames with water until well after the fire is out. Specific methods In the event of fire, cool tanks with water spray. Use water spray to cool unopened containers.

Section 6: Accidental Release Measures

Personal precautions: Keep unnecessary personnel away. Keep upwind. Keep out of low areas. Keep people away from and upwind of spill/leak. Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Environmental precautions: Prevent further leakage or spillage if safe to do so. Do not contaminate water. Methods for containment: ELIMINATE all ignition. Stop the flow of material, if this is without risk. Dike the spilled material, where this is possible. Use water spray to reduce vapors or divert vapor cloud drift. Prevent entry into waterways, sewer, basements or confined areas. Small Spill: Wipe up with absorbent material (e.g. cloth, fleece). Clean surface thoroughly to remove residual contamination. Following product recovery, flush area with water. Large Spill: Dike far ahead of spill for later disposal. Use a non-combustible material like vermiculite, sand or earth to soak up the product and place into a container for later disposal.

Section 7: Handling and Storage

Handling: Use only with adequate ventilation. Wash thoroughly after handling. Do NOT handle, store or open near an open flame, sources of heat or sources of ignition. Protect material from direct sunlight. Do not get this material in contact with eyes. Avoid contact with skin. Avoid prolonged exposure. Avoid release to the environment. Storage: Keep away from heat, sparks, and open flame. Store in a well- ventilated place. Keep container tightly closed. Keep away from food, drink, and animal feedingstuffs. Keep out of reach of children. Keep container tightly closed. Keep container in a cool, well-ventilated area. Hygroscopic. Use care in handling/storage.

Section 8: Exposure Controls/Personal Protection

Exposure Limits TWA: 10 (mg/m3) from ACGIH (TLV) [United States] [1999] Inhalation Total. TWA: 15 (mg/m3) from OSHA (PEL) [United States] Inhalation Total. TWA: 10 STEL: 20 (mg/m3) [Canada] TWA: 5 (mg/m3) from OSHA (PEL) [United States] Inhalation Respirable.

Engineering Controls	Engineering controls: Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below recommended exposure limits. General hygiene considerations: Do not get in eyes. Avoid contact with skin. Keep away from food and rink. Handle in accordance with good industrial hygiene and safety practice.
Personal Protection	Eye/face protection: Do not get in eyes. Chemical goggles are recommended. Skin protection: Wear appropriate chemical resistant clothing. Chemical resistant gloves. Respiratory protection: When workers are facing concentrations above the exposure limit they must use appropriate certified respirators.

Section 9: Physical and Chemical Properties

Appearance	Clear colorless liquid. (Viscous (Syrupy) liquid.)
Odor	Mild/Bland.
Odor Threshold	Not available.

Melting Point	20°C (68°F)	**pH**	Not available.
Freezing Point	Not available.	**Vapor Pressure**	<=0.008 mm Hg at 68°F (20°C)
Boiling Point/Range	>= 550.4 °F (>= 288 °C) at 7	**Vapor Density**	3.17 (Air = 1)
Decomposition temperature	Not available.	**Viscosity**	1300 mPa*s @ 20°C
Partition Coefficient: n-octanol/water	-2.6	**Evaporation Rate**	Not available.
Flash Point	>= 390.2 °F (>= 199 °C) Pen	**Autoignition temperature**	739 F (392.8 C)
Flammability	Not available.	**Flammability or Explosive Limits:**	
		Lower	Not available.
		Upper	Not available.

Solubility(ies)	Miscible in cold water, hot water and alcohol. Partially soluble in acetone. Very slightly soluble in diethyl ether (ethyl ether).Limited solubility in ethyl acetate.
Other	Relative density 1.262 at 25/25 C

Section 10: Stability and Reactivity

Reactivity	Not available.
Chemical Stability	The product is stable.
Hazardous Polymerization	Non-corrosive in presence of glass. Hazardous polymerization does not occur. Thermal decomposition may release acrolein.
Conditions to Avoid	Avoid contact with incompatible materials, excess heat and ignition, sources, moisture.
Incompatible Materials	Highly reactive with oxidizing agents (e.g. nitric acid, peroxides, and chromates).
Hazardous Decomposition Products	Does not decompose up to 400° F (204° C). Thermal decomposition may release acrolsin.

