Toxicological profile for

delta-Decalactone

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

6-Pentyloxan-2-one (PubChem)

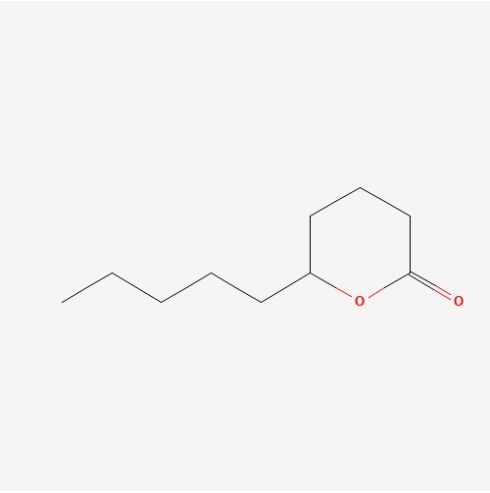
1.2. Synonyms

DELTA-DECALACTONE; 6-Pentyltetrahydro-2H-pyran-2-one; 5-Decanolide; 2H-Pyran-2-one, tetrahydro-6-pentyl-; 6-pentyloxan-2-one; Decan-5-olide; delta-Decanolactone; Decanolide-1,5; 5-Decalactone; Amyl-delta-valerolactone; 5-Pentyl-5-pentanolide; 5-Hydroxydecanoic acid delta-lactone; FEMA No. 2361; delta-Pentyl-delta-valerolactone; Delta Decalactone; Tetrahydro-6-pentyl-2H-pyran-2-one; .delta.-Decalactone; 5-Amyl-5-hydroxypentanoic acid lactone; AI3-36028; Tetrahydro-6-pentylpyran-2-one; CHEBI:87327; Decanoic acid, 5-hydroxy-, .delta.-lactone; EINECS 211-889-1; UNII-CNA0S5T234; .delta.-Decanolide; delta-Caprinolactone; 6-pentylvalerolactone; delta-Amylvalerolactone; (+/-)-delta-Pentyl-delta-valerolactone; Nat. Delta Decalactone; (+/-)-6-Pentyltetrahydro-2H-pyran-2-one; 5-Hydroxydecanoic lactone; .DELTA.-LACTONE-; Amyl-.delta.-valerolactone; (R)-(+)delta-Decalactone; laquo deltaRaquo -decanolide; laquo deltaRaquo -decalactone; SCHEMBL114875; 5-Hydroxydecanoic acid lactone; laquo deltaRaquo -decanolactone; CHEMBL3182189; DTXSID0044496; 6-pentyl-tetrahydro-pyran-2-one; laquo deltaRaquo -amylvalerolactone; Amyl-laquo deltaRaquo -valerolactone; LT laquo deltaRaquo GT -decalactone; 5-Hydroxydecanoic Acid Delta Lactone; AC2158; 6-Pentyltetrahydro-2H-pyran-2-one #; AKOS015914990; 5-Hydroxydecanoic acid .delta.-lactone; 5-Decanolide (laquo deltaRaquo -decalactone); CAPRIC ACID, .DELTA.-HYDROXY-, LACTONE; 5-Hydroxydecanoic acid laquo deltaRaquo -lactone; Decanoic acid, 5-hydroxy-, laquo deltaRaquo -lactone (PubChem)*1.3. Molecular formula*

1.3. Molecular formula

C10H18O2 (PubChem)

1.4. Structural Formula



(PubChem)

1.5. Molecular weight (g/mol)

170.25 (PubChem)

1.6. CAS registration number

705-86-2

1.7. Properties

1.7.1. Melting point

(°C): -27 to -25 (ChemSpider)

1.7.2. Boiling point

(°C): 117-120 at 0.02 mmHg (489.747-495.3638 at 760 mmHg) (ChemSpider; EPISuite, 2017); 267.2 (ChemSpider)

1.7.3. Solubility

Insoluble in water; 393.8 mg/L at 25°C (estimated) (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 106.1 or 145 (ChemSpider)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

0.00475 mmHg at 25°C (estimated) (EPISuite, 2017); 0.0 ± 0.5 mmHg at 25°C (estimated) (ChemSpider)

1.7.11. log Kow

3.102 (ChemSpider)

2. General information

2.1. Exposure

SOURCES OF EXPOSURE			
Cosmetics	Yes (Cosing)	**Food**	Yes (Burdock GA, 2010; CoE, 2000)
Environment	No evidence (Merck, 2013)	Pharmaceuticals	No evidence (Martindale 1999; Merck, 2013)

The per capita intake in the USA in 1987 was estimated, by NAS, to be 31 µg/kg bw/day (Adams et al. 1998).

EFSA estimated the daily intake per person to be 7200 µg in Europe, based on the MSDI (Maximised Survey-derived Daily Intakes) approach (EFSA, 2012).

delta-Decalactone is used as a fragrance and perfuming agent in cosmetics in the EU. As taken from CosIng (Cosmetic substances and ingredients database). Available at https://ec.europa.eu/growth/tools-databases/cosing/.

delta-Decalactone is listed as a fragrance ingredient by International Fragrance Association IFRA and on the US EPA InertFinder Database (2023).

Upper use level 5 and 20 mg/kg in beverages and food, respectively (CoE, 2000).

Reported estimated individual intake from use as a flavouring: 0.03064 mg/kg bw/day.

As taken from Burdock, 2010

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for (FEMA no. 2361)

Baked Goods	14/26
Beverages, Non-Alcoholic	5/14
Beverages, Alcoholic	15/20
Chewing Gum	15/100
Confections and Frostings	0.5/5
Fats and Oils	9/20
Frozen Dairy	19/37
Gelatins and Puddings	15/30
Gravies	4/8
Hard Candy	0.3/5
Soft Candy	13/26
Sweet Sauces	0.1/0.1

As taken from Cohen SM et al. 2020. Food Technology 74(3), 44-65. Available at https://www.femaflavor.org/sites/default/files/2020-03/GRAS%2029.pdf

delta-Decalactone is listed as an ingredient in personal care (at <1% where stated), inside the home and auto products by the CPID.

delta-Decalactone (CAS RN 705-86-2) is used as a flavour enhancer in oral non-medicinal natural health products (Health Canada, 2021).

“Tobacco heating products (THPs) are designed to heat tobacco to produce a nicotine-containing aerosol without combustion. Although THP aerosol is less complex mixture than cigarette smoking, there are a few reports related to the relationship between its chemical composition and its taste and aroma profiles. This study, therefore, aims to characterise the chemical composition of particulate phase aerosol (PPA) in a model THP system heated at different temperatures (100 °C; 150 °C; 200 °C; 240 °C; and 290 °C). The particulate phase of collected THP aerosol was extracted and analysed by GC × GC-TOFMS. The bidimensional total ion current (TIC)

chromatograms were analysed using the SIMPLISMA algorithm, together with Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) to extract key compounds. The semi-quantification was carried out based on similarity (Match), reverse match factor (R. Match) and linear retentions index (LRI). A total of 123 compounds responsible for important taste and aroma characteristics were identified and quantified in the model THP PPA. Although the concentration of compounds increased with the heating temperature, their release rate could be clustered into six groups based on HCA. Four of them, representing 92% of evaluated compounds showed a maximum release ratio at 290 °C, with distinct release behaviours that could fit into either linear, second order polynomial and exponential models. The purposed approach is useful for characterizing volatile compounds related to the taste and aroma profile in the THP system. In addition, these findings suggested that selecting a suitable heating temperature is a critical factor in THP system design in order to optimise tobacco taste and aroma." As taken from Schwanz TC et al. 2020. Microchemical Journal 159, 105578. Available at https://www.sciencedirect.com/science/article/abs/pii/S0026265X20318750

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%**
unknown	37907805	1.41	67124799	1.92
unknown	88012158	3.28	34249435	1.12
delta-decalactone	2520603833	94.04	2960886000	96.88
unknown	33754459	1.26	48046745	1.57
Total ion chromatogram	2678925317	100	3060302229	100

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

Ingredients CAS Number	Chemical Class	Mol. Wt. (M) Bp or Mp (^{0}C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (nü¡g)
delta-Decalactone CAS 705-86-2	Delta-Lactone	M=170 Bp 117-120 at 0.02 mm Hg	5	98	deltaDecalactone 97.5 delta-Tetradecalactone? 0.8 Heptanal 0.5 Methoxyethyl acetate? 0.4 delta-Octalactone 0.4 gamma-Undecalatone 0.2	2 0.02 0.01 0.01 0.01 0.005 0.005

					Octanal 0.2	

2.3. Ingredient(s) from which it originates

Natural occurrence: Reported found in rum, coconut, raspberry, apricot, bilberry, peach, strawberry, Swiss cheese, other cheeses, butter, milk, milk powder, mutton fat, mango and nectarine. (Burdock GA, 2010).

Food	**Mg/kg**
Raspberry	0.005-1.4
Other fruits:	Up to 0.15
butter	0.85 -7.95
coconut	0.1-97
Wine (white)	0.06
Rum	0.02

As taken from CoE, 2000

3. Status in legislation and other official guidance

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives delta-DECALACTONE	
COE No.:	621
FEMA No.:	2361
JECFA No.:	232
Chemical names:	5-DECANOLIDE; 6-PENTYLTETRAHYDRO-2-PYRONE
Synonyms:	DECA-1,5-LACTONE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1997
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 884-JECFA 49/42
Specifications:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/138 (2000)
Tox monograph:	FAS 40-JECFA 49/231
Previous status:	1998, COMPENDIUM ADDENDUM 6/FNP 52 Add. 6/172. N

As taken from JECFA, 2001 evaluation of delta-decalactone available at http://www.inchem.org/documents/jecfa/jeceval/jec_500.htm

STATUS IN LEGISLATION AND OTHER OFFICIAL GUIDANCE						
States approving use in tobacco	Germany, France, Belgium and UK					
Food	UK	No	EU	Yes	USA	GRAS

						172.515
ADI / TDI	No formal ADI identified. JECFA (1998) considered there to be no safety concern at the current level of use of delta-decalactone as a flavouring (estimated intake 8400 or 1900 ug/person/day in Europe and the USA respectively), noting that the substance is metabolised to innocuous products. The Council of Europe has classed delta-decalactone in flavourings Category B, as provisionally acceptable in foods, with a request for further data (CoE 2000). The UK Food Additives and Contaminants Committee, in an old review of flavourings, considered delta-decalactone to be acceptable for use in foodstuffs, subject to a concentration limit of 20 ppm (MAFF 1976).					
Codex Alim.	Not listed					
C of E no.	621			FEMA no.	2361	
TLV / OEL	Not listed					
Cosmetics (UK)	Not listed (Schedule 1).					

delta-Decalactone is included on the FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is covered under 21 CFR section 172.515 (synthetic flavoring substances and adjuvants) (FDA, 2022, 2023).

There is a REACH dossier on decan-5-olide (ECHA, undated).

Decan-5-olide (CAS RN 705-86-2) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

2H-Pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). 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.

US EPA 2020 CDR List. US EPA TSCA inventory.

delta-Decalactone (CAS RN 705-86-2) is listed in the US EPA InertFinder Database (2023) as approved for fragrance use pesticide products.

delta-Decalactone is included on the US EPA's list of Safer Chemical Ingredients and it's marked as the chemical that has met Safer Choice Criteria for its functional ingredient-class, but has some hazard profile issues. Specifically, a chemical with this code is not associated with a low level of hazard concern for all human health and environmental endpoints.(US EPA, 2023).

Decano-1,5-lactone (CAS RN 705-86-2) is authorised for use as a flavouring substance in all categories of flavoured food in the EU under (EU) legislation no 872/2012 (European Commission).

delta-Decalactone has been given GRAS (generally recognized as safe) status by FEMA (FEMA no. 2361) (Hall and Oser, 1965).

2H-Pyran-2-one, tetrahydro-6-pentyl- CAS 705-86-2 is listed on Australian Inventory of Industrial Chemicals (AICIS, formerly NICNAS).

As taken from AICIS

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“Linear saturated 5-hydrocarboxylic acids are converted, via acetyl coenzyme A, to hydroxythioesters which then undergo beta-oxidation and cleavage to yield an acetyl CoA fragment and a new beta-hydroxythioester reduced by 2 carbons. Even-numbered carbon acids continue to be oxidised and cleaved to yield acetyl CoA which enters the citric acid cycle directly.”

“delta-decalactone is metabolized to innocuous products.”

As taken from WHO Food Additives Series 40, available at http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm

4.2. Absorption, distribution and excretion

“Lactones are generally formed by acid-catalysed intramolecular cyclization of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as urine, the lactone ring is favoured. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract.”

As taken from WHO Food Additives Series 40, available at http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
rat	LD	oral	> 4300mg/kg (4300mg/kg)		National Technical Information Service. Vol. AD-A053-896,

As taken from ChemIDplus, available via PubChem

Both the acute oral LD50 value in rats and the acute dermal LD50 value in rabbits exceeded 5 g/kg bw (Opdyke, 1976).

5.2. Repeated dose toxicity

No adverse effects were seen in a 49-week study in rats and a 38-week study in dogs fed a mixture of delta-decalactone and delta-dodecalactone in the diet at levels providing average intakes of 150 and 75 mg delta-decalactone/kg bw/day respectively (Adams et al. 1998).

There are sufficient repeated dose toxicity data on δ-decalactone. In a GLP/OECD 407-compliant subchronic study, 6 Sprague Dawley rats/sex/dose were administered δ-decalactone via gavage at doses of 0, 250, 500, and 1000 mg/kg/day for 28 days. An additional 6 Sprague Dawley rats/sex/dose at 0 and 1000 mg/kg/day were maintained as recovery groups for 2 weeks after the treatment period. No mortality occurred throughout the study period. No treatment-related effects were observed on clinical signs, body weights, bodyweight gains, food consumption, ophthalmology, hematology, clinical biochemistry, urinalysis, behavior, organ weights, gross pathology, or histopathology. Based on no toxicologically relevant effects seen up to the highest dose, the NOAEL for this study was determined to be 1000 mg/kg/day. A default safety factor of 3 was used (ECHA, 2012) when deriving a NOAEL from an OECD 407 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. Thus, the derived NOAEL for the repeated dose toxicity data is 1000/ 3, or 333 mg/kg/day. Therefore, the δ-decalactone MOE for the repeated dose toxicity endpoint can be calculated by dividing the δ-decalactone NOAEL in mg/ kg/day by the total systemic exposure to δ-decalactone, 333/0.0016, or 208125. In addition, the total systemic exposure to δ-decalactone (1.6 µg/kg/ day) is below the TTC (30 µg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use. *The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

As taken from Api AM et al. (2021)

5.3. Reproduction toxicity

There are sufficient reproductive toxicity data on δ-decalactone that can be used to support the reproductive toxicity endpoint. An OECD 421/GLP reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered test material δ-decalactone via oral gavage in corn oil at doses of 0, 250, 500, or 1000 mg/kg/day. Males were dosed for 37 days (2 weeks prior to mating, through the mating period, and until termination), while females were dosed for approximately 62 days (2 weeks prior to mating, during mating, during post coitum, and up to lactation day 13). No treatment-related mortality was observed in any dose group. No changes were observed in mean body weights and organ weights (both relative and absolute). No treatment-related effects were seen with respect to any fertility parameters for males and females. Pups did not show any clinical signs or external anomalies throughout the lactation period. No treatmentrelated changes in pup weights or ano-genital distance ratio were observed in any groups. The NOAEL for developmental toxicity and fertility was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013). Therefore, the δ-decalactone MOE for the developmental toxicity and fertility endpoints can be calculated by dividing the δ-decalactone NOAEL in mg/kg/day by the total systemic exposure to δ-decalactone, 1000/0.0016, or 625000. In addition, the total systemic exposure to δ-decalactone (1.6 µg/kg/ day) is below the TTC (30 µg/kg/day; Kroes, 2007) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

As taken from Api AM et al. (2021)

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which delta-decalactone was part of the test mixture include EMT980416 and EMT000302 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

“Electronic nicotine delivery systems (ENDS) are regulated tobacco products and often contain flavor compounds. Given the concern of increased use and the appeal of ENDS by young people, evaluating the potential of flavors to induce DNA damage is important for health hazard identification. In this study, alternative methods were used as prioritization tools to study the

genotoxic mode of action (MoA) of 150 flavor compounds. In particular, clastogen-sensitive (γH2AX and p53) and aneugen-sensitive (p-H3 and polyploidy) biomarkers of DNA damage in human TK6 cells were aggregated through a supervised three-pronged ensemble machine learning prediction model to prioritize chemicals based on genotoxicity. In addition, in silico quantitative structure-activity relationship (QSAR) models were used to predict genotoxicity and carcinogenic potential. The in vitro assay identified 25 flavors as positive for genotoxicity: 15 clastogenic, eight aneugenic and two with a mixed MoA (clastogenic and aneugenic). Twenty-three of these 25 flavors predicted to induce DNA damage in vitro are documented in public literature to be in e-liquid or in the aerosols produced by ENDS products with youth-appealing flavors and names. QSAR models predicted 46 (31%) of 150 compounds having at least one positive call for mutagenicity, clastogenicity or rodent carcinogenicity, 49 (33%) compounds were predicted negative for all three endpoints, and remaining compounds had no prediction call. The parallel use of these predictive technologies to elucidate MoAs for potential genetic damage, hold utility as a screening strategy. This study is the first high-content and high-throughput genotoxicity screening study with an emphasis on flavors in ENDS products." As taken from Hung PH et al. 2020. J. Appl. Toxicol. 40(11), 1566-1587. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32662109/

The mutagenic activity of δ-decalactone has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. Salmonella typhimurium strains TA98, TA100, TA1535, TA102, and TA97a were treated with δ-decalactone in dimethyl sulfoxide (DMSO) at concentrations up to 5 mg/plate (5000 µg/plate). No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2001a). Under the conditions of the study, δ-decalactone was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of δ-decalactone; however, read-across can be made to hydroxynonanoic acid, δ-lactone (CAS # 3301-94-8; see Section VI).

The clastogenic activity of hydroxynonanoic acid, δ-lactone was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with hydroxynonanoic acid, A.M. Api et al. Food and Chemical Toxicology 153 (2021) 112142 4 δ-lactone in DMSO at concentrations up to 1562.3 µg/mL in a dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1562.3 µg/mL in the presence and absence of metabolic activation. Hydroxynonanoic acid, δ-lactone did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (RIFM, 2015). Under the conditions of the study, hydroxynonanoic acid, δ-lactone was considered to be non-clastogenic in the in vitro micronucleus test, and this can be extended to δ-decalactone.

Based on the data available, hydroxynonanoic acid, δ-lactone does not present a concern for genotoxic potential, and this can be extended to δ-decalactone.