Section 11: Toxicological Information

RTECS MA8050000

Acute Toxicity

WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE. Acute oral toxicity (LD50): 4090 mg/kg [Mouse]. Acute dermal toxicity (LD50): 10000 mg/kg [Rabbit]. Acute toxicity of the mist (LC50): >570 mg/m3 1 hours [Rat]. May cause damage to the following organs: kidneys. Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation. TDL (rat) - Route: Oral; Dose: 100 mg/kg 1 day prior to mating. TDL (human) - Route: Oral; Dose: 1428 mg/kg Glycerin is transferred across the plancenta in small amounts. May cause adverse reproductive effects based on animal data (Paternal Effects (Rat): Spermatogenesis (including genetic material, sperm morphology, motility, and count), Testes, epididymis, sperm duct). May affect genetic material. Acute Potential Health Effects: Low hazard for normal industrial handling or normal workplace conditions. Skin: May cause skin irritation. May be absorbed through skin Eyes: May cause eye irritation with stinging, redness, burning sensation, and tearing, but no eye injury. Ingestion: Low hazard. Low toxicity except with very large doses. When large doses are ingested, it can cause gastrointestinal tract irritation with thirst (dehydration), nausea or vomiting diarrhea. It may also affect behavior/central nervous system/nervous system (central nervous system depression, general anesthetic, headache, dizziness, confusion, insomnia, toxic psychosis, muscle weakness, paralysisconvulsions), urinary system/kidneys(renal failure, hemoglobinuria), cardiovascular system (cardiac arrhythmias), liver. It may also cause elevated blood sugar. Inhalation: Due to low vapor pressure, inhalation of the vapors at room temperature is unlikely. Inhalation of mist may cause respiratory tract irritation. Chronic Potential Health Effects: Ingestion: Prolonged or repeated ingestion may affect the blood(hemolysis, changes in white blood cell count), endocrine system (changes in adrenal weight), respiratory system, and may cause kidney injury.

Skin Corrosion/Irritation
Not available.

Serious Eye Damage/Irritation
Not available.

Respiratory or Skin Sensitization
Not available.

Germ Cell Mutagenicity
Not available.

Carcinogenicity
Not available.

Reproductive Toxicity
Not available.

Routes of Entry
Absorbed through skin. Eye contact.

Symptoms Related to Exposure
Not available.

Potential Health Effects
Not available.

Target Organ(s) Not available.

Section 12: Ecological Information

Ecotoxicity
Ecotoxicity in water (LC50): 58.5 ppm 96 hours [Trout]. Even though this product is readily biodegradable, it must not be indiscriminately discarded into the environment. Partition coefficient: -2.6. 1,2,3- PROPANETRIOL (56 -81 -5) LC50 Rainbow trout,donaldson trout (Oncorhynchus mykiss): 51000 - 57000 mg /l 96.00 hours Estimates for product may be based on additional component data not shown. Ecotoxicity Components of this product have been identified as having potential environmental concerns. Environmental effects An environmental hazard cannot be excluded in the event of unprofessional handling or disposal. Persistence / degradability Even though this product is readily biodegradable, it must not be indiscriminately discarded into the environment.

Persistance and Degradability
Not available.

Bioaccumulative Potential
Not available.

Mobility in Soil
Not available.

Other Adverse Effects
Not available.

Section 13: Disposal Considerations

Waste Disposal
Waste must be disposed of in accordance with federal, state and local environmental control regulations. Do not allow this material to drain into sewers /water supplies. This product, in its present state, when discarded or disposed of, is not a hazardous waste according to Federal regulations (40 CFR 261.4 (b)(4)). Under RCRA, it is the responsibility of the user of the product to determine, at the time of disposal, whether the product meets RCRA criteria for hazardous waste. Dispose in accordance with all applicable regulations.

Disposal of Container
Not available.

Other Considerations
Not available.

Section 14: Transport Information

DOT Classification

Not a DOT controlled material (United States). This material is not classified dangerous good according to international transportation regulations (ADR/RID-IMDG-ICAO/IATA).

Section 15: Regulatory Information

Regulations

US Federal Regulations This product is not knon to be a "Hazardous Chemical" as defined by the OSHA Hazard Communication Standard, 29 CFR 1910.1200. All components are on the U.S. EPA TSCA Inventory List. CERCLA/SARA Hazardous Substances - Not applicable. Drug Enforcement Administration (DEA). List 2, Essential Chemicals (21 CFR 1310.02(b) and 1310.04(f)(2): Not regulated. DEA Essential Chemical Code Number: Not regulated DEA List 1 & 2 Exempt Chemical Mixtures (21 CFR 1310.12(c)): Not regulated. DEA Exempt Chemical Mixtures Code Number: Not regulated. Superfund Amendments and Reauthorization Act of 1986 (SARA) Hazard categories: Immediate Hazard - Yes. Delayed Hazard - Yes. Fire Hazard - No. Pressure Hazard - No. Reactivity Hazard - No. Inventory Status Country(s) or Region Inventory Name On inventory (yes/no)* Australia Australian Inventory of Chemical Substances (AICS) Yes Canada Domestic Substances List (DSL) Yes China Inventory of Existing Chemical Substances in China (IECSC) Yes Europe European Inventory of Existing Commercial Chemical Substances (EINECS) Yes Europe European List of Notified Chemical Substances (ELINCS) No Japan Inventory of Existing and New Chemical Substances (ENCS) Yes Korea Existing Chemicals List (ECL) Yes New Zealand New Zealand Inventory Yes Philippines Philippine Inventory of Chemicals and Chemical Substances (PICCS) Yes Switzerland Switzerland FOPH No United States & Puerto Rico Toxic Substances Control Act (TSCA) Inventory Yes *A "Yes" indicates that all components of this product comply with the inventory requirements administered by the governing countries. State regulations: This product does not contain a chemical known to the state of California to cause cancer, birth defects or other reproductive harm. US - New Jersy Community RTK (EHS Survey): Reportable threshold. US - Pennsylvania RTK - Hazardous Substances: Listed substance. 1,2,3-propanetriol (CAS 56-81-5) Listed.

Other

Not available.

Section 16: Other Information

Not available.