Hydroxynonanoic acid, δ-lactone (CAS # 3301-94-8) was used as a read-across analog for the target material δ-decalactone (CAS # 705-86-2) for the genotoxicity endpoint.

o The target material and the read-across analog are structurally similar and belong to a class of δ lactones. o The target material and the read-across analog share a δ lactone substructure.

o The key difference between the target material and the read-across analog is that the target material has methyl substitution on the 4 position, which the read-across analog lacks. One more structural difference is that the target material is a lactone of octanoic acid, while the read-across analog is a lactone of nonanoic acid. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.

o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties. o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the readacross analog.

o The read-across analog and the target material have an alert of containing lactone type reacting functional group under the oncologic classification scheme by OECD QSAR Toolbox. Lactones are cyclic esters that may open to serve as an acylating agent. In general, the ability to open the ring is dependent on the size of the ring. Gamma and δ lactones are considerably weaker acylating agents with some carcinogenicity potential, only if unsaturation is present in the ring α-β to the carbonyl group. The ring in the target material, as well as the read-across analog, is saturated. The data on the read-across analog confirm that the material does not pose a concern for genetic toxicity. Therefore, based on the structural similarity between the target material and the read-across analog and the data present on the read-across analog, the predictions are superseded by the data.

o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator. o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

As taken from Api AM et al. (2021)

5.5. Cytotoxicity

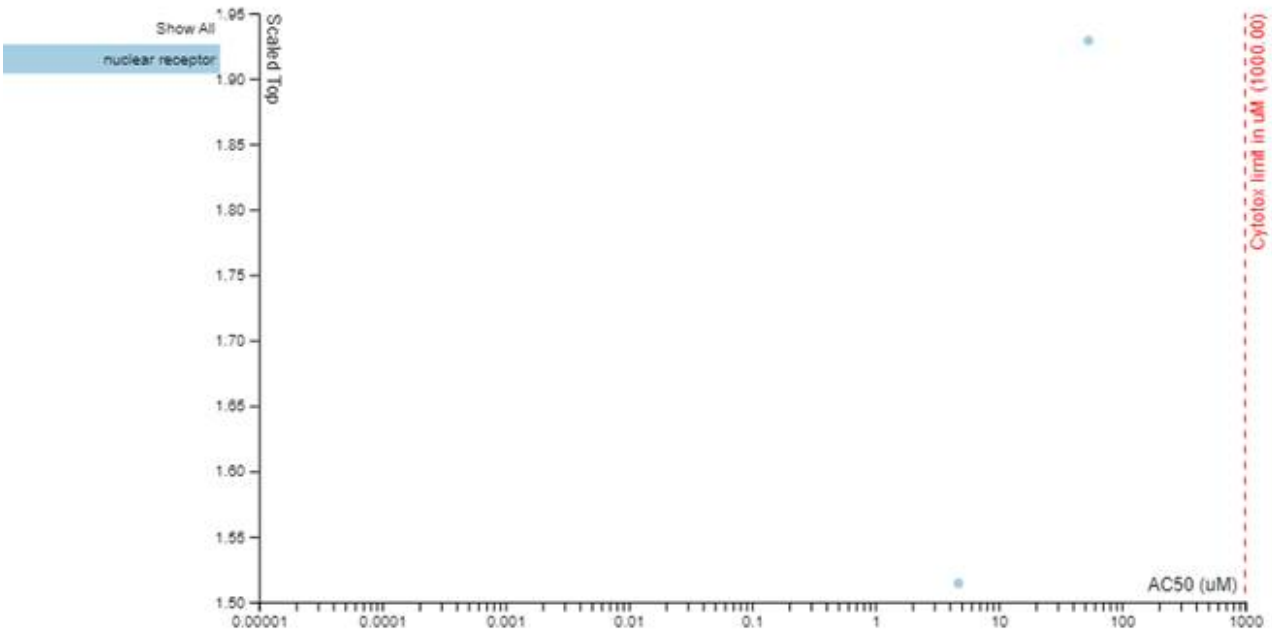
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 6-pentyltetrahydro-2H-pyran-2-one in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

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

A summary of the ToxCast assay data on 6-pentyltetrahydro-2H-pyran-2-one is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

A 500 mg exposure for 24 hours on rabbit skin produced only a mild irritant effect as did a 100mg exposure in rabbits eyes.

As taken from RTECS, 1997

Delta decalactone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was slightly irritating (Levenstein, 1975). Tested at 1% in petrolatum it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1975) (As taken from Opdyke, 1976).

Sensitization

No sensitization reactions were induced in 25 volunteers following a maximization test at a concentration of 1% in petrolatum (Opdyke, 1976).

Limited skin sensitization studies are available for δ-decalactone. Based on read-across material δ-octalactone (CAS # 698-76-0; see Section VI), δ-decalactone is not considered a skin sensitizer. The chemical structures of these materials indicate that they would be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). The read-across material δ-octalactone was found to be negative in an in vitro direct peptide reactivity assay (DPRA) and KeratinoSens test (ECHA, 2019). In guinea pig maximization tests, δ-decalactone and the read-across material did not present reactions indicative of sensitization (ECHA, 2019; RIFM, 1981). In human maximization tests, no skin sensitization reactions were observed with δ-decalactone and read-across material δ-octalactone (RIFM, 1975; RIFM, 1977). Additionally, in a confirmatory Confirmation of No Induction in Humans test (CNIH) with 1.25% or 969 µg/cm2 of δ-decalactone in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 38 volunteers (RIFM, 1972). Based on the weight of evidence (WoE) from structural analysis, animal and human studies, and read-across material δ-octalactone, δ-decalactone does not present a concern for skin sensitization under the current, declared levels of use.

δ-Octalactone (CAS # 698-76-0) was used as a read-across analog for the target material δ-decalactone (CAS # 705-86-2) for the skin sensitization endpoint.

o The target material and the read-across analog are structurally similar and belong to a class of δ lactones.

o The target material and the read-across analog share a δ lactone substructure.

o The key difference between the target material and the read-across analog is that the target material has methyl substitution on the 4 position, which the read-across analog lacks. One more structural difference is that the target material is a lactone of octanoic acid, while the read-across analog is a lactone of octanoic acid. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.

o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties. o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the readacross analog.

o The read-across analog and the target material have an alert of direct acylating agent for the skin sensitization endpoint by several models. Lactones are cyclic esters that may open to serve as an acylating agent. The chemical may have an assumptive weak sensitization effect as a result of protein acylation by lactones. In general, the ability to open the ring is dependent on the size of the ring. Gamma and δ lactones are considerably weaker acylating agents, only if unsaturation is present in the ring α-β to the carbonyl group. The ring in the target material, as well as the read-across analog, is saturated. The data on the read-across analog confirm that the material does not pose a concern for skin sensitization. Therefore, based on the structural similarity between the target material and the read-across analog and the data present on the readacross analog, the predictions are superseded by the data.

o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator. o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

As taken from Api AM et al. (2021)

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Delta-Decalactone 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 Delta-Decalactone 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	494	JTI KB Study Report(s)
In vitro cytotoxicity	494	JTI KB Study Report(s)

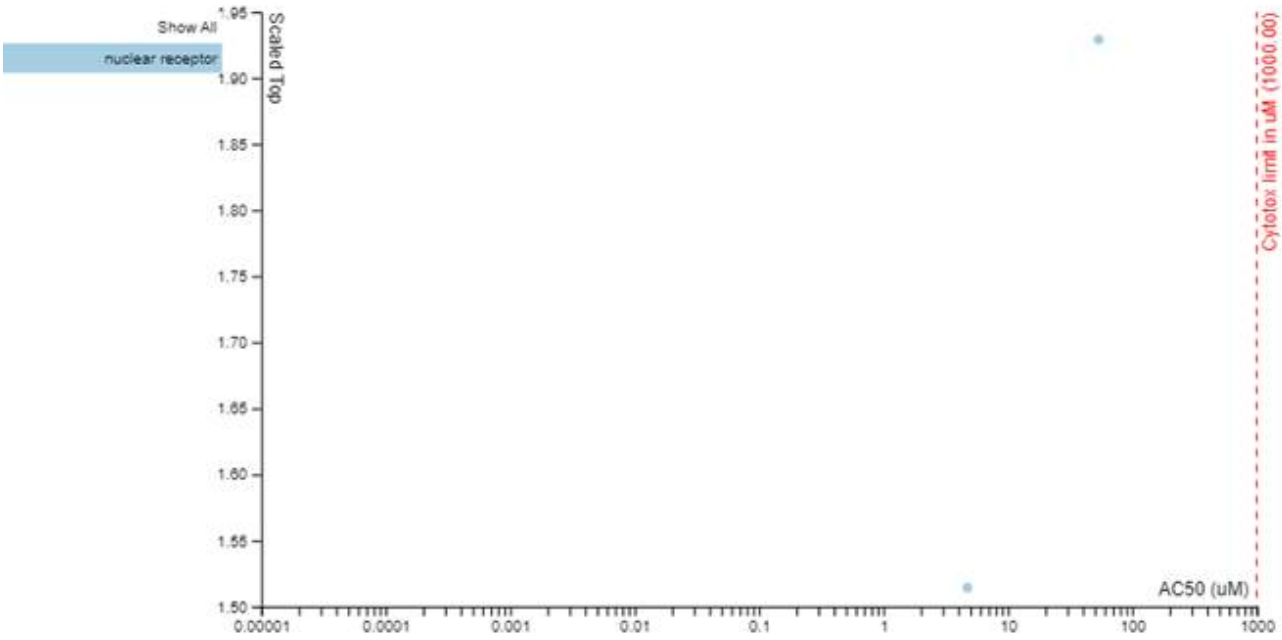
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 6-pentyltetrahydro-2H-pyran-2-one in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

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

A summary of the ToxCast assay data on 6-pentyltetrahydro-2H-pyran-2-one is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA’s CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

No data available to us at this time.

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

No data available to us at this time.

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 delta-Decalactone 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 delta-Decalactone. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	6	Carmines, 2002 & Rustemeier et al. 2002
	18	Baker et al. 2004a
	6.5 70	JTI KB Study Report(s)
	21	Roemer et al., 2014
In vitro genotoxicity	6	Carmines, 2002 & Roemer et al. 2002
	18	Baker et al. 2004c
	6.5	Renne et al. 2006
	6.5 70	JTI KB Study Report(s)
	12	fGLH Study Report (2010)
	21	Roemer et al., 2014
In vitro cytotoxicity	6	Carmines, 2002 & Roemer et al. 2002
	18	Baker et al. 2004c
	70	JTI KB Study Report(s)
	12	fGLH Study Report (2010)
	21	Roemer et al., 2014
Inhalation study	6	Carmines, 2002 & Vanscheeuwijck et al. 2002
	18	Baker et al. 2004c
	6.5	Renne et al. 2006
	6.5 70	JTI KB Study Report(s)
	21	Schramke et al., 2014
Skin painting	6.5	JTI KB Study Report(s)
In vivo genotoxicity	21 70	Schramke et al., 2014 JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Delta-Decalactone 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 Delta-

Decalactone 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	494	JTI KB Study Report(s)
In vitro cytotoxicity	494	JTI KB Study Report(s)

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

Endpoint	Tested level	Reference
Aerosol chemistry	0.0001 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro genotoxicity	0.0001 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro cytotoxicity	0.0001 mg/(tobacco portion; 310 mg)	Logic (2019)
In vivo genotoxicity	0.0001 mg/(tobacco portion; 310 mg)	Logic (2019)
Inhalation study	0.0001 mg/(tobacco portion; 310 mg)	Logic (2019)

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

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	1,000	Labstat International Inc. (2021)
In vitro genotoxicity	1,000	Labstat International Inc. (2022)
In vitro cytotoxicity	1,000	Labstat International Inc. (2022)

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

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.0017	Labstat International Inc. (2021a)
In vitro genotoxicity	0.0017	Labstat International Inc. (2021b)
In vitro cytotoxicity	0.0017	Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2) is not persistent in the environment:

Media of concern leading to Categorization	Water-Soil
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	8.67
MITI probability of biodegradation	0.9148
TOPKAT probability of biodegradation	0.519
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.7962

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

EPISuite provides the following information:
Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	5.62E-004 atm-m3/mole (5.69E+001 Pa-m3/mole)
Group Method:	2.39E-004 atm-m3/mole (2.42E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.702E-006 atm-m3/mole (2.738E-001 Pa-m3/mole) VP: 0.00475 mm Hg (source: MPBPVP) WS: 394 mg/L (source: WSKOWWIN)

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

Log Kow used:	2.57 (KowWin est)
Log Kaw used:	-1.639 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	4.209
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.9491
Biowin2 (Non-Linear Model) :	0.9985
Biowin3 (Ultimate Survey Model):	3.2615 (days-weeks)
Biowin4 (Primary Survey Model) :	4.1001 (days)
Biowin5 (MITI Linear Model) :	0.8191
Biowin6 (MITI Non-Linear Model):	0.9148
Biowin7 (Anaerobic Linear Model):	0.3940
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):	0.633 Pa (0.00475 mm Hg)
Log Koa (Koawin est):	4.209
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	4.74E-006 3.97E-009

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.000171
Mackay model:	0.000379
Octanol/air (Koa) model:	3.18E-007

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

OVERALL OH Rate Constant =	13.4335 E-12 cm3/molecule-sec
Half-Life =	0.796 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	9.555 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.000275 (Junge-Pankow, Mackay avg) 3.18E-007 (Koa method)	

Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	218.7 L/kg (MCI method)
Log Koc:	2.340 (MCI method)
Koc :	191 L/kg (Kow method)
Log Koc:	2.281 (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: 0.000239 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	4.528 hours
Half-Life from Model Lake:	158.8 hours (6.617 days)

Removal In Wastewater Treatment:

Total removal:	13.12 percent
Total biodegradation:	0.10 percent
Total sludge adsorption:	2.99 percent
Total to Air:	10.03 percent

(using 10000 hr Bio P,A,S)
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	3.83	19.1	1000
Water	24.9	208	1000
Soil	71.1	416	1000
Sediment	0.205	1.87e+003	0

Persistence Time: 248 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	21.2
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	21.2
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	12.769
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	40.457
Toxicity to fish (LC50 in mg/l) as predicted by Aster	12.551614
Toxicity to fish (LC50 in mg/l) as predicted by PNN	225.8968
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	40.4
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	40.963
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.23E+001

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

ECOSAR Version 1.11 reports the following aquatic toxicity data for 705-86-2:

Values used to Generate ECOSAR Profile

Log Kow: 2.567 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 393.8 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class

Esters

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Esters :	Fish	96-hr	LC50	10.616
Esters :	Daphnid	48-hr	LC50	20.767
Esters :	Green Algae	96-hr	EC50	8.079
Esters :	Fish		ChV	0.709
Esters :	Daphnid		ChV	12.062
Esters :	Green Algae		ChV	2.490
Esters :	Fish (SW)	96-hr	LC50	15.598
Esters :	Mysid	96-hr	LC50	11.601
Esters :	Fish (SW)		ChV	2.476

Esters :	Mysid (SW)		ChV	268.692	
Neutral Organic SAR :	Fish	96-hr	LC50	43.281	
(Baseline Toxicity) :	Daphnid	48-hr	LC50	25.896	
	Green Algae	96-hr	EC50	23.954	
	Fish		ChV	4.500	
	Daphnid		ChV	2.922	
	Green Algae		ChV	7.049	

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR Version 1.11 reports the following terretrial toxicity data for 705-86-2:

Values used to Generate ECOSAR Profile

Log Kow: 2.567 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 393.8 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	1456.906 *

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.

“The enantiomerically enriched γ- and δ-decalactones (4a and 4b) were prepared from corresponding racemic primary-secondary 1,4- and 1,5-diols (1a and 1b), as products of enzymatic oxidation catalyzed by different alcohol dehydrogenases. The results of biotransformations indicated that the oxidation processes catalyzed by alcohol dehydrogenase (HLADH), both isolated from horse liver and recombinant in Escherichia coli, were characterized by the highest degree of conversion with moderate enantioselectivity of the reaction. Useful, environmentally friendly extraction procedure of decalactones (4a and 4b) based on hydrodistillation using a Deryng apparatus was developed. Both racemic lactones (4a and 4b), as well as their enantiomerically enriched isomers, were tested for feeding deterrent activity against Myzus persicae. The effect of these compounds on probing, feeding and settling behavior of M. persicae was studied in vivo. The deterrent activity of decalactones (4a and 4b) against aphids depended on the size of the lactone ring and the enantiomeric purity of the compounds. δ-Decalactone (4b) appeared inactive against

M. persicae while γ-decalactone (4a) restrained aphid probing at ingestional phase. Only (-)-(S)-γ-decalactone (4a) had strong and durable (i.e. lasting for at least 24 hours) limiting effect, expressed at phloem level." As taken from Boraty+äski F et al. 2016. PLoS One 11(1), e0146160. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/26741824

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2) is not bioaccumulative in the environment:

Log Kow predicted by KowWin	2.57
Log BAF T2MTL predicted by Gobas	1.39019032384435
Log BCF 5% T2LTL predicted by Gobas	1.29866715380139
Log BCF Max predicted by OASIS	2.05854509264126
Log BCF predicted by BCFWIN	1.277

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

EPISuite provides the following information: **Bioaccumulation Estimates (BCFBAF v3.01):**

Log BCF from regression-based method:	1.361 (BCF = 22.95 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.2678 days (HL = 0.05397 days)
Log BCF Arnot-Gobas method (upper trophic):	1.125 (BCF = 13.33)
Log BAF Arnot-Gobas method (upper trophic):	1.125 (BAF = 13.33)
log Kow used:	2.57 (estimated)

"The enantiomerically enriched γ- and δ-decalactones (4a and 4b) were prepared from corresponding racemic primary-secondary 1,4- and 1,5-diols (1a and 1b), as products of enzymatic oxidation catalyzed by different alcohol dehydrogenases. The results of biotransformations indicated that the oxidation processes catalyzed by alcohol dehydrogenase (HLADH), both isolated from horse liver and recombinant in Escherichia coli, were characterized by the highest degree of conversion with moderate enantioselectivity of the reaction. Useful, environmentally friendly extraction procedure of decalactones (4a and 4b) based on hydrodistillation using a Deryng apparatus was developed. Both racemic lactones (4a and 4b), as well as their enantiomerically enriched isomers, were tested for feeding deterrent activity against Myzus persicae. The effect of these compounds on probing, feeding and settling behavior of M. persicae was studied in vivo. The deterrent activity of decalactones (4a and 4b) against aphids depended on the size of the lactone ring and the enantiomeric purity of the compounds. δ-Decalactone (4b) appeared inactive against M. persicae while γ-decalactone (4a) restrained aphid probing at ingestional phase. Only (-)-(S)-γ-decalactone (4a) had strong and durable (i.e. lasting for at least 24 hours) limiting effect, expressed at phloem level." As taken from Boratynski F et al. 2016. PLoS One 11(1), e0146160. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/26741824

11. References

- Adams TB et al. (1998). The FEMA GRAS assessment of lactones used as flavour ingredients. Food and Chemical Toxicology 36, 249-278. Available at https://www.femaflavor.org/sites/default/files/Adams et al%2C 1998_cleaned.pdf
- AICIS (Undated). Australian Government. Department of Health. Australian Inventory of Industrial Chemicals. Record for CAS 705-86-2. Available at https://www.industrialchemicals.gov.au/chemicals/2h-pyran-2-one-tetrahydro-6-pentyl
- Api AM et al. (2021).RIFM fragrance ingredient safety assessment,δ-decalactone, CAS Registry Number 705-86-2. Food and Chemical Toxicology; 2021. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/705-86-2.pdf
- Baker R and Bishop L. (2004). The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrolysis 71, 223–311. DOI.org/10.1016/S0165-2370(03)00090-1. Available at: https://www.sciencedirect.com/science/article/pii/S0165237003000901?via%3Dihub
- Baker R et al. (2004a). The effect of tobacco ingredients on smoke chemistry.Part I: Flavourings and additives. Food and Chemical Toxicology 42s, S3-S37. DOI: 10.1016/S0278-6915(03)00189-3. Available at: https://pubmed.ncbi.nlm.nih.gov/15072836/
- Baker R et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food and Chemical Toxicology 42s, S53-S83. DOI.org/10.1016/j.fct.2004.01.001. Available at: https://www.sciencedirect.com/science/article/pii/S0278691504000043 Boratynski F et al. (2016). Chemo-Enzymatic Synthesis of Optically Activeγ- andδ-Decalactones and Their Effect on Aphid Probing, Feeding and Settling Behavior. PLoS One 11(1), e0146160. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/26741824
- Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.
- Carmines E (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1. Cigarette design, testing approach, and review of results. Food and Chemical Toxicology, 40, 77-91. DOI: 10.1016/s0278-6915(01)00084-9. Available at: https://pubmed.ncbi.nlm.nih.gov/11731038/
- CD-ROM 1, JTI Submission, 2002
- ChemIDplus. Record for delta-Decalactone (CAS RN 705-86-2) AvailablePubChem via
- ChemSpider. Record for2361 (CAS RN 705-86-2).Undated.. Available at
- CoE (2000). Chemically-defined flavouring substances. Partial agreement in the Social and Public Health Field, Strasbourg.4th Edition, revised. Council of Europe.
- Cohen SM et al. (2020). GRAS 29 Flavoring Substances. Food Technology 74(3), 44-65. Available at https://www.femaflavor.org/sites/default/files/2020-03/GRAS 29.pdf
- CosIng. Cosmetic Substances and Ingredients database. Record for delta-decalactone. Undated.. Available at https://ec.europa.eu/growth/tools-databases/cosing/
- CPID (undated). Consumer Product Information Database. Record for delta-decalactone (CAS RN 705-86-2).Available at https://www.whatsinproducts.com/
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes.Available at http://legacy.library.ucsf.edu/tid/thy03c00
- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes.Available at http://legacy.library.ucsf.edu/tid/wzp67e00
- ECHA (2023). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 8 March 2023. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECHA undated). European Chemicals Agency. Information on Chemicals. Record for decan-5-olide. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances
- ECOSAR (undated). Record for 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2). (ECOSAR content has not been updated since 2012, version 1.11.) Available to download,

through EPISuite, at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EFSA (2012)Scientific Opinion on Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3): Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30. EFSA Journal, 10, 2563. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2563/epdf
- EPISuite (2017). Record for 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- EPISuite (undated). Record for 2H-pyran-2-one, tetrahydro-6-pentyl- (CAS RN 705-86-2). Accessed May 2017. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- European Commission. Database of Food Flavourings. Record for decano-1,5-lactone. Last modified 17 September 2012. . Available at: https://ec.europa.eu/food/food-feed-portal/screen/food-flavourings/search
- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 13 October 2022. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances
- FDA (2023). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR), Title 21 Food and Drugs. Current as of 6 March 2023. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- fGLH Study Report (2010)
- Hall RL and Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. III. GRAS substances. Food Technology 19(2), 151. Available at http://www.femaflavor.org/sites/default/files/3. GRAS Substances(2001-3124)_0.pdf
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Record for delta-decalactone (CAS RN 705-86-2). Last updated 12 July 2021. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=2061&lang=eng
- Hung PH et al. (2020). In vitro and in silico genetic toxicity screening of flavor compounds and other ingredients in tobacco products with emphasis on ENDS. J. Appl. Toxicol. 40(11), 1566-1587. DOI: 10.1002/jat.4020. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32662109/
- IFRA (undated). International Fragrance Association. IFRA Transparency List. . Available at: https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
- JECFA (2001). Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. delta-Decalactone. Dated 12 November 2001. Accessed April 2021. Available at http://www.inchem.org/documents/jecfa/jeceval/jec_500.htm
- JTI KB Study Report (s).
- JTI Study Report (s).
- Labstat International Inc. (2021). Characterization of E-cigarette Aerosol.Analytical Test Report.
- Labstat International Inc. (2021a). Characterization of Heat-not-Burn Emissions.Analytical Test Report(s).
- Labstat International Inc. (2021b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products.Biological Activity Test Report(s).

- Labstat International Inc. (2022). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Collected Matter (ACM) and Mainstream Gas Vapor Phase (GVP) of Electronic Cigarette Products.Biological Activity Test Report.
- Logic (2019). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- MAFF (1976). Food Additives and Contaminants Committee Report on the Review of Flavourings in Food, FAC/REP/22, HMSO, London.
- Martindale (1999). The Extra Pharmacopoeia. Edited by Kathleen parfitt.Thirty-second edition. The Pharmaceutical Press. ISBN 0-85369-429-X.
- Merck (2013). The Merck Index.. An encyclopaedia of chemicals, drugs and biologicals. Fifteenth edition. Ed. O'Neil MJ et al. Royal Society of Chemistry, Cambridge, UK.
- OECD (undated). Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). 2H-Pyran-2-one, tetrahydro-6-pentyl-. Accessed May 2017. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Opdyke DLJ. (1976). Fragrance Raw Materials Monograph for delta decalactone.Food Cosmet. Toxicol. 14, 739.
- PubChem (2023). Record for delta-decalactone (CAS RN 705-86-2). Created 27 March 2005. Last modified4 March 2023. Available at https://pubchem.ncbi.nlm.nih.gov/compound/12810
- Renne RA et al. (2006). Effects of flavoring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. Inhal Toxicol. 18(9), 685-70. PubMed 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/16864559?dopt=AbstractPlus
- Roemer E et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: in vitro genotoxicity and cytotoxicity. Food Chem Toxicol. 40(1), 105-11. PubMed 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/11731040?dopt=AbstractPlus
- Roemer E et al., (2014). Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity.Regulatory Toxicology and Pharmacology 70; S66-80.
- RTECS (1997). Registry of Toxic Effects of Chemical Substances. Record for 2H-pyran-2-one, tetrahydro-6-pentyl- (705-86-2).Last updated January 1997. .
- Rustemeier K et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: chemical composition of mainstream smoke. Food Chem Toxicol. 40(1), 93-104. PubMed 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/11731039?dopt=AbstractPlus
- Schramke H et al. (2014). Toxicological assessment of kretek cigarettes Part 7: The impact of ingredients added to kretek cigarettes on inhalation toxicity. Regulatory Toxicology and Pharmacology 70; S81-89. DOI.org/10.1016/j.yrtph.2014.09.014. Available at: https://www.sciencedirect.com/science/article/pii/S0273230014002190 Schwanz TC et al. (2020). GC × GC-TOFMS and chemometrics approach for comparative study of volatile compound release by tobacco heating system as a function of temperature.Microchemical Journal 159, 105578.DOI: 0.1016/j.microc.2020.105578.Available at https://www.sciencedirect.com/science/article/abs/pii/S0026265X20318750
- US EPA (2023). Safer Chemical Ingredients List. Last updated 12 January 2023. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA 2020 CDR list (Chemical Data Reporting Rule).Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/advancedsearch/externalSearch.do?p_type=SRSITN&p_value=68494
- US EPA InertFinder Database (2023). Last updated 28 February 2023. Available at:: https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA ToxCast. Available via US EPA CompTox Chemistry Dashboard at https://comptox.epa.gov/dashboard

- US EPA TSCA inventory. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/advancedsearch/externalSearch.do?p_type=SRSITN&p_value=68494
- Vanscheeuwijck PM et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: subchronic inhalation toxicity. Food Chem Toxicol. 2002 Jan;40(1), 113-31. PubMed, 2014, available at: http://www.ncbi.nlm.nih.gov/pubmed/11731041?dopt=AbstractPlus
- WHO Food Additives Series 40, (2006). available at: http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm

12. Other information

No data available to us at this time.

13. Last audited

March 2023

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

delta-DECALACTONE

COE No.:	621
FEMA No.:	2361
JECFA No.:	232
Chemical names:	5-DECANOLIDE; 6-PENTYLTETRAHYDRO-2-PYRONE
Synonyms:	DECA-1,5-LACTONE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1997
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 884-JECFA 49/42
Specifications:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/138 (2000)
Tox monograph:	FAS 40-JECFA 49/231
Previous status:	1998, COMPENDIUM ADDENDUM 6/FNP 52 Add. 6/172. N

12 Nov 01

See Also:
Toxicological Abbreviations

WHO Technical Report Series
884

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Geneva 1999

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Rome, 17–26 June 1997

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.

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 International Programme on Chemical Safety (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 met in Rome from 17 to 26 June 1997. The meeting was opened by Dr H. de Haen, Assistant Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr de Haen emphasized the importance of the work of the Committee and its impact on international trade and stressed the necessity for transparency in its deliberations. Dr de Haen highlighted the significance of accurate, comprehensive and concise reporting of the proceedings and deliberations of the Committee's meetings. He also stated that the scientific committees that advise the Codex Alimentarius Commission must have adequate expertise and experience to enable them to perform accurate and scientifically sound evaluations. FAO and WHO make every effort to ensure that the process for the selection of Committee members is open and transparent, and that this process results in the selection of highly qualified scientists in the fields of competence needed to make important decisions that affect the safety of the world's food supply.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been 48 previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the forty-sixth meeting (Annex 1, reference *122*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
— to undertake toxicological evaluations of certain food additives, food ingredients, flavouring agents and contaminants (sections 3, 4 and 5 and Annex 2);
— to review and prepare specifications for selected food additives and flavouring agents (sections 3, 4 and 6 and Annex 2);
— to assess the risks associated with aflatoxin contamination (section 5); and
— to consider approaches to evaluate the intake of food additives in the light of the recommendations of the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) (section 2.4.2).

2.1 Modification of the agenda

The flavouring agents isoamyl alcohol, isoamyl formate and isoamyl acetate were removed from the agenda for toxicological evaluation because they had been evaluated at the forty-sixth meeting of the Committee (Annex 1, reference *122*). The flavouring agent geranyl 2-ethylbutanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-ethylbutanoate) was on the agenda under the esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids; however, it was evaluated with the esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids. The flavouring agent 4-hydroxy-3-methyloctanoic acid γ-lactone was added for toxicological evaluation under the aliphatic lactones.

Sixty flavouring agents included on the agenda under the saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids and the aliphatic lactones were not considered for specifications at the present meeting. The specifications for these substances will be reviewed at a future meeting of the Committee at which food additives and contaminants are considered.

The Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) reviewed the specifications of identity and purity of selected food additives that had been prepared at the forty-sixth meeting of the Expert Committee (Annex 1, reference *122*). The Codex Committee referred back to the Expert Committee the specifications of nine compounds: citric acid, propylene glycol, allyl cyclohexane propionate (2-propenyl cyclohexane propanoate), ethyl nonanoate, ethyl octanoate, isoamyl acetate (3-methylbutyl acetate), isoamyl butyrate (3-methylbutyl butanoate), isoamyl isobutyrate (3-methylbutyl 2-methylpropanoate) and isoamyl isovalerate (3-methylbutyl 3-methylbutanoate); these were added to the agenda of the present meeting.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, food ingredients, flavouring agents and contaminants, 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 subsequently at meetings of the Committee (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116* and *122*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) embraces the major observations, comments

and recommendations on the safety assessment of food additives and contaminants contained, up to the time of its publication, in the reports of the Committee and other associated bodies. The Committee noted that the document 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 modern technical advances.

2.2.1 *Procedure for the Safety Evaluation of Flavouring Agents*

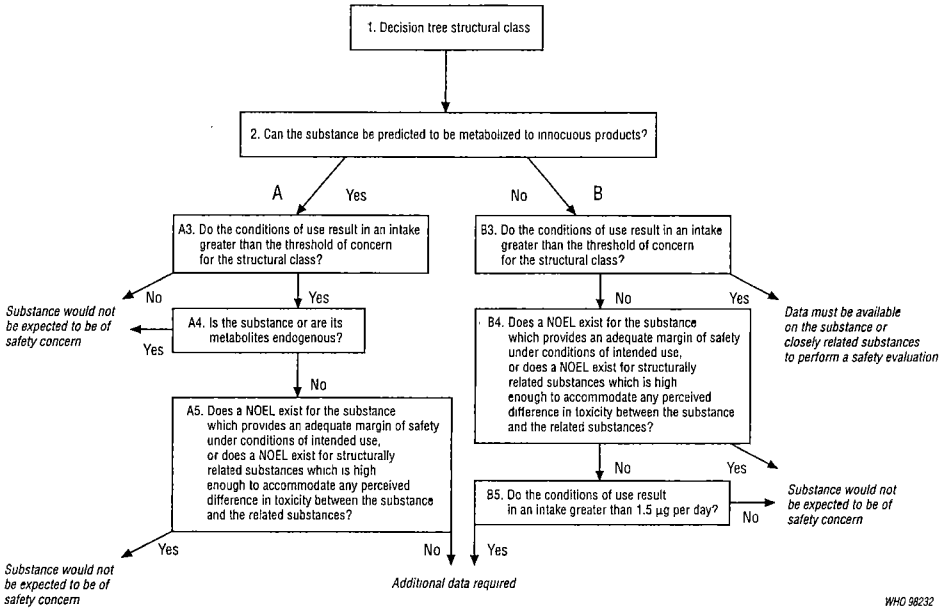
At its forty-sixth meeting (Annex 1, reference *116*), the Committee evaluated three groups of flavouring agents using a procedure based on that reviewed at its forty-fourth meeting (Annex 1, reference *122*). At the forty-sixth meeting, the Committee did not fully discuss the application of the last step on the right-hand side of the Procedure ("Do the conditions of use result in an intake greater than 1.5 μg per day?") to flavouring agents and this step was not considered. At that time, the Committee recommended that this step be considered at a future meeting at which food additives and contaminants were evaluated.

At its present meeting, the Committee considered the numbering of the various steps in the Procedure for the Safety Evaluation of Flavouring Agents. Concern was expressed about the numbering system used in the Procedure at the forty-sixth meeting, which implied that the two sides of the Procedure were parallel. To avoid this confusion, the Committee decided to prefix the steps on the left- and right-hand sides of the Procedure with the letters "A" and "B" respectively.

At its present meeting, the Committee also considered a paper that will be published in the WHO Food Additives Series (*3*) that provided further information relating to the derivation of the value of 1.5 μg per person per day. The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 μg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the conditions of use result in an intake greater than 1.5 μg per day?") (Fig. 1). The Committee

Figure 1

Procedure for the Safety Evaluation of Flavouring Agents adopted by the Committee at its present meeting



recognized that flavouring agents with unresolved toxicity problems could be evaluated by steps B3, B4 and B5 of the Procedure, but reaffirmed its view, expressed at the forty-sixth meeting, that as with any scheme, its application calls for judgement, and it should not replace expert opinion; the Committee therefore reserved the right to use alternative approaches when data on specific flavouring agents warranted such action.

During its present meeting, the Committee used the Procedure for the Safety Evaluation of Flavouring Agents given in Fig. 1.

In applying the Procedure at the present meeting, a number of general issues were raised.

Consideration of flavouring agents as groups

The flavouring agents were evaluated by the Committee in groups of structurally and/or metabolically related substances. Systematic changes in structure are the basis for understanding the effect of structure on the chemical and biological properties of a substance (Annex 1, reference *117*, Annex 5). When the Procedure is being used, the evaluation of flavouring agents in groups facilitates the consideration of common pathways of metabolism which apply to

members of a group. For substances which share common metabolites, consideration should be given to the total intake from all related substances. The total intake of such related flavouring agents, either from within the same group or across groups, should be evaluated where assessment of the intake data and toxicological profile warrant a combined appraisal. Total intakes should be considered for substances which produce the same potentially toxic metabolite (for example the esters of allyl alcohol considered at the forty-sixth meeting of the Committee (Annex 1, reference *122*)). The Committee concluded that flavouring agents which produce metabolites that are innocuous and endogenous would not be of safety concern, providing that the total intake from all related substances was judged not to give rise to perturbations outside the normal physiological range. Evaluations of such theoretical combined intakes should also take into account, where possible, the likelihood of the different flavouring agents being consumed together.

Natural occurrence

Some substances used as flavouring agents also occur as natural constituents of food; the influence of natural occurrence on the application of the Procedure was considered at the forty-fourth meeting of the Committee (Annex 1, reference *117*, Annex 5). Intakes from use as flavouring agents should be considered relative to the intakes from natural sources. This is a complex issue and interpretation of data on natural occurrence is dependent on:

— the nature of the flavouring substance and its structural class (step 1);
— the natural source of the substance;
— the intake from natural sources; and
— the proportion of the total intake accounted for by its use as a flavouring agent.

As a consequence the intake of such naturally occurring substances should be considered on a case-by-case basis.

Data on intakes of flavouring agents

The only estimates of intake of flavouring agents available to the Committee were derived from surveys in Europe and the USA. The Committee would welcome additional information on intakes of flavouring agents from other geographical regions. The Committee noted that the evaluations performed to date using the Procedure have been based on intake estimates available at the meeting at which the flavouring agents were considered and that changes in intake might warrant re-evaluation of a flavouring agent. The Committee

recommended that information on intakes be updated periodically to ensure the validity of the safety evaluations, particularly for flavouring agents for which the annual volume of production is variable.

The Committee considered that the estimation of intakes based on production data is a practical and realistic approach. Further consideration of intake should be given to flavouring agents for which there are high reported levels of use in some foods or beverages, but low intakes when calculated from production data. This would be particularly important for flavouring agents with intakes calculated to be only slightly below one of the threshold criteria in the evaluation process (i.e. steps A3, B3 or B5). However, the Committee recognized that estimation of intakes of flavouring agents based on reported levels of use would require detailed information on the specific products in which the flavouring agent is used, the actual levels of use and the intake of the individual food products containing the flavouring agent by consumers of both average and above average quantities of these foods.

2.2.2 *Role of the Committee in the risk analysis process of the Codex Alimentarius Commission*

The Committee welcomed the acknowledgement by the Codex Alimentarius Commission of the continuing need for the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues to provide risk assessments to the Codex Alimentarius Commission (*4*). The Committee wished to emphasize the essential role of the expert scientific judgements provided by scientific committees as the basis for risk assessment. Such expertise will be of increasing importance for future developments in understanding the scientific basis of risk assessment, such as mechanisms of toxicity, differences between test species and humans, and human diversity and genetic variability. The Committee will continue to welcome developments in methods of risk assessment which will provide a more sound scientific basis to the advice that it provides to the Codex Alimentarius Commission.

2.3 Principles governing the establishment and revision of specifications

2.3.1 *Significance of identity and purity requirements*

The Committee noted that the specifications monographs contain requirements under two subheadings, "identity tests" and "purity tests", and it emphasized that substances should meet all require-

ments listed under these headings. The meanings of "identification" and "purity" are both clearly defined in FAO Food and Nutrition Paper No. 5, Rev. 2 (Annex 1, reference *100*).

2.3.2 *Limits for arsenic, lead and other heavy metals*

In keeping with its previously stated intentions (Annex 1, references *116* and *122*) regarding limits for arsenic, lead and other heavy metals, the Committee considered four issues:

— the lack of specificity in the current limit test for heavy metals (expressed as lead) and the potential loss of metals during the dry ashing procedure, both of which compromise the validity of the test;
— the need to replace the general test for heavy metals with tests for specific metals, particularly for lead, cadmium, mercury and arsenic;
— the need for more specific and sensitive analytical methods, such as atomic absorption spectroscopy (including the graphite furnace technique) and for improved methods of sample preparation; and
— the need to consider the amount of a food additive consumed and its potential for contamination by specific heavy metals.

In order to address these issues in greater depth the Committee will seek information on the specific content of heavy metals in food additives as well as on methods of analysis. At the present meeting, the Committee decided to delete specifications for limits for heavy metals (expressed as lead) from specifications monographs where a specification for lead was available.

2.3.3 *Analytical methods*

Alternative methods

The Committee's policy has been to describe only one analytical method for an analyte with a limit in the specifications. However, the Committee has now concluded that in some instances the inclusion of alternative methods may be desirable for reasons of economy or simplicity. For example, the use of nuclear magnetic resonance spectroscopy has been presented as an alternative to gas chromatography for the method of assay in the specifications monograph on salatrim, because of the comparative simplicity of the sample preparation and instrumental analysis.

Methods of determining residual solvents and other volatile substances

The Committee observed that a number of specifications require analysis for volatile substances such as residual solvents and reaction

by-products. However, many of the analytical methods are out of date. The Committee wishes to encourage the use of improved techniques such as headspace gas chromatography, and in the future will expect descriptions of such methods to be supplied as part of information for revised or new specifications.

References to standard methods

During the revision of existing specifications the Committee noted that several references have been made to published standard analytical procedures (e.g. American Society for Testing and Materials (ASTM) methods) instead of providing full descriptions of the methods. One such reference was also included in a new monograph prepared at this meeting. The Committee concluded that in future such references should be replaced by a complete description of the methods.

2.3.4 *Enzymes derived from genetically modified organisms*

The Committee designated as "tentative" new specifications for two enzyme preparations derived from genetically modified organisms. This decision was made because Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*, section 2.3.4), referred to in the specifications, is itself tentative. The Committee decided to review Appendix B at its next meeting in 1998 and subsequently to re-evaluate the specifications for enzyme preparations which refer to Appendix B.

The Committee requested comments on Appendix B, to be reviewed in 1998.

2.3.5 *Flavouring agents*

The Committee was asked to consider the specifications for 224 flavouring agents at the present meeting, of which 60 were transferred to the agenda for a future meeting. It decided to modify the tabular format for the specifications for flavouring agents adopted at the forty-sixth meeting (Annex 1, reference *124*). Special methods of analysis and spectra will continue to be included as appendices to the table of flavouring agents. Where a substance has a function in addition to that of a flavouring agent, a standard specification format will be included.

The common names, chemical names and synonyms will be used to index the substances. A new identification number was allocated to

each substance for the convenience of the Committee and an identification number will also be allocated to each substance evaluated in the future. The Committee proposed to develop a classification by chemical group, for ease of future safety evaluations.

2.4 Principles governing intake assessments of food additives and contaminants

The Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standards Issues (*4*) recognized that assessments of the intake of food additives, contaminants and residues of pesticides and veterinary drugs should be considered an integral part of the risk assessment process for these substances. At its present meeting, the Committee also recognized the importance of assessments of dietary intake in characterizing any potential risks posed by food additives and contaminants.

2.4.1 *Methods for assessing dietary intake*

The Committee agreed with the conclusion of the FAO/WHO Consultation on Food Consumption and Exposure Assessment to Chemicals (*5*) that, in principle, the estimation of potential dietary intakes is the same for all food chemicals. However, because of differences in their occurrence in the food supply, assessments of intake of food additives and contaminants may be based on different types of data on consumption of food and on occurrence of chemicals in food. The procedures developed for pesticide residues are applicable to assessments of the dietary intake of contaminants which also occur in raw agricultural commodities (*6*). These procedures were employed in the assessment of the intake of aflatoxins (section 5).

In most cases, assessments of the intake of food additives are conducted using data on consumption of processed foods. For certain food additives, "poundage" data may be used to estimate per capita intake. Five main approaches for assessing dietary intake of food additives have been used by countries at the national level (*7*, *8*). These approaches usually overestimate chronic (long-term) daily intake, and this could compensate for potential differences in intake between population subgroups and for day-to-day fluctuations in individuals. The five approaches are summarized below.

The budget method

This method takes into account the physiological requirements for energy and fluid in estimating consumption of foods in solid and liquid form. The Committee emphasized that the budget method is

not a procedure for estimating food additive intake *per se*. However, because of its simplicity it is generally accepted as an appropriate screening tool at the international or national level for the identification of food additives that require further assessment.

The "poundage" approach
This approach uses statistics on usage of food additives, adjusted for imports, exports and non-food uses.

The food balance sheet/household survey approach
This method is based on food available for consumption either at a national or household level. Food balance sheets are adjusted for the proportion of each raw commodity that is processed and likely to contain the food additive being studied.

The model diet approach
This method is used to construct a model diet from information on food consumption for a selected population subgroup.

Individual dietary records
These methods use data from representative national surveys of individual food consumption.

These approaches are listed in the order of their increasing ability to accurately predict dietary intake. However, increased accuracy requires more comprehensive data on food consumption and use of food additives as well as greater resources for the assessment of dietary intake. Other approaches for assessing intake such as duplicate diet studies and total diet studies may also be used.

2.4.2 *Assessment of dietary intake of specific additives*

The Committee was requested at the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) to perform assessments of the intake of five food additives: benzoic acid and its salts, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and sulfites. These food additives have been identified for priority assessment by the Codex Committee because initial assessments indicate that intakes of these food additives may approach or exceed the ADIs established by the Expert Committee.

Information on national assessments of the intake of these five food additives will be requested from countries based on procedures developed by the Committee. The Committee recommended that an evaluation of intake assessments from various countries should be

undertaken at the next meeting of the Committee at which food additives and contaminants are considered. The likely intakes of these substances on a global basis will be assessed to respond to the request of the Codex Committee to provide guidance on the potential for the intakes of these food additives to exceed their ADIs.

3. Specific food additives and food ingredients

The Committee evaluated three food additives and one food ingredient for the first time and re-evaluated several food additives and aflatoxins considered at previous meetings. In addition, the Committee evaluated a large number of flavouring agents using the Procedure for the Safety Evaluation of Flavouring Agents (section 2.2.1). Information on the evaluations and on specifications is summarized in Annex 2.

3.1 Antioxidant: *tert*-butylhydroquinone (TBHQ)

tert-Butylhydroquinone (TBHQ) was previously evaluated by the Committee at its nineteenth, twenty-first, thirtieth, thirty-seventh and forty-fourth meetings (Annex 1, references *38*, *44*, *73*, *94* and *116*). At the forty-fourth meeting, the previously established temporary ADI of 0–0.2 mg/kg of body weight was extended, pending results from long-term toxicity studies in rodents. This ADI was derived from a no-observed-effect level (NOEL) of 1500 mg/kg in the diet (equivalent to 37.5 mg/kg of body weight per day) in a 117-week feeding study in dogs on the basis of haematological changes observed at the next highest dose level of 5000 mg/kg in the diet. At its present meeting, the Committee reviewed the results of the long-term toxicity studies in mice and rats. In addition, new information relating to the metabolism of TBHQ, its effects on the induction of enzymes, and its short-term and reproductive toxicity in rodents was available for review. The results from the long-term study in dogs and the genotoxicity studies relating to the clastogenic potential of TBHQ were also re-evaluated.

In studies reviewed at earlier meetings of the Committee (Annex 1, references *39* and *117*), TBHQ was shown to be extensively absorbed and rapidly excreted following ingestion by rats, dogs and humans. The major urinary metabolites in all three species were the *O*-sulfate and *O*-glucuronide conjugates, with the former predominating. In numerous *in vitro* studies, TBHQ was shown to induce the activity of phase II enzymes, including glucuronosyl transferase and glutathione transferase, by a mechanism independent of the *Ah* receptor.

Induction of hepatic glutathione transferase activity was also demonstrated following short-term administration of TBHQ in the diet of female mice.

TBHQ also undergoes redox cycling with the corresponding quinone, accompanied by the production of reactive oxygen species. In a study reviewed at the present meeting, three glutathione conjugates of TBHQ were identified in the bile of male rats and sulfur-containing metabolites of TBHQ were detected in the urine. In other studies, glutathione conjugates of TBHQ demonstrated higher redox cycling activity than unconjugated TBHQ, and were toxic to the kidney and bladder when administered intravenously to male rats.

In a new 13-week feeding study conducted in mice, significant treatment-related effects were noted in both sexes. These effects were decreased body-weight gain and hyperplasia of the mucosal epithelium of the forestomach. The latter effect was noted only at very high doses of TBHQ; 20 g/kg in the diet (equal to 4000 mg/kg of body weight per day) and above. The NOEL was 870 mg/kg of body weight per day. In a 13-week feeding study conducted in rats continuously exposed to TBHQ, starting *in utero*, treatment-related haemosiderin pigmentation of the spleen was noted in both sexes. In addition, there was a treatment-related increase in atrophy of the red pulp of the spleen in female rats receiving 2.5 and 5 g/kg in the diet. Bone marrow and haematological parameters were not altered at these doses. Hyperplasia of the forestomach was not observed in this study, even at the highest dose tested; 10 g/kg in the diet (equal to 800 mg/kg of body weight per day), although it has been noted in another study in adult rats following short-term administration of TBHQ at 20 g/kg in the diet. Treatment with TBHQ had no effect on the estrous cycle or on the histological appearance of the reproductive organs. Because pigmentation of the spleen was noted in female rats at the lowest dose tested, a NOEL could not be established, but the lowest-observed-effect level (LOEL) was 190 mg/kg of body weight per day. Irritation and hyperplasia observed in the nasal epithelium of both rats and mice and on the skin of mice were considered to be the consequence of direct contact with TBHQ from the diet.

The results of two recently conducted carcinogenicity studies were reviewed. In female mice, TBHQ induced an increase in the incidence of hyperplasia of the thyroid follicular cells at all dose levels. A non-significant increase in follicular cell adenomas was reported at the highest dose of 5 g/kg in the diet (equal to 600 mg/kg of body weight per day) but the incidence was within the range of historical controls. No follicular cell carcinomas were observed. Decreased body-weight

gains were also observed at the highest dose in both sexes. Since the Committee was aware that hydroquinone (the unsubstituted parent compound) induces thyrotoxicity in mice, but not rats (*9*), it considered that the hyperplasia of the follicular cells observed with TBHQ in this study might be a toxicologically significant effect. Consequently, it concluded that a NOEL could not be identified in this study and that the lowest dose of 1.25 g/kg in the diet, equal to 130 mg/kg of body weight per day, represented the LOEL. In the study in rats, toxicologically significant effects were noted only at the highest dose tested, 5 g/kg in the diet (equal to 220 mg/kg of body weight per day); these effects were an increase in the incidence of hyperplasia of transitional cells and suppurative inflammation of the kidneys of male rats and haemosiderin pigmentation of the spleen of the females. The Committee considered that 2500 mg/kg in the diet, equal to 110 mg/kg of body weight per day, represented the NOEL and that TBHQ was not carcinogenic in mice or rats.

The 117-week study on dogs in which the temporary ADI had been based was re-evaluated in the light of supplementary information requested from its authors. On the basis of actual intake data, nominal levels of 500, 1580 and 5000 mg/kg in the diet were equal to doses of 21, 72 and 260 mg/kg of body weight per day. In dogs of both sexes given the highest dose, statistically significant reductions in haemoglobin concentrations and erythrocyte volume fractions were observed at several sampling intervals throughout the study, although the values were within the ranges in historical controls. Red blood cell counts were also significantly decreased in male and female dogs in the highest-dose group at week 112, the only time at which measurement of this parameter was reported. Increases in the reticulocyte count (as a percentage of red blood cells) and the presence of immature red blood cell forms in the peripheral blood of animals from all treated groups, reported to occur late in the study, were not dose-related nor were they accompanied by changes in red blood cell parameters at 500 and 1580 mg/kg in the diet. On the basis of this re-evaluation, the Committee confirmed that the NOEL for long-term toxicity in dogs was 1580 mg/kg in the diet, equal to 72 mg/kg of body weight per day.

In view of the conflicting results of the genotoxicity assays reviewed at previous meetings of the Committee, many of the studies of TBHQ were re-evaluated at the present meeting with respect to the validity of the protocol and interpretation of data. The conclusions of a number of the studies could no longer be supported. The results of the well conducted studies indicated that TBHQ was clastogenic *in vitro* in the absence or presence of metabolic activation, but did not induce

the formation of micronuclei *in vivo*. In sister chromatid exchange assays, TBHQ was positive in mice *in vivo* and in an *in vitro* system. The results from several studies suggested that damage to DNA resulting from exposure to TBHQ, including chromosome loss and breakage, was secondary to the production of reactive oxygen species. In the light of this information, and the fact that TBHQ was not carcinogenic in rats or mice, the Committee concluded that TBHQ was unlikely to be genotoxic *in vivo* under conditions of use as an antioxidant, and that further genotoxicity studies were unnecessary.

The results of four reproductive toxicity studies in rats were evaluated. Taken together, the results of these studies indicated an adverse effect of TBHQ on the survival and/or body weight of pups at levels of 5 g/kg in the diet or higher. The effect on pup body weight occurred late in the lactation period. The NOEL was 2.5 g/kg in the diet, equivalent to 125 mg/kg of body weight per day.

On the basis of the data reviewed at the present meeting, the Committee concluded that TBHQ was not carcinogenic in rats or mice. After reviewing the long-term toxicity studies in mice, rats and dogs and the reproductive toxicity studies in rats, the Committee concluded that the most sensitive species was the dog. The Committee allocated an ADI of 0–0.7 mg/kg of body weight for TBHQ, based on the NOEL of 72 mg/kg of body weight per day and a safety factor of 100. The NOEL was rounded to one significant figure, as is the usual practice.

A toxicological monograph incorporating new information and relevant information from the earlier toxicological monographs and monograph addenda was prepared. The existing specifications were revised, with minor changes.

3.2 Emulsifiers

3.2.1 *Microcrystalline cellulose*

Microcrystalline cellulose was evaluated at the fifteenth, seventeenth and nineteenth meetings of the Committee (Annex 1, references *26*, *32* and *38*). At the nineteenth meeting, an ADI "not specified" was allocated. In the light of concern about possible persorption and consequential adverse effects of fine particles, the substance was re-evaluated at the present meeting.

In early studies persorption of microcrystalline cellulose was reported in various species including rats. A recent study in which a special

preparation of fine particle size of microcrystalline cellulose (median diameter 6 μm) was administered orally to rats (5 g/kg of body weight per day) for 90 days has failed to confirm the earlier observations. In this study precautions were taken to ensure that there was no cross-contamination of the tissues with fine particulate matter at autopsy.

In various acute toxicity studies in animals given microcrystalline cellulose parenterally there have been signs consistent with a tissue response to foreign particles. Similarly microcrystalline cellulose has been associated with the formation of granulomas in human lung when it has been injected intravenously by drug abusers. No such lesions have been described as a consequence of oral ingestion of microcrystalline cellulose by rats or humans.

In 90-day toxicity tests during which microcrystalline cellulose was administered to rats at concentrations of 25 g/kg to 500 g/kg in the diet, increased consumption of food to compensate for the low energy content of this material was observed. Although this may have some adverse effects on mineral absorption there was, in general, no compound-related systemic toxicity. The NOEL was 50 g/kg in the diet, equal to 3.8 g/kg of body weight per day.

A 2-year study in rats, which were fed microcrystalline cellulose in the diet, was brought to the attention of the Committee. Despite a lack of evidence of toxic effects, the Committee considered that the execution and reporting of the study were not adequate to identify a NOEL. *In vitro* and *in vivo* genotoxicity studies were negative.

In a 3-generation reproductive toxicity study in rats that had been reviewed by the Committee at its fifteenth meeting (Annex 1, reference *27*), there were some effects in animals given microcrystalline cellulose at 300 g/kg in the diet; these were considered to be a consequence of the quantity of material reducing the energy density of the diet. In recent embryotoxicity and teratogenicity studies in rats, there was no evidence of treatment-related effects at levels of up to 50 g/kg in the diet (equal to 4.6 g/kg of body weight per day), given on days 6 to 15 of pregnancy.

In some studies in humans there have been reports of alterations in gastrointestinal function following ingestion of microcrystalline cellulose. The changes do not appear to be related to systemic toxicity. The Committee concluded that the toxicological data from humans and animals provided no evidence that the ingestion of microcrystalline cellulose can cause toxic effects in humans when used in foods according to good manufacturing practice.

It is recognized that small particles of other materials may be persorbed and that the extent of persorption is greater with very small particles (<1 μm in diameter). Despite the absence of any demonstrated persorption of microcrystalline cellulose in the recent study in rats, the Committee, as a precautionary measure, revised the specifications for microcrystalline cellulose at the present meeting to limit the content of particles less than 5 μm in diameter. The Committee retained the ADI "not specified" for microcrystalline cellulose conforming to these specifications.

A toxicological monograph that incorporated the updated earlier monograph and summaries of studies reviewed for the first time at the present meeting was prepared. The existing specifications were revised and the requirement for particle size was changed from "greater than 5 μm" to "not more than 10% of the material has a particle size of less than 5 μm".

3.2.2 *Sucrose esters of fatty acids and sucroglycerides*

Sucrose esters of fatty acids and sucroglycerides were previously reviewed by the Committee at its thirteenth, seventeenth, twentieth, twenty-fourth, thirty-fifth, thirty-ninth and forty-fourth meetings (Annex 1, references *19*, *32*, *41*, *53*, *88*, *101* and *116*). At the forty-fourth meeting, the NOEL from a new long-term toxicity/carcinogenicity study in rats was used as the basis for the ADI. The sucrose ester formulations used in this study contained no monoglycerides or diglycerides and the highest dose tested was 50 g/kg in the diet, equal to 1970 mg/kg of body weight per day. Because the results from a tolerance study in humans raised some concerns about potential laxative effects and related abdominal symptoms, a temporary group ADI of 0–20 mg/kg of body weight was established for the sucrose ester content of sucrose esters of fatty acids and sucroglycerides, based on the long-term toxicity study in rats and a safety factor of 100. The results of a well designed and conducted tolerance study in humans were requested for review in 1997.

In the previous study in humans, single doses of 1.5–3.0 g, or divided doses of 3.0–4.5 g per day for 5–7 days, induced laxation and related abdominal symptoms. The results from a new study in humans indicated an absence of effects of sucrose esters of fatty acids on the frequency and appearance of faeces and related abdominal symptoms in men and women ingesting a divided daily dose of 1.5 g of sucrose esters of fatty acids (equal to 27 and 29 mg/kg of body weight per day, respectively) for 5 days. Even though the deficiencies in design noted in the earlier study, i.e. the small number of subjects and the lack of

proper controls, were corrected in the new study only a single dose level was used, which was below that previously associated with gastrointestinal disturbances. Consequently, it was not possible to confirm that the effects observed in the previous study were the result of treatment with sucrose esters of fatty acids.

The Committee noted that no systemic effects were observed in a well conducted long-term toxicity study in rats up to the highest dose tested, 1970 mg/kg of body weight per day. Consequently, a group ADI of 0–30 mg/kg of body weight for sucrose esters of fatty acids and sucroglycerides was allocated on the basis of the new study in humans without the application of a safety factor.

An addendum to the toxicological monograph was prepared. The existing specifications for sucrose esters of fatty acids and sucroglycerides were revised, with minor changes.

3.3 Enzyme preparations

3.3.1 *α-Acetolactate decarboxylase*

α-Acetolactate decarboxylase is an enzyme produced by submerged fermentation of *Bacillus subtilis* carrying the gene coding for α-acetolactate decarboxylase from *B. brevis*. It is used as a processing aid in the brewing and alcohol industry to avoid formation of the unpleasant tasting α-diacetyl from α-acetolactate during fermentation.

α-Acetolactate decarboxylase expressed in *B. subtilis* has not been previously evaluated by the Committee.

Data reviewed by the Committee included information on the pathogenicity of the source and donor organisms as well as short-term toxicity and genotoxicity studies on the enzyme preparation.

Two forms of α-acetolactate decarboxylase have been used in the toxicity studies, namely, an unstabilized form and a glutaraldehyde-stabilized form, which is the form used in the final commercial product.

The available data indicate that both the source organism, *B. subtilis*, and the donor organism, *B. brevis*, are considered to be non-pathogenic species. *B. subtilis* was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. The vector, pUB110, is a plasmid commonly used in the construction of recombinant microorganisms in the production of enzymes and was not considered to be of toxicological concern.

The pathogenicity of four strains of *B. subtilis* involved either in the construction of the recombinant strain or in the production of α-acetolactate decarboxylase was tested in a study in mice given a single dose by the intraperitoneal route. There were no clinical symptoms related to treatment, and no pathological changes were noted at the end of the study that could be associated with treatment.

The Committee considered the recombinant DNA procedures used, and concluded that the recombinant strain of *B. subtilis* should be regarded as a safe source of α-acetolactate decarboxylase.

Administration of α-acetolactate decarboxylase in the diet to rats in a 14-day and a 13-week study was not associated with any signs of toxicity at dietary levels equivalent to 2500 mg/kg in the feed (14-day study) or 500 mg/kg in the feed (13-week study) for either the unstabilized or stabilized enzyme. No long-term toxicity studies were available. In genotoxicity studies, negative results were obtained with both unstabilized and stabilized α-acetolactate decarboxylase in *in vitro* gene mutation assays in bacteria and mammalian cells and in a chromosomal aberration assay in human lymphocytes.

On the basis of the toxicological data, the Committee concluded that α-acetolactate decarboxylase is an enzyme of low toxicity and that no further studies are required to assess its safety.

The Committee established a temporary ADI "not specified" for α-acetolactate decarboxylase from this recombinant strain of *B. subtilis* when the preparation is used in accordance with good manufacturing practice. A temporary ADI was allocated, pending consideration of the "tentative" qualification of the specifications.

A toxicological monograph was prepared. New specifications were prepared and designated as "tentative" because of the tentative qualification of Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*, section 2.3.4).

3.3.2 *Maltogenic amylase*

The enzyme under evaluation is a maltogenic amylase produced by submerged fermentation of a non-pathogenic and non-toxicogenic strain of *Bacillus subtilis* which contains the *amy*M gene from *B. stearothermophilus* coding for maltogenic amylase.

Maltogenic amylase expressed in *B. subtilis* has not been previously evaluated by the Committee.

Formulations of maltogenic amylase are used in the baking and starch industry. It is an exo-acting maltogenic amylase enzyme (EC 3.2.1.133, glucan 1,4-α-maltohydrolase), which catalyses the hydrolysis of 1,4-α-glucosidic linkages in amylose, amylopectin and related glucose polymers. Maltose units are successively removed from the non-reducing end of the polymer chain until the molecule is degraded or, in the case of amylopectin, until a branch point is reached. The Committee noted that the human intake of this recombinant maltogenic amylase resulting from its intended use in the baking and starch industry would be low and that the material consumed would not be the active maltogenic amylase but a heated, denatured material.

The data reviewed included the genetic modification procedures employed, characterization of the producing organisms, the fermentation process, acute and short-term toxicity studies in animals, and genotoxicity studies.

The Committee noted that well documented non-pathogenic and non-toxicogenic strains of microorganisms (*Bacillus subtilis*, *Escherichia coli* K12 and *B. stearothermophilus*) had been used in the genetic modification procedures. The final vector used (pUB110) is well characterized and has been used for several years as a cloning vehicle for *B. subtilis*. The plasmid construct pDN1413, containing the *amy*M gene, was introduced into *B. subtilis* (a derivative of strain 168) using standard transformation procedures. Although the plasmid pDN1413 carries the gene for kanamycin resistance, it is unlikely that this gene can be transferred, since it is well integrated into the host genome and no plasmid DNA could be detected in the end-product. The entire DNA sequence of pDN1413 was determined, which confirmed that genes coding for shiga-like toxins are not present.

B. subtilis was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation.

From the evaluation of the recombinant DNA procedures being employed, the Committee concluded that the final construct should be regarded as a safe source of maltogenic amylase.

The product tested in the toxicological studies was a concentrated material with an enzyme activity of 35900 units/g. It was produced according to the standard production process except that the formulation/standardization step was omitted and the product was lyophilized.

In a 90-day study in which the lyophilized test compound was administered in the diet of rats, the highest dose, 50 g/kg in the diet, caused a significant reduction in body-weight gain accompanied by a slight decrease in food consumption in both males and females. A significant decrease in thyroid weights was also seen in both males and females. At the next dose, 15 mg/kg in the diet, no statistically significant treatment-related findings were observed. The NOEL in this study was 15 g/kg in the diet, equal to 1200 mg/kg of body weight per day.

The test compound had no effects in *in vitro* gene mutation studies in bacteria or mammalian cells, and the results of chromosomal aberration tests *in vivo* and *in vitro* were consistently negative.

The test compound did not cause skin or eye irritation in rabbits and did not produce skin sensitization in a delayed-contact hypersensitivity assay in guinea-pigs.

The Committee allocated a temporary ADI "not specified" to maltogenic amylase derived from this recombinant strain, pending deletion of the "tentative" qualification of the specifications.

A toxicological monograph was prepared. New specifications were prepared and designated as "tentative" because of the tentative qualification of Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*, section 2.3.4).

3.4 Flavouring agent: *trans*-anethole

trans-Anethole was previously reviewed by the Committee at its eleventh, twenty-third, twenty-seventh, twenty-eighth, thirty-first, thirty-third, thirty-seventh and thirty-ninth meetings (Annex 1, references *14*, *50*, *62*, *66*, *77*, *83*, *94* and *101*). At the thirty-seventh meeting, a temporary ADI of 0–0.6 mg/kg of body weight was allocated on the basis of the results of a long-term toxicity/carcinogenicity study in Sprague–Dawley rats. In this study, a dose-related increase in the incidence of non-neoplastic proliferative lesions in the liver was observed in both males and females at all doses. In addition, a clear increase in the incidence of hepatocellular adenomas and carcinomas was observed in female rats receiving *trans*-anethole at the highest level, 10 g/kg in the diet (equal to 550 mg/kg of body weight per day). In male animals a slight increase in the incidence of hepatocellular adenomas but not carcinomas was observed at 10 g/kg in the diet (equal to 400 mg/kg of body weight per day).

At the thirty-ninth meeting, the Committee was informed that comparative metabolic studies in mice and rats, studies on the effects of long-term dietary administration of *trans*-anethole on hepatic enzyme induction and cell proliferation in these species and on enzyme induction in humans, and *in vitro* cytotoxicity and genotoxicity studies were in progress. The temporary ADI was extended to 1997, pending the completion of these studies and the results of a long-term study in mice at appropriate levels to establish a no-effect level.

Not all of the requested studies were available for review at the present meeting. The Committee was informed that further studies would be available by 1998.

The Committee extended the previously allocated temporary ADI of 0–0.6 mg/kg of body weight until 1998, pending the submission of the results of the remaining studies.

No toxicological monograph was prepared. The specifications were revised and, as *trans*-anethole is used only as a flavouring agent, they were transferred to the list of flavouring agents in the *Compendium of food additive specifications, Addendum 5* (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997).

3.5 Glazing agent: hydrogenated poly-1-decene

Hydrogenated poly-1-decene has not been previously evaluated by the Committee. It is used as a glazing and releasing agent.

The Committee considered that the data available from a 28-day range-finding study and a 90-day study in which hydrogenated poly-1-decene was administered in the feed of rats were inadequate to support the use of this product as a food additive. Considering the potentially high intake from its use, the Committee concluded that adequate data are required to establish that oily coats observed in rats fed hydrogenated poly-1-decene are not the result of systemic absorption. In addition, data in humans that clearly demonstrate the lack of absorption of this substance should be provided. In the absence of these data, long-term toxicity and reproductive toxicity studies and information on the metabolism, distribution and excretion of hydrogenated poly-1-decene would be required.

A toxicological monograph was not prepared. New specifications were prepared.

3.6 Sweetening agent: maltitol syrup

At the present meeting, the Committee reviewed a request for the amendment of the specifications for maltitol syrup in the context of its

toxicological implications. The current specifications (Annex 1, reference *124*) to which the ADI "not specified" applies (Annex 1, references *83* and *107*) require that maltitol syrup has a maltitol content of no less than 50%, a sorbitol content of no more than 8%, a maltotriitol content of no more than 25% and a content of hydrogenated polysaccharides containing more than three glucose or glucitol units of no more than 30%. An ADI "not specified" was allocated to maltitol syrup produced from glucose syrups that meet these specifications at the thirty-third meeting of the Committee (Annex 1, reference *83*) and confirmed at the forty-first meeting (Annex 1, reference *107*).

The proposed amendment would support the use of a broader range of starch hydrogenation products than are currently permitted. By deletion of the specification tests for hydrogenated saccharides other than maltitol, while still requiring a maltitol content of no less than 50.0% and a total polyol content of no less than 99.0%, the content of any of these components in malititol syrup (sorbitol, maltotriitol and higher-order polyols) could theoretically be as high as 49%. Since an ADI "not specified" has been established for both sorbitol and maltitol, the toxicological review concentrated on the consequences of high concentrations of the higher-order hydrogenated saccharides.

The results of metabolic studies in rats and humans indicated that the higher-order polyol components in hydrogenated starch hydrolysates of differing composition were efficiently hydrolysed in the gastrointestinal tract to glucose and a small amount of maltitol. Maltitol was hydrolysed less readily by endogenous enzymes and a considerable portion undergoes fermentation in the lower gastrointestinal tract. The small amount that is absorbed is rapidly excreted unchanged in the urine.

Studies in animals treated with maltitol syrup composed of up to 41% higher-order polyols were reviewed at the twenty-ninth meeting of the Committee (Annex 1, reference *70*). At its present meeting, the Committee reviewed the toxic potential of two materials that contain more than 49% of the hydrogenated polysaccharides, the first containing 10% sorbitol, 8% maltitol and 82% higher-order polyols and the second containing 100% hydrogenated dextrin, were evaluated in studies in which they were fed to animals, and the mutagenic potential of hydrogenated dextrin was also examined in bacterial assays. A study in rats showed that ingestion of up to 5.2 g/kg of body weight per day of hydrogenated dextrin for 13 weeks did not result in any treatment-related effects. No treatment-related toxicity was seen in

rats or dogs when the material containing 10% sorbitol, 8% maltitol and 82% higher-order polyols was administered in the diet at dosages of up to 18 and 43 g/kg of body weight per day, respectively, for 90 days. Hydrogenated dextrin was not mutagenic in either *Salmonella typhimurium* or *Escherichia coli* strains in the absence or presence of rat S9 microsomal fraction.

On the basis of the above considerations, the Committee confirmed the previous ADI "not specified" and concluded that it could be applied to maltitol syrup meeting the revised specifications.

An addendum to the toxicological monograph was prepared. In reviewing the specifications for maltitol syrup, the Committee considered the specifications for "hydrogenated saccharides other than maltitol" to be unnecessary and deleted the reference and respective purity test.

3.7 Miscellaneous substance: salatrim (short- and long-chain acyltriglyceride molecules)

Salatrim has not been previously reviewed by the Committee. Salatrim is a family of structured triglycerides containing, on average, one or two long-chain fatty acid moieties (usually stearic acid), the remainder being short-chain fatty acids. It is intended for use as a reduced-calorie replacement for conventional fats and oils.

The Committee evaluated studies on the caloric value of salatrim, being aware that short-chain fatty acids supply fewer kilocalories per gram than long-chain fatty acids. However, the claim of poor absorption of stearic acid from salatrim has not been proven for humans. Because there is no specific formulation for salatrim, it is not possible to assign a single caloric value to this product. The Committee noted that the specifications for salatrim that were elaborated at the present meeting permit formulations that include up to 0.87 g of stearate per g of fat. The biological data available do not provide information on materials with such compositions. If future studies determine that stearic acid is poorly absorbed from such formulations, the Committee considered that the consequences of this will need to be determined.

In evaluating the safety of salatrim, the Committee considered various studies. An *in vitro* study with porcine pancreatic lipase demonstrated that a wide range of the salatrim triacylglycerides are hydrolysed rapidly. In rats, the *in vivo* metabolism of a specific salatrim formulation indicated that it was metabolized in an analogous manner to triolein.

Salatrim products do not contain any structural features suggestive of potential mutagenicity, and no evidence of genotoxicity was observed in an adequate range of *in vitro* or *in vivo* studies.

Five 90-day studies in rats, each using a different salatrim formulation administered at concentrations of up to 100 g/kg in the diet, showed no toxicologically significant effects. A 28-day study in minipigs of a specific salatrim formulation was carried out using dose levels of 30, 60 and 100 g/kg in the diet, and also showed no toxicologically significant effects. These studies were not designed to detect potential nutritional effects, and the study in minipigs was of insufficient duration. The Committee concluded that these limitations meant that the studies did not provide an adequate basis for a nutritional or toxicological evaluation.

Because of the high projected intake of salatrim products (90th percentile levels for "all ages" and for 3–5-year-olds are 37 and 26 g per day, respectively) and given that no systemic effects were seen in animal studies, the Committee paid particular attention to the results of the five studies in humans. Of these, one was a free-living trial, the other four were clinic-based with different experimental designs.

In the four clinic-based studies the experimental protocols provided intakes of up to 60 g of salatrim per person per day for periods of 1, 4 or 7 days. Although these studies provided some indication that the consumption of salatrim in the diet was associated with an increased incidence of mild gastrointestinal symptoms and significantly elevated serum enzymes, the treatment periods were short and the numbers of study participants were few.

The free-living study was a randomized, double-blind, multiple-dose, parallel comparison between diets in which the fat was replaced by salatrim oil (23SO, 4SO or 43SO) and control diets in which the fat was soy oil. At least 12 women and 12 men were recruited for each of the two control groups and each of the five treatment groups which received 30, 45 or 60 g per day of 23SO, 60 g per day of 4SO or 60 g per day of 43SO. The total duration of the study was 6 weeks. Subjects in the treatment groups received soy oil during weeks 1 and 6 and salatrim during weeks 2–5, while those in the control groups received soy oil throughout the study.

A total of 183 subjects started the study; 34 dropped out, four of whom were controls. Of those who dropped out, 20 had received salatrim and recorded adverse effects as the reason for leaving the study. The Committee noted inconsistencies between the published

and unpublished reports of the study in that there were differences in the recorded numbers of subjects dropping out.

The consumption of 60 g of salatrim per day was associated with more reports (compared to controls) of stomach cramps and nausea in a substantial number of subjects. Transient elevations of the levels of alanine aminotransferase and aspartate aminotransferase were recorded. Due to the short duration of the study, the high drop-out rate and the modest number of participants, the Committee concluded that it was not possible to determine whether these observations were clinically significant.

The Committee concluded that the available studies did not provide an adequate basis for evaluating the safety and nutritional effects of salatrim. The Committee recommended that additional appropriately designed studies be performed to assess fully both the safety and nutritional consequences of ingestion of salatrim.

A monograph addressing the safety and nutritional effects of salatrim, which included an analysis of the caloric content of the salatrim family, was prepared.

The Committee considered salatrim to be a replacement for conventional fats and oils and therefore regarded it as a food ingredient and not as a food additive. At the request of the Codex Committee on Food Additives and Contaminants the Committee nevertheless prepared new specifications to describe this food ingredient.

4. Substances evaluated using 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 modified at the present meeting (section 2.2.1 and Fig. 1).

The Committee noted that in applying the Procedure, the substance is first assigned to a structural class as identified at the forty-sixth meeting of the Committee (Annex 1, reference *122*). The structural classes are as follows:

- Class I. Substances that have simple chemical structures and efficient modes of metabolism which would suggest a low order of oral toxicity.
- Class II. Substances 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. Substances 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.

The Committee first considered the metabolic pathways common to the groups of flavouring agents evaluated at the present meeting.

Hydrolysis of esters

Linear alkyl esters are hydrolysed rapidly to their component alcohols and carboxylic acids in the intestinal tract, blood and liver, and most tissues throughout the body. Hydrolysis is catalysed by classes of enzymes recognized as carboxylesterases or esterases. For simple linear esters, as considered at this meeting, the rate of hydrolysis increases with increased chain length of either the acid or alcohol component. The rate of hydrolysis of straight-chain esters is approximately 100 times that of branched-chain esters. The rates of hydrolysis of the alkenyl esters citronellyl acetate (3,7-dimethyl-6-octen-1-yl acetate) and citronellyl phenylacetate, by artificial pancreatic juice, were similar to the rates for simple branched-chain esters.

Oxidation of alkyl primary alcohols and aldehydes

Most linear and branched-chain, saturated and unsaturated primary alcohols are oxidized rapidly to their corresponding aldehydes by alcohol dehydrogenase. The rate of oxidation increases with increased chain length, and the presence of a double bond.

The subsequent oxidation of the aldehydes to their corresponding acids is catalysed by dehydrogenase and oxidase enzymes. The most active is a NAD^+/NADH-dependent aldehyde dehydrogenase present in the cytosol, the activity of which increases with increasing relative molecular mass of the aldehyde substrate. Aldehydes may also be reduced to alcohols or conjugated with sulfhydryl-containing substances, such as glutathione. Aldehyde dehydrogenase-catalysed oxidation of aldehydes of low relative molecular mass requires glutathione, which suggests that the free aldehyde may be conjugated rapidly with glutathione *in vivo* to form a thiohemiacetal that is subsequently oxidized to the corresponding acid. Branched-chain aldehydes are rapidly oxidized by aldehyde dehydrogenase, and the rate of oxidation of 2-methylpropanal is similar to that of acetaldehyde.

Oxidation of linear saturated carboxylic acids

Aliphatic linear saturated carboxylic acids are metabolized in the fatty acid β-oxidation pathway, the tricarboxylic acid cycle, or the C_1-tetrahydrofolate pathway. Oxidation of formic acid to carbon dioxide and water occurs primarily in the liver and is catalysed by tetrahydrofolate in humans and other primates.

Other carboxylic acids are condensed with coenzyme A (CoA) to yield thioesters that undergo β-cleavage to acetyl CoA. Carboxylic acids containing an even number of carbon atoms give acetyl CoA, whereas those containing an odd number yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly, whereas propionyl CoA is first converted to succinyl CoA.

Oxidation of branched-chain saturated carboxylic acids

Short-chain (containing six or fewer carbon atoms) branched-chain saturated aliphatic acids undergo β-oxidation preferentially in the longer chain, followed by cleavage to yield linear acid fragments that are metabolized via the fatty acid pathway or the tricarboxylic acid cycle. Isobutyric acid (2-methylpropanoic acid), isovaleric acid (3-methylbutanoic acid) and 2-methylbutyric acid (2-methylbutanoic acid) are formed during the oxidative deamination of endogenous branched-chain amino acids and are metabolized by normal pathways of intermediary metabolism. At high dose levels, longer branched-chain acids may undergo ω-oxidation to yield diacids that undergo further oxidation and cleavage.

Acids with a methyl substituent are extensively metabolized to carbon dioxide via β-oxidation, unless the methyl group is located at the β-position (e.g. 3-methylpentanoic acid), in which case α-oxidation

occurs, yielding short-chain acid fragments capable of being completely metabolized.

The presence of a 2-ethyl substituent prevents the β-oxidation of aliphatic carboxylic acids, and these compounds undergo ω-oxidation and ω-1 oxidation to yield polar metabolites that are excreted primarily in the urine. Saturation of this ω-oxidation pathway may lead to formation of the 2-substituted carboxylic acid that may be excreted as the glucuronic acid conjugate.

4.1 Allyl 2-furoate

Twenty-one allyl esters used as flavouring agents in food were evaluated by the Committee at its forty-sixth meeting using the Procedure for the Safety Evaluation of Flavouring Agents modified at that meeting (Annex 1, reference *122*). The Committee concluded that the use of 20 of the 21 allyl esters that were evaluated posed no safety concerns at their estimated levels of intake. The evaluation of one of the flavouring agents in the group, allyl 2-furoate (2-propenyl furan-2-carboxylate), was postponed pending consideration of the last step on the right-hand side of the Procedure in which a decision criterion of 1.5 μg per person per day is applied to a substance for which adequate data on metabolism and toxicity are lacking. At the present meeting, allyl 2-furoate was evaluated in accordance with the Procedure inclusive of this step (see Fig. 1).

Intake data

On the basis of a reported annual volume of production of 1 kg in Europe (International Organization of the Flavor Industry, personal communication, 1995) and <0.01 kg in the USA (*10*), the estimated daily per capita intake of allyl 2-furoate is 0.14 μg in Europe and <0.01 μg in the USA. These estimates were calculated assuming under-reporting of the poundage data and consumption by 10% of the population as described in Annex 5 of WHO Food Additives Series, No. 35 (Annex 1, reference *117*).

Information on absorption, metabolism and elimination

No data on the metabolism of allyl 2-furoate, an ester of allyl alcohol and 2-furoic acid, were available. The Committee recognized that allyl esters are generally hydrolysed to allyl alcohol and their corresponding carboxylic acids. At its forty-sixth meeting, however, the Committee determined that there was insufficient evidence to conclude that allyl 2-furoate would be rapidly and completely hydrolysed in humans.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to allyl 2-furoate, the Committee assigned the compound to structural class III (*11*).

Step 2. No data on the metabolism of allyl 2-furoate were available and the substance could not be predicted to be metabolized to innocuous products. Accordingly, the right-hand side of the decision tree was further considered.

Step B3. The intake estimates for allyl 2-furoate were below the threshold for class III (90 μg per day).

Step B4. There were no toxicity data on allyl 2-furoate or on a structurally related substance to provide a NOEL to indicate whether an adequate margin of safety exists under conditions of intended use.

Step B5. The conditions of use of allyl 2-furoate do not result in an intake greater than 1.5 μg per day. The estimated intake is approximately one-tenth of this value.

Conclusion

No multiple-dose toxicity studies on allyl 2-furoate were available. At its forty-sixth meeting, the Committee considered the available toxicity data on the other allyl esters as inapplicable to the evaluation of allyl 2-furoate, because they were all expected to be rapidly and completely hydrolysed in humans.

If hydrolysis of allyl 2-furoate were assumed, the ADI for allyl alcohol and knowledge of the metabolism of 2-furoic acid would support a conclusion of no safety concern for this substance. In accordance with the Procedure, the Committee concluded that allyl 2-furoate would not be expected to present a safety concern at the estimated level of current intake.

A toxicological monograph was not prepared. The tentative specifications prepared at the forty-sixth meeting of the Committee were maintained.

4.2 Saturated aliphatic acyclic linear primary alcohols, aldehydes and acids

The Committee evaluated a group of 38 flavouring agents that included selected saturated aliphatic acyclic linear primary alcohols, aldehydes and acids of chain length C_{1-18} (Table 1) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 1
Summary of the results of safety evaluations of 38 saturated aliphatic acyclic linear primary alcohols, aldehydes and acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on levels of current intake
Formic acid[c]	0079	No Europe: 800 USA: 160	NR	Formic acid is produced endogenously in humans and is a normal component of intermediate metabolism	No safety concern
Acetaldehyde	0080	Yes Europe: 11000 USA: 9700	Yes	Acetaldehyde is oxidized to acetic acid which is metabolized via the citric acid cycle; acetaldehyde can also be reduced to ethanol	
Acetic acid[c]	0081	Yes Europe: ND USA: 360000	Yes	Acetic acid is metabolized to carbon dioxide; it acetylates amines and can be incorporated into proteins	
Propyl alcohol	0082	Yes Europe: 420 USA: 2700	Yes	Propyl alcohol is oxidized to propionaldehyde which is oxidized to propionic acid; propionic acid is metabolized via the citric acid cycle	
Propionaldehyde	0083	No Europe: 33 USA: 140	NR	Propionaldehyde is oxidized to propionic acid which is metabolized via the citric acid cycle	

Propionic acid[c]	0084	Yes Europe: 1100 USA: 5200	Yes	Propionic acid is metabolized via the citric acid cycle	No safety concern
Butyl alcohol	0085	Yes Europe: 1900 USA: 8100	Yes	Butyl alcohol is oxidized to butyraldehyde which is oxidized to butyric acid; butyric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Butyraldehyde	0086	No Europe: 26 USA: 17	NR	Butyraldehyde is oxidized to butyric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Butyric acid	0087	Yes Europe: 10000 USA: 5900	Yes	Butyric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Amyl alcohol	0088	No Europe: 97 USA: 44	NR	Amyl alcohol is oxidized to valeraldehyde which is rapidly oxidized to valeric acid; valeric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Valeraldehyde	0089	Yes Europe: 3000 USA: 8.8	Yes	Valeraldehyde is rapidly oxidized to valeric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Valeric acid	0090	No Europe: 140 USA: 850	NR	Valeric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexyl alcohol	0091	Yes Europe: 1900 USA: 800	Yes	Hexyl alcohol is oxidized to hexanal which is rapidly oxidized to hexanoic acid; hexanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexanal	0092	No Europe: 780 USA: 260	NR	Hexanal is rapidly oxidized to hexanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexanoic acid	0093	Yes Europe: 3500 USA: 1300	Yes	Hexanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	

Table 1 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Heptyl alcohol	0094	No Europe: 12 USA: 7	NR	Heptyl alcohol is oxidized to heptanal which is rapidly oxidized to heptanoic acid; heptanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
Heptanal	0095	No Europe: 200 USA: 3.2	NR	Heptanal is rapidly oxidized to heptanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Heptanoic acid	0096	No Europe: 170 USA: 5.3	NR	Heptanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
1-Octanol	0097	No Europe: 230 USA: 32	NR	1-Octanol is oxidized to octanal which is rapidly oxidized to octanoic acid; octanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Octanal[c]	0098	No Europe: 170 USA: 90	NR	Octanal is rapidly oxidized to octanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Octanoic acid	0099	Yes Europe: 3800 USA: 650	Yes	Octanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Nonyl alcohol	0100	No Europe: 8.1 USA: 2.1	NR	Nonyl alcohol is oxidized to nonanal which is rapidly oxidized to nonanoic acid; nonanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Nonanal[c]	0101	No Europe: 130 USA: 17	NR	Nonanal is rapidly oxidized to nonanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	

Nonanoic acid	0102	No Europe: 64 USA: 63	NR	Nonanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
1-Decanol	0103	No Europe: 290 USA: 7	NR	1-Decanol is oxidized to decanal which is rapidly oxidized to decanoic acid; decanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Decanal	0104	No Europe: 288 USA: 61	NR	Decanal is rapidly oxidized to decanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Decanoic acid	0105	No Europe: 1400 USA: 980	NR	Decanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways; at high concentrations, it undergoes ω-oxidation	
Undecyl alcohol	0106	No Europe: 0.9 USA: 11	NR	Undecyl alcohol is oxidized to undecanal which is rapidly oxidized to undecanoic acid; undecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Undecanal	0107	No Europe: 480 USA: 1.5	NR	Undecanal is rapidly oxidized to undecanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Undecanoic acid	0108	No Europe: 4.6 USA: 8.8	NR	Undecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Lauryl alcohol	0109	No Europe: 170 USA: 80	NR	Lauryl alcohol is oxidized to lauric aldehyde which is rapidly oxidized to lauric acid; lauric acid is metabolized via the fatty acid and tricarboxylic acid pathways	

Table 1 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Lauric aldehyde	0110	No Europe: 52 USA: 21	NR	Lauric aldehyde is rapidly oxidized to lauric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
Lauric acid	0111	No Europe: 590 USA: 1200	NR	Lauric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Myristaldehyde	0112	No Europe: 9.4 USA: 25	NR	Myristaldehyde is rapidly oxidized to myristic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Myristic acid	0113	No Europe: 160 USA: 72	NR	Myristic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
1-Hexadecanol	0114	No Europe: 3.6 USA: 0.2	NR	1-Hexadecanol is oxidized to hexadecanal which is rapidly oxidized to hexadecanoic acid; hexadecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Palmitic acid	0115	No Europe: 89 USA: 234	NR	β-oxidation of palmitic acid yields 2-carbon units which enter the tricarboxylic acid cycle	
Stearic acid	0116	Yes Europe: 58 USA: 1900	Yes	β-oxidation of stearic acid yields 2-carbon units which enter the tricarboxylic acid cycle	

NR: Not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the procedure. ND: no intake data reported

[a] *Step 1*: All of the substances in this group are in structural class I, the human intake threshold of which is 1800 μg per day.
Step 2: All of the substances in this group are metabolized to innocuous products.

[b] All intake values are expressed in μg per day.

[c] The ADI for this substance was maintained.

Several substances in the group have been evaluated previously by the Committee. At the seventeenth meeting, group ADIs "not limited" were allocated to acetic acid and its potassium and sodium salts and to propionic acid (propanoic acid) and its calcium, potassium and sodium salts, and an ADI of 0–3 mg/kg of body weight was allocated to formic acid (Annex 1, reference *32*), which was made a group ADI with ethyl formate at the twenty-third meeting (Annex 1, reference *50*). A group ADI of 0–0.1 mg/kg of body weight was established for octanal and nonanal, singly or in combination, at the twenty-eighth meeting (Annex 1, reference *66*). An ADI "not limited" was allocated to the aluminium, ammonium, calcium, magnesium, potassium and sodium salts of myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acids at the seventeenth meeting (Annex 1, reference *32*). At the twenty-ninth meeting, the Committee did not establish ADIs for these acids due to lack of information on the manufacture and use of the food-grade material, but noted that they are normal constituents of coconut oil, butter and other edible oils. ADIs have not been allocated to butyl alcohol (1-butanol), decanal or propyl alcohol (1-propanol) because the data were considered to be inadequate (Annex 1, references *38*, *14* and *56* respectively).

The intake of one substance, ethyl alcohol (ethanol), which is structurally related to the group was considered at the forty-sixth meeting (Annex 1, reference *122*), when the Committee evaluated ethyl esters used as flavouring agents. At that time, the Committee concluded that ethyl alcohol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.

Intake data

The total annual volume of production of the 38 substances from their use as flavouring agents is approximately 2100 tonnes in the USA. Approximately 90% of the total volume is accounted for by acetic acid, which includes the amount produced for uses (acidulant or solvent) in food other than as a flavouring agent. No specific data were available on the use of acetic acid as a flavouring agent in Europe. Disregarding acetic acid, the total reported annual volume of production of the remaining 37 aliphatic substances used as flavouring agents is approximately 300 tonnes in Europe and 200 tonnes in the USA.

According to production statistics and derived estimated intakes of flavouring agents in Europe and the USA, acetaldehyde (ethanal), butyl alcohol and butyric acid (butanoic acid) are the most important substances in this group. Acetaldehyde and butyric acid account for

about 50% of the daily per capita intake in Europe, and acetaldehyde and butyl alcohol account for about 46% of the daily per capita intake in the USA. Other flavouring agents in this group with high intake levels (i.e. >1800 μg per person per day) include octanoic acid, hexanoic acid, valeraldehyde (pentanal), butyl alcohol and hexyl alcohol (1-hexanol) in Europe and butyric acid, propionic acid, propyl alcohol and stearic acid in the USA (Table 1).

Saturated linear aliphatic alcohols, aldehydes and acids are ubiquitous in nature. Alcohols and acids of low relative molecular mass have been detected in almost every known fruit and vegetable. There are a limited number of reports of the natural occurrence of the corresponding aldehydes. In the USA, the available quantitative data indicate that the dietary consumption of saturated linear aliphatic alcohols, aldehydes and acids from naturally occurring sources exceeds their consumption from use as flavouring substances.

Information on absorption, metabolism and elimination

Linear aliphatic acyclic alcohols, aldehydes and carboxylic acids are absorbed through the gastrointestinal tract. Their half-lives in plasma are difficult to measure since many alcohols of low relative molecular mass (e.g. ethyl alcohol), aldehydes and carboxylic acids (e.g. acetic acid and propionic acid) are endogenous in humans. Acetaldehyde has been detected in whole blood (<0.2 mg/l) and acetate is a blood buffer.

The flavouring agents in this group of selected saturated aliphatic linear alcohols, aldehydes and acids are all metabolized via the fatty acid and tricarboxylic acid pathways (see pages 26–27).

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic linear alcohols, aldehydes and acids, the Committee assigned all 38 substances to structural class I.

Step 2. All of the flavouring agents in this group are known to be, or can be readily predicted to be, efficiently metabolized to innocuous substances. Accordingly, the left-hand side of the decision tree was further considered.

Step A3. The intake estimates for 27 substances in this group fall below the threshold for human intake for class I (1800 μg per day); therefore, these substances were considered to be of no safety concern.

Step A4. The intake estimates for 11 substances in this group exceeded the threshold for human intake for class I. In all cases, the substances could be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways. In the opinion of the Committee, the endogenous levels of metabolites from these substances would not give rise to perturbations outside the physiological range. Therefore, these 11 substances were also considered to be of no safety concern.

Table 1 summarizes the evaluation of the 38 saturated aliphatic acyclic linear primary alcohols, aldehydes and acids using the Procedure.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 38 substances in this group were consumed simultaneously on a daily basis, the estimated daily per capita intake in Europe and the USA (excluding intakes of acetic acid and propionic acid which have ADIs "not limited") would be approximately 40 mg and 30 mg respectively, i.e. above the threshold for human intake for substances in class I.

All of the substances in this group and their metabolites are innocuous and endogenous and their combined intake was judged by the Committee not to give rise to perturbations outside the physiological range.

Conclusion

The Committee concluded that the substances in this group would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that the toxicity data that were available were consistent with the results of using the Procedure. In cases where ADIs had been established previously, they were maintained at the present meeting.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.3 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

The Committee evaluated a group of 25 flavouring agents that included selected saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids (see Table 2) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 2
Summary of the results of safety evaluations of 25 saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids[a]

Substance	No.	*Step A3* Does intake exceed the threshold for human intake?[b]	Conclusion based on current levels of intake
Structural class I: methyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Isobutyl alcohol	0251	No Europe: 530 USA: 290	
Isobutyraldehyde	0252	No Europe: 130 USA:100	
Isobutyric acid	0253	No Europe: 820 USA: 140	
2-Methylbutyraldehyde	0254	No Europe: 4.9 USA: 370	
2-Methylbutyric acid	0255	No Europe: 1200 USA: 480	
3-Methylbutyraldehyde	0258	No Europe: 110 USA: 140	
Isovaleric acid	0259	No Europe: 480 USA: 96	
2-Methylpentanal	0260	No Europe: 12 USA: 8.5	No safety concern
2-Methylvaleric acid	0261	No Europe: 680 USA: 2.3	
3-Methylpentanoic acid	0262	No Europe: 2.9 USA: 8.8	
3-Methyl-1-pentanol	0263	No Europe: 5.9 USA: 4.2	
4-Methylpentanoic acid	0264	No Europe: 1.6 USA: 55	
2-Methylhexanoic acid	0265	No Europe: 15 USA: 2.3	
5-Methylhexanoic acid	0266	No Europe: 0.0 USA: 8.6	
3,5,5-Trimethyl-1-hexanol	0268	No Europe: 13 USA: 0.76	

Table 2 (*continued*)

Substance	No.	*Step A3* Does intake exceed the threshold for human intake?[b]	Conclusion based on current levels of intake
3,5,5-Trimethylhexanal	0269	No Europe: 0.29 USA: 150	
2-Methyloctanal	0270	No Europe: 0.14 USA: 0.95	
4-Methyloctanoic acid	0271	No Europe: 11 USA: 0.10	
3,7-Dimethyl-1-octanol	0272	No Europe: 94 USA: 2.9	No safety concern
2,6-Dimethyloctanal	0273	No Europe: 0.01 USA: 6.7	
4-Methylnonanoic acid	0274	No Europe: 1.00 USA: 1.5	
2-Methylundecanal	0275	No Europe: 0.61 USA: 0.10	
Structural class II: ethyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
2-Ethylbutyraldehyde[c]	0256	No Europe: 0.57 USA: 0.17	
2-Ethylbutyric acid[c]	0257	No Europe: 60 USA: 31	No safety concern
2-Ethyl-1-hexanol[c, d]	0267	No Europe: 86 USA: 40	

[a] *Step 2:* All of the substances in this group are metabolized to innocuous products.
[b] The thresholds for human intake for classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.
[c] The 2-ethyl substituent inhibits the β-oxidation of aliphatic alcohols, aldehydes and carboxylic acids. These compounds undergo ω- and ω-1-oxidation to yield polar metabolites which are primarily excreted in urine.
[d] The ADI for this substance was maintained.

Twenty-two of these substances contain one or more methyl substituents and the remaining three have ethyl substituents in the α-position.

Two of the substances have been evaluated previously by the Committee. Isobutyl alcohol (2-methyl-1-propanol) was considered at the twenty-third meeting, when an ADI was not allocated because of a

lack of information (Annex 1, reference *50*). An ADI of 0–0.5 mg per kg of body weight was allocated to 2-ethyl-1-hexanol at the forty-first meeting (Annex 1, reference *107*).

Intake data

The total annual volume of production of the 22 methyl-substituted saturated aliphatic branched-chain primary alcohols, aldehydes and acids from their use as flavouring substances is approximately 29 tonnes in Europe and 9.8 tonnes in the USA. In Europe, more than 85% of the total annual volume is accounted for by five substances (isobutyl alcohol, isobutyric acid, 2-methylbutyric acid, isovaleric acid and 2-methylvaleric acid (2-methylpentanoic acid)). In the USA more than 80% of the total annual volume is accounted for by seven substances (isobutyl alcohol, isobutyraldehyde (2-methylpropanal), isobutyric acid, 2-methylbutyraldehyde (2-methylbutanal), 2-methylbutyric acid, 3-methylbutyraldehyde (3-methylbutanal) and isovaleric acid).

The total reported annual volume of production of the three 2-ethyl substituted substances for use as flavouring agents is 1000 kg in Europe and 370 kg in the USA.

Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have been detected as natural components of a wide variety of foods such as cheese, fruits, vinegar and alcoholic beverages. Quantitative data on the natural occurrence of these flavouring agents has been reported for 11 of the 25 substances in the group and their total annual consumption in food is estimated at 1.5 million kg per year.

Information on absorption, metabolism and elimination

The metabolism of methyl- and ethyl-substituted saturated aliphatic acyclic branched-chain alcohols, aldehydes and carboxylic acids is described on pages 26–27.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, the Committee assigned all 22 methyl-substituted substances to structural class I. The three ethyl-substituted substances (2-ethylbutyraldehyde (2-ethylbutanal), 2-ethylbutyric acid (2-ethylbutanoic

acid) and 2-ethyl-1-hexanol) contain sterically hindered functional groups and were therefore assigned to structural class II.

Step 2. At their current levels of intake from use as flavouring agents (see Table 2), the 22 methyl-substituted alcohols, aldehydes and carboxylic acids and the three ethyl-substituted alcohols, aldehydes and carboxylic acids would not be expected to saturate the metabolic pathways and all the compounds were predicted to be metabolized to innocuous products.

Step A3. The intake estimates for all the 22 methyl-substituted substances in this group in both Europe and the USA are below the threshold for human intake for class I (1800 μg per day). Therefore, these substances were considered to be of no safety concern when used as flavouring agents at current estimated levels of intake.

The intake estimates for the three ethyl-substituted substances (2-ethylbutyraldehyde, 2-ethylbutyric acid and 2-ethyl-1-hexanol) in this group in both Europe and the USA are below the threshold for human intake for class II (540 μg per day). Therefore, these substances were also considered to be of no safety concern when used as flavouring agents at current estimated levels of intake.

Table 2 summarizes the evaluation of the 25 saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and carboxylic acids using the Procedure.

Consideration of combined intake

In the unlikely event that all foods containing all 22 methyl-substituted alcohols, aldehydes and acids as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these substances would be 410 μg in Europe and 1900 μg in the USA.

In the unlikely event that all foods containing all three ethyl-substituted alcohols, aldehydes and acids were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these three substances would be less than 145 μg in Europe and less than 71 μg in the USA.

The Committee judged that the combined intake of substances in this group is of no safety concern, since all the substances are expected to be efficiently metabolized and the combined level of intake is not expected to saturate metabolic pathways.

Conclusion

The Committee concluded that the use of the above-mentioned substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that where toxicity data were available they were consistent with the results of the Procedure. The ADI established previously for 2-ethyl-1-hexanol was maintained.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.4 Aliphatic lactones

The Committee evaluated a group of 35 aliphatic lactones used as flavouring agents in food (Table 3) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Two substances in the group, γ-nonalactone and γ-undecalactone, were previously evaluated by the Committee at its eleventh meeting, when ADIs of 0–1.25 mg/kg of body weight were established for each substance (Annex 1, reference *14*).

Intake data

The total annual volume of production of the 35 substances from their use as flavouring agents is approximately 160 tonnes in Europe and 27 tonnes in the USA. The estimated total daily per capita intakes of all aliphatic lactones resulting from their use as flavouring agents are 30.3 mg in Europe and less than 5.3 mg in the USA. In Europe, γ-decalactone and δ-dodecalactone account for two-thirds of the daily per capita intake of lactones used as flavouring agents. In the USA, four substances (γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone) account for most of the daily per capita intake of aliphatic lactones used as flavouring agents.

The four lactones that are α,β-unsaturated (5-hydroxy-2,4-decadienoic acid δ-lactone, 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone, and a mixture of 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone and 5-hydroxy-2-tetradecenoic acid δ-lactone) and the two hydroxyfuranones (5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone and 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one) are estimated to have very low total daily per capita intakes. The combined estimated per capita intakes of these six substances from their use in food is 27 μg in Europe and less than 9 μg in the USA.

Table 3
Summary of the results of safety evaluations of 35 aliphatic lactones

Substance	No.	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
Structural class I						
4-Hydroxybutyric acid lactone (γ-butyrolactone)	0219	Yes	No Europe: 130 USA: 100	NA	NA	
γ-Valerolactone	0220	Yes	No Europe: 140 USA: 57	NA	NA	
γ-Hexalactone	0223	Yes	No Europe: 190 USA: 19	NA	NA	No safety concern
δ-Hexalactone	0224	Yes	No Europe: 380 USA: 2.5	NA	NA	
γ-Heptalactone	0225	Yes	No Europe: 190 USA: 41	NA	NA	

Table 3 (*continued*)

Substance	No.	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
γ-Octalactone	0226	Yes	No Europe: 490 USA: 90	NA	NA	No safety concern
δ-Octalactone	0228	Yes	No Europe: 270 USA: 17	NA	NA	
γ-Nonalactone[b]	0229	Yes	No Europe: 1200 USA: 470	NA	NA	
Hydroxynonanoic acid δ-lactone	0230	Yes	No Europe: 150 USA: 11	NA	NA	
γ-Decalactone	0231	Yes	Yes Europe: 1800 USA: 370	No	Yes	
δ-Decalactone	0232	Yes	Yes Europe: 8400 USA: 1900	No	Yes	
ε-Decalactone	0241	Yes	No Europe: 0.01 USA: 0	NA	NA	
γ-Undecalactone[b]	0233	Yes	No Europe: 1400 USA: 550	NA	NA	

5-Hydroxyundecanoic acid δ-lactone	0234	Yes	No Europe: 350 USA: 180	NA	NA	No safety concern
γ-Dodecalactone	0235	Yes	No Europe: 220 USA: 110	NA	NA	
δ-Dodecalactone	0236	Yes	Yes Europe: 6800 USA: 1140	No	Yes	
ε-Dodecalactone	0242	Yes	No Europe: 0.01 USA: 0.17	NA	NA	
δ-Tetradecalactone	0238	Yes	No Europe: 120 USA: 2.5	NA	NA	
ω-Pentadecalactone	0239	Yes	No Europe: 84 USA: 51	NA	NA	
4-Hydroxy-3-pentenoic acid lactone	0221	Yes	No Europe: NR USA: 4.8	NA	NA	
5-Hydroxy-7-decenoic acid δ-lactone	0247	Yes	No Europe: 0.26 USA: 0.10	NA	NA	

Table 3 (*continued*)

Substance	No.	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
5-Hydroxy-8-undecenoic acid δ-lactone	0248	Yes	No Europe: 0.01 USA: 8.6	NA	NA	No safety concern
1,4-Dodec-6-enolactone	0249	Yes	No Europe: 0.01 USA: 8.6	NA	NA	
ω-6-Hexadecenlactone	0240	Yes	No Europe: 6 USA: 0.10	NA	NA	
4,4-Dibutyl-γ-butyrolactone	0227	Yes	No Europe: 0.14 USA: 0.10	NA	NA	
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	0244	Yes	No Europe: 0.04 USA: 0.1	NA	NA	
4-Hydroxy-3-methyloctanoic acid γ-lactone	0437	Yes	No Europe: 0 USA: 8.6	NA	NA	
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	Yes	No Europe: 0.1 USA: 0	NA	NA	
γ-Methyldecalactone	0250	Yes	No Europe: 0 USA: 43	NA	NA	

Structural class III						
5-Hydroxy-2-decenoic acid δ-lactone	0246	No	No Europe: 12 USA: 0.10	NA	Insufficient information	Not evaluated[c]
5-Hydroxy-2,4-decadienoic acid δ-lactone	0245	No	No Europe: 0.33 USA: 0.10	NA	Insufficient information	
Mixture of 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone and 5-hydroxy-2-tetradecenoic acid δ-lactone	0276	No	— Europe: 0 USA: 2	NA	Insufficient information	
5-Hydroxy-2-dodecenoic acid δ-lactone	0438	No	— Europe: 0 USA: 8.6	NA	Insufficient information	
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	0222	No	No Europe: 13 USA: 6.1	NA	Yes	No safety concern
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	No	No Europe: 2.1 USA: 0.1	NA	Yes	

NA: not applicable.
[a] The thresholds for human intake for classes I and III are 1800 μg per day and 90 μg per day, respectively. All intake values are expressed in μg per day.
[b] The ADI for this substance was maintained.
[c] Evaluation deferred, pending consideration of other α,β-unsaturated compounds.

Most of the aliphatic lactones have been reported to occur naturally in traditional foods. The four aliphatic lactones that are used most as flavouring agents (γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone) are ubiquitous in food, occurring mainly in fruits, alcoholic beverages, meats and dairy products.

Information on absorption, metabolism and elimination

Lactones are generally formed by acid-catalysed intramolecular cyclization of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as urine, the lactone ring is favoured. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract.

The aliphatic lactones in this group can be divided into three subgroups on the basis of their predicted metabolism, namely, lactones derived from linear and branched-chain hydroxycarboxylic acids, lactones which are α,β-unsaturated, and the two hydroxyfuranones. The metabolism of the members of each of these subgroups is discussed below.

Lactones derived from linear saturated 5-hydroxycarboxylic acids. Linear saturated 5-hydroxycarboxylic acids (formed from δ-lactones) are converted, via acetyl CoA, to hydroxythioesters which then undergo β-oxidation and cleavage to yield an acetyl CoA fragment and a new β-hydroxythioester reduced by two carbons. Acids containing even numbers of carbon atoms continue to be oxidized and cleaved to yield acetyl CoA while those containing odd numbers of carbon atoms yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly while propionyl CoA is transformed into succinyl CoA which then enters the citric acid cycle.

Lactones derived from linear saturated 4- or 6-hydroxycarboxylic acids. Linear saturated 4- or 6-hydroxycarboxylic acids (formed from γ- or ε-lactones) participate in the same pathway as described above for linear saturated 5-hydroxycarboxylic acids; however, loss of an acetyl CoA fragment produces an α-hydroxythioester which undergoes α-oxidation and α-decarboxylation to yield a linear carboxylic acid and eventually carbon dioxide. γ-Butyrolactone, the only lactone in this group formed from a primary alcohol, may participate in an alternative oxidation pathway, namely, oxidation by alcohol dehydrogenase and succinate-semialdehyde dehydrogenase to succinate which then enters the citric acid cycle.

Lactones derived from linear unsaturated hydroxycarboxylic acids. If the lactone is formed from a linear hydroxycarboxylic acid which is unsaturated, cleavage of acetyl CoA units continues along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl CoA fragmentation will eventually yield a 3-enoyl CoA which is converted to the *trans*-Δ^2-enoyl CoA before entering the fatty acid pathway. If unsaturation begins at an even-numbered carbon, acetyl CoA fragmentation yields a Δ^2-enoyl CoA product which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, hydration yields (*R*)-3-hydroxyacyl CoA which is isomerized to (*S*)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering the fatty acid pathway.

Lactones derived from branched-chain hydroxycarboxylic acids. For branched-chain hydroxycarboxylic acids, the principal metabolic pathways utilized for detoxication are influenced by the chain length and the position and size of the alkyl substituents. Short-chain (< six carbon atoms) branched aliphatic hydroxycarboxylic acids may be excreted unchanged as the glucuronic acid conjugate, or undergo α- or β-oxidation followed by cleavage and complete metabolism to carbon dioxide via the fatty acid pathway and the tricarboxylic acid cycle. Alternatively, as chain length, substitution and lipophilicity increase, the hydroxycarboxylic acid may undergo a combination of ω-, ω-1 and β-oxidation to yield polar hydroxyacid, ketoacid and hydroxydiacid metabolites which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces. These metabolites are considered to be innocuous.

α,β-Unsaturated lactones. For the four substances which are α,β-unsaturated there was no direct evidence of hydrolysis. While hydrolysis to the corresponding ring-opened α,β-unsaturated hydroxycarboxylic acids may occur, no information was available on the four substances considered to enable the Committee to predict that this is the major route of metabolism. If hydrolysis to the corresponding ring-opened form occurs, condensation of the α,β-unsaturated hydroxycarboxylic acid with acetyl CoA would yield a Δ^2-enoyl CoA product, which is a substrate in the fatty acid pathway. Since the stereochemistry of the double bond in a lactone is *cis*, hydration would yield (*R*)-3-hydroxyacyl CoA, which is then isomerized to (*S*)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering the fatty acid pathway.

Alternatively, the lactones which are α,β-unsaturated may conjugate with glutathione and be excreted as cysteine or mercapturic acid

derivatives. Evidence for this alternative pathway comes from two structurally related lactones which are α,β-unsaturated. The Committee considered that further information was required in order to clarify the metabolic route(s) of these substances.

Hydroxyfuranones. There was no direct evidence available of hydrolysis of the two hydroxyfuranones (nos 0222 and 0243, see Table 3) to the corresponding ring-opened compound. The Committee considered that alternative metabolic pathways are likely and that no prediction of a metabolic route is possible for these substances.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the aliphatic lactones, the Committee assigned 29 substances to structural class I; the four α,β-unsaturated substances and the two hydroxyfuranones were assigned to structural class III.

Step 2. The available data indicate that for the 29 lactones in class I derived from saturated linear and branched-chain hydroxycarboxylic acids, the corresponding aliphatic hydroxycarboxylic acids are metabolized via the fatty acid pathway. For these substances, the evaluation should proceed via the left-hand side of the decision-tree. For the four lactones in class III which are α,β-unsaturated, metabolism may occur either via hydrolysis followed by β-oxidation or via conjugation with glutathione. There was insufficient information available to predict the route of metabolism of these four substances with confidence. The Committee considered that further information on their metabolism was required and that they should be evaluated together with other α,β-unsaturated substances and that their evaluation should therefore be deferred. No information was available to indicate the route of metabolism for the two lactones in class III which are hydroxyfuranones and therefore the evaluation of these two substances should proceed via the right-hand side of the decision-tree.

Step A3/B3. For the 29 lactones derived from saturated linear and branched-chain hydroxycarboxylic acids in class I, three lactones (γ-decalactone, δ-decalactone and δ-dodecalactone) had intake estimates equal to or greater than the threshold for class I (1800 μg per day). The evaluation of these three substances therefore proceeded to step A4. For the other 26 lactones of similar structure the intake estimates are below the threshold for class I and they would therefore not be expected to be of safety concern. For the two hydroxyfuranones in class III, the intake estimates are well below the

threshold for class III (90 μg per day). The evaluation of these substances therefore proceeded to step B4.

Step A4. None of the three lactones derived from saturated linear hydroxycarboxylic acids (γ-decalactone, δ-decalactone and δ-dodecalactone) are known to be endogenous or to be metabolized to endogenous substances. The safety evaluation of these substances therefore proceeded to step A5.

Step A5. Although adequate studies on which to base a NOEL for the three lactones derived from saturated linear hydroxycarboxylic acids were not available, the following NOELs have been reported for structurally related lactones in 2-year studies in rats: 250 mg/kg of body weight per day for γ-nonalactone and γ-undecalactone and 110 mg/kg of body weight per day for γ-butyrolactone. In a 2-year study in mice with γ-butyrolactone a NOEL of 260 mg/kg of body weight per day was found. The studies on γ-nonalactone and γ-undecalactone were considered previously by the Committee and ADIs were established at the eleventh meeting (Annex 1, reference *14*). Although these studies were not conducted according to modern standards, the results are considered to be valid. These NOELs provide an adequate margin of safety (>1000) for γ-decalactone, δ-decalactone and δ-dodecalactone and therefore these substances would not be expected to be of safety concern.

Step B4. The Committee considered the results of studies on the two hydroxyfuranones in class III. In a 90-day study in rats administered 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone in the diet, the NOEL was 1.3 mg/kg of body weight per day, and in a 1-year study in rats administered 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one in the diet, the NOEL was 46 mg/kg of body weight per day. These NOELs provide an adequate margin of safety (>1000) for these substances and therefore they would not be expected to be of safety concern.

Table 3 summarizes the evaluation of the 35 aliphatic lactones using the Procedure.

Consideration of combined intakes

From the available data, the 29 lactones derived from linear and branched-chain hydroxycarboxylic acids would be expected to be efficiently metabolized via commonly known biochemical pathways to innocuous substances. In the unlikely event that all foods containing all 29 substances as flavouring agents were consumed simultaneously on a daily basis, the estimated daily per capita consumption in Europe and the USA would exceed the threshold for human intake for substances in class I but, in the opinion of the Committee, this

would not give rise to perturbations outside the physiological range.

For the two hydroxyfuranones whose route of metabolism is unknown, their combined estimated intake was very low (15 μg per day) compared to the known NOELs for each of these substances and was not considered to present a safety concern.

Conclusions

The Committee concluded that the evaluation of the four substances which are α,β-unsaturated should be deferred, pending consideration of other α,β-unsaturated substances. The safety evaluation of the two hydroxyfuranones proceeded because of the existence of supporting data from toxicity studies.

On the basis of the results of the evaluation of the 29 lactones derived from linear and branched-chain hydroxycarboxylic acids and the substances 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone and 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one, the Committee concluded that the use of these substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

In using the Procedure, the Committee noted that where toxicity data were available, they were consistent with the results of the safety evaluation.

The ADIs for γ-nonalactone and γ-undecalactone were maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.5 Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids

The Committee evaluated a group of 32 flavouring agents that included selected esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids (Table 4) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

The Committee had previously evaluated one member of the group, ethyl isovalerate, at its eleventh meeting (Annex 1, reference *14*), but because of a lack of data, was unable to allocate an ADI.

Intake data

The total annual volume of production of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids from their use as flavouring substances is approximately 32 tonnes in Europe and 16 tonnes in the USA. In Europe, more than 90% of the

Table 4

Summary of the results of safety evaluations of 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Methyl isobutyrate	0185	No Europe: 23 USA: 270	NR	No safety concern
Ethyl isobutyrate	0186	No Europe: 750 USA: 470	NR	
Propyl isobutyrate	0187	No Europe: 15 USA: 0.08	NR	
Butyl isobutyrate	0188	No Europe: 2.7 USA: 1.9	NR	
Hexyl isobutyrate	0189	No Europe: 3.00 USA: 0.57	NR	
Heptyl isobutyrate	0190	No Europe: 0.00 USA: 3.0	NR	
trans-3-Heptenyl 2-methylpropanoate	0191	No Europe: 0.01 USA: 2.3	NR	
Octyl isobutyrate	0192	No Europe: 11 USA: 5.0	NR	
Dodecyl isobutyrate	0193	No Europe: 50 USA: 0.76	NR	
Isobutyl isobutyrate	0194	No Europe: 65 USA: 2.3	NR	
Methyl isovalerate	0195	No Europe: 7.8 USA: 110	NR	
Ethyl isovalerate	0196	No Europe: 760 USA: 540	NR	
Propyl isovalerate	0197	No Europe: 2.00 USA: 0.10	NR	
Butyl isovalerate	0198	No Europe: 94 USA: 500	NR	
Hexyl 3-methyl-butanoate	0199	No Europe: 2.3 USA: 3.1	NR	
Octyl isovalerate	0200	No Europe: 7.3 USA: 0.57	NR	
Nonyl isovalerate	0201	No Europe: 0.01 USA: 0.08	NR	

Table 4 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
3-Hexenyl 3-methylbutanoate	0202	No Europe: 9.4 USA: 30	NR	No safety concern
2-Methylpropyl 3-methylbutyrate	0203	No Europe: 78 USA: 130	NR	
2-Methylbutyl 3-methylbutanoate	0204	No Europe: 0.86 USA: 0.95	NR	
Methyl 2-methylbutyrate	0205	No Europe: 390 USA: 69	NR	
Ethyl 2-methylbutyrate[c]	0206	Yes Europe: 2200 USA: 560	Yes	
n-Butyl 2-methylbutyrate	0207	No Europe: 26 USA: 0.02	NR	
Hexyl 2-methylbutanoate	0208	No Europe: 4.9 USA: 8.6	NR	
Octyl 2-methylbutyrate	0209	No Europe: 0.01 USA: 0.10	NR	
Isopropyl 2-methylbutyrate	0210	No Europe: 4.9 USA: 0.10	NR	
3-Hexenyl 2-methylbutanoate	0211	No Europe: 5 USA: 8.8	NR	
2-Methylbutyl 2-methylbutyrate	0212	No Europe: 3.6 USA: 0.04	NR	
Methyl 2-methylpentanoate	0213	No Europe: 0.17 USA: 0.02	NR	
Ethyl 2-methylpentanoate	0214	No Europe: 7.6 USA: 320	NR	
Ethyl 3-methylpentanoate	0215	No Europe: 0.31 USA: 5.90	NR	
Methyl 4-methylvalerate	0216	No Europe: 0.03 USA: 0.10	NR	

NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the procedure.

[a] *Step 1*: All of the esters in this group are in structural class I.
Step 2: All of the esters in this group are metabolized to innocuous products.

[b] The threshold for human intake for class I is 1800 µg per day. All intake values are expressed in µg per day.

[c] The components ethanol and 2-methylbutyric acid are endogenous. The acid is an intermediate in the metabolism of the amino acid.

total annual volume is accounted for by ethyl isobutyrate (ethyl 2-methylpropanoate), ethyl isovalerate (ethyl 3-methylbutanoate), ethyl 2-methylbutyrate (ethyl 2-methylbutanoate) and methyl 2-methylbutyrate (methyl 2-methylbutanoate). In the USA, approximately 67% of the total annual volume is accounted for by ethyl isobutyrate, ethyl isovalerate, butyl isovalerate (butyl 3-methylbutanoate) and ethyl 2-methylbutyrate.

Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids have been detected as natural components of a wide variety of foods. The available quantitative data on the natural occurrence of these esters indicate that the total intake from natural food sources is approximately 14 tonnes per year. This estimated intake is approximately equal to the estimated intake from their use as flavouring substances. In the USA, the consumption of isobutyrate esters from natural food sources is equivalent to their consumption from use as flavouring substances. The consumption of isovalerate esters and 2-methylbutyrate esters from natural food sources is several orders of magnitude higher than that from their use as flavouring agents.

Information on absorption, metabolism and elimination

It is expected that the esters in this group will be readily hydrolysed to their component alcohols and carboxylic acids in the intestinal tract, blood and liver. The metabolism of the hydrolysis products is discussed on pages 26–27.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids, the Committee assigned all 32 substances to structural class I.

Step 2. At the estimated current levels of intake (see Table 4), these esters would not be expected to saturate the metabolic pathways, and they were all predicted to be metabolized to innocuous products. The left-hand side of the decision-tree was therefore considered.

Step A3. The estimated daily per capita intakes of all but one of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids in Europe and the USA were below the threshold for class I (1800 μg per day), so that they were considered to be of no safety concern when used at estimated current levels of

intake as flavouring agents. Only ethyl 2-methylbutyrate has an estimated intake greater than the threshold for class I.

Step A4. Ethyl 2-methylbutyrate is expected to be hydrolysed to ethyl alcohol and 2-methylbutyric acid, which are endogenous. Therefore, this substance was determined to be of no safety concern on the basis of its structural class and known metabolism.

Table 4 summarizes the evaluation of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids used as flavouring substances.

Consideration of combined intakes

In the unlikely event that all foods containing all of the substances in this group as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake would be 4.6 mg in Europe and 3 mg in the USA. The estimated daily per capita intake of the branched-chain acids (i.e. isobutyric acid, isovaleric acid and 2-methylbutyric acid) formed via hydrolysis of these esters is 3.1 mg in Europe and 2 mg in the USA.

These estimated combined intakes would exceed the threshold for class I. Since all the 32 substances in this group are expected to be efficiently metabolized, they would not be expected to saturate the metabolic pathways. On the basis of the evaluation of the collective data, the Committee concluded that combined intake of these substances would not be expected to be of safety concern.

Conclusion

The Committee concluded that the substances in this group would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for application of the Procedure. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.6 Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids

The Committee evaluated a group of 67 esters of aliphatic linear and branched-chain saturated and monounsaturated primary alcohols

with aliphatic linear saturated carboxylic acids (see Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

One member of the group, butyl acetate, had been previously evaluated at the eleventh meeting of the Committee, but no ADI was established due to a lack of data (Annex 1, reference *14*).

Intake data

The total annual volume of production of the 67 esters in this group for use as flavouring agents is approximately 65 tonnes in Europe and 19 tonnes in the USA. In Europe, more than 75% of the total annual volume of production is accounted for by butyl butyrate (*n*-butyl *n*-butanoate), *n*-amyl butyrate (pentyl butanoate) and the acetate esters of methyl alcohol (methanol), butyl alcohol, hexyl alcohol, *cis*-3-hexenol and isobutyl alcohol. In the USA, more than 70% of the total annual volume of production is accounted for by *n*-amyl butyrate, *cis*-3- and *trans*-2-hexenyl propionate (*cis*-3- and *trans*-2-hexenyl propanoate) and the acetate esters of propyl alcohol, isobutyl alcohol and 2-methylbutyl alcohol (2-methylbutanol). On the basis of the reported annual volume of production in Europe and the USA, the total estimated daily per capita intake of the 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids from their use as flavouring agents is 9.2 mg in Europe and 3.8 mg in the USA. The use of seven of the esters (heptyl formate, octyl propionate (octyl propanoate), decyl propionate (decyl propanoate), decyl butyrate (decyl butanoate), butyl heptanoate, butyl laurate (butyl dodecanoate), and *cis*-3- and *trans*-2-hexenyl propionate) has been reported in the USA but not in Europe.

Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids are principal components of alcoholic beverages and of a wide variety of fruits. Quantitative data on the natural occurrence in food for 37 substances in the group have been reported from the USA, which indicate that the intake of these substances from natural sources exceeds the intake from their use as flavouring agents.

Information on absorption, metabolism and elimination

In general, it is expected that esters of aliphatic linear and branched-chain primary alcohols with aliphatic linear saturated carboxylic acids would be hydrolysed to their component alcohols and carboxylic acids. The metabolism of the saturated acids and alcohols is described on pages 26–27.

Esters of the three monounsaturated alcohols in this group are expected to be oxidized via their corresponding aldehydes to carboxylic

Table 5
Summary of the results of safety evaluations of 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Propyl formate	0117	No Europe: 5.0 USA: 0.38	NA	
Butyl formate	0118	No Europe: 21 USA: 0.17	NA	
n-Amyl formate	0119	No Europe: 29 USA: 110	NA	
Hexyl formate	0120	No Europe: 8.7 USA: 8.0	NA	
Heptyl formate	0121	No Europe: 0.00 USA: 0.10	NA	
Octyl formate	0122	No Europe: 0.14 USA: 0.95	NA	
cis-3-Hexenyl formate	0123	No Europe: 43 USA: 1.7	NA	
Isobutyl formate	0124	No Europe: 4.7 USA: 1.5	NA	
Methyl acetate	0125	No Europe: 460 USA: 110	NA	No safety concern
Propyl acetate	0126	No Europe: 180 USA: 440	NA	
Butyl acetate	0127	No Europe: 1200 USA: 170	NA	
Hexyl acetate	0128	Yes Europe: 3200 USA: 160	Yes[c]	
Heptyl acetate	0129	No Europe: 56 USA: 2.3	NA	
Octyl acetate	0130	No Europe: 83 USA: 9.5	NA	
Nonyl acetate	0131	No Europe: 6.6 USA: 2.5	NA	
Decyl acetate	0132	No Europe: 7.3 USA: 21	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[a] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Lauryl acetate	0133	No Europe: 9.3 USA: 0.57	NA	No safety concern
cis-3-Hexenyl acetate	0134	No Europe: 640 USA: 57	NA	
trans-3-Heptenyl acetate	0135	No Europe: 0.24 USA: 0.76	NA	
10-Undecen-1-yl acetate	0136	No Europe: 0.83 USA: 0.10	NA	
Isobutyl acetate	0137	No Europe: 1200 USA: 1300	NA	
2-Methylbutyl acetate	0138	No Europe: 130 USA: 360	NA	
2-Ethylbutyl acetate	0140	No Europe: 4.0 USA: 0.17	NA	
Methyl propionate	0141	No Europe: 9.3 USA: 30	NA	
Propyl propionate	0142	No Europe: 9.6 USA: 44	NA	
Butyl propionate	0143	No Europe: 10 USA: 1.1	NA	
Hexyl propionate	0144	No Europe: 5.7 USA: 3.0	NA	
Octyl propionate	0145	No Europe: 0.00 USA: 0.02	NA	
Decyl propionate	0146	No Europe: 0.00 USA: 0.95	NA	
cis-3-Hexenyl propionate and *trans*-2-hexenyl propionate	0147	No Europe: 0.00 USA: 430	NA	Not evaluated[c]
Isobutyl propionate	0148	No Europe: 12 USA: 6.5	NA	No safety concern
Methyl butyrate	0149	No Europe: 220 USA: 44	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Propyl butyrate	0150	No Europe: 75 USA: 38	NA	
Butyl butyrate	0151	No Europe: 390 USA: 63	NA	
n-Amyl butyrate	0152	No Europe: 450 USA: 200	NA	
Hexyl butyrate	0153	No Europe: 110 USA: 27	NA	
Heptyl butyrate	0154	No Europe: 6.0 USA: 3.8	NA	
Octyl butyrate	0155	No Europe: 16 USA: 0.38	NA	
Decyl butyrate	0156	No Europe: 0.00 USA: 0.08	NA	
cis-3-Hexenyl butyrate	0157	No Europe: 160 USA: 4.8	NA	
Isobutyl butyrate	0158	No Europe: 47 USA: 7.4	NA	No safety concern
Methyl valerate	0159	No Europe: 30 USA: 11	NA	
Butyl valerate	0160	No Europe: 3.7 USA: 0.10	NA	
Propyl hexanoate	0161	No Europe: 14 USA: 0.17	NA	
Butyl hexanoate	0162	No Europe: 15 USA: 1.9	NA	
n-Amyl hexanoate	0163	No Europe: 8.7 USA: 8.8	NA	
Hexyl hexanoate	0164	No Europe: 150 USA: 13	NA	
cis-3-Hexenyl hexanoate	0165	No Europe: 42 USA: 1.3	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Isobutyl hexanoate	0166	No Europe: 6.1 USA: 1.7	NA	
Methyl heptanoate	0167	No Europe: 5.7 USA: 0.10	NA	
Propyl heptanoate	0168	No Europe: 0.14 USA: 0.38	NA	
Butyl heptanoate	0169	No Europe: 0.00 USA: 4.4	NA	
n-Amyl heptanoate	0170	No Europe: 0.61 USA: 0.02	NA	
Octyl heptanoate	0171	No Europe: 0.21 USA: 0.38	NA	
Isobutyl heptanoate	0172	No Europe: 0.01 USA: 1.9	NA	
Methyl octanoate	0173	No Europe: 9.7 USA: 0.17	NA	
n-Amyl octanoate	0174	No Europe: 3.4 USA: 1.9	NA	No safety concern
Hexyl octanoate	0175	No Europe: 1.3 USA: 0.95	NA	
Heptyl octanoate	0176	No Europe: 0.71 USA: 0.95	NA	
Octyl octanoate	0177	No Europe: 0.03 USA: 2.3	NA	
Nonyl octanoate	0178	No Europe: 0.14 USA: 0.95	NA	
Methyl nonanoate	0179	No Europe: 0.86 USA: 2.3	NA	
Methyl laurate	0180	No Europe: 5.1 USA: 0.76	NA	
Butyl laurate	0181	No Europe: 0.00 USA: 0.10	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Isoamyl laurate	0182	No Europe: 0.14 USA: 0.57	NA	No safety concern
Methyl myristate	0183	No Europe: 62 USA: 46	NA	
Butyl stearate	0184	No Europe: 5.1 USA: 5.5	NA	

[a] *Step 1*: All of the esters in this group are in structural class I except 2-ethylbutyl acetate, which is in structural class II.
Step 2: Evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed. All of the other substances in this group are metabolized to innocuous products.
[b] The thresholds for human intake for classes I and II are 1800 μg per day and 540 μg per day, respectively. All intake values are expressed in μg per day.
[c] Hexanoic acid, the metabolite of the component hexyl alcohol, and acetic acid are endogenous in humans.
[d] Evaluation postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

acids, which then undergo β-oxidation in the fatty acid and other well-known metabolic pathways.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids, the Committee assigned all but one of the 67 esters to structural class I. 2-Ethylbutyl acetate contains a sterically hindered functional group and was therefore assigned to structural class II.

Step 2. At this step the evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

The available data indicate that the remaining esters in this group would be hydrolysed in humans to their component alcohols and carboxylic acids. The aliphatic acyclic primary alcohols are oxidized to their corresponding carboxylic acids, which are either conjugated and excreted in the urine, or undergo β-oxidation and cleavage. The aliphatic linear saturated carboxylic acids are endogenous in humans. At the current levels of per capita intake these esters would not be expected to saturate the metabolic pathways. Therefore, the remaining 66 esters of aliphatic acyclic primary alcohols with aliphatic linear

saturated carboxylic acids were predicted to be metabolized to innocuous products. Accordingly, the Committee considered the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of all but one of the remaining 65 class I esters in this group in Europe and the USA were below the threshold for human intake (1800 μg). Only hexyl acetate had an estimated daily per capita intake greater than the threshold for class I. The estimated daily per capita intake of 2-ethylbutyl acetate in Europe and the USA was below the threshold for human intake for class II (540 μg).

Step A4. This step was considered only for hexyl acetate which was the only substance in this group for which the estimated level of intake exceeded the threshold for class I. The component hexyl alcohol is oxidized to hexanoic acid which is endogenous as it is an intermediary metabolite in the fatty acid pathway, and acetate is a component of the tricarboxylic acid cycle. In the opinion of the Committee the endogenous levels of these two metabolites would not give rise to perturbations outside the physiological range. Therefore, hexyl acetate was also determined to be of no safety concern on the basis of its structural class and known metabolism.

Table 5 summarizes the evaluation of the 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids used as flavouring substances.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 66 esters evaluated as flavouring substances were consumed simultaneously on a daily basis, the estimated daily per capita intake in Europe and the USA would exceed the threshold for human intake for substances in class I. All the flavouring agents in this group of esters are expected to be metabolized via well-known biochemical pathways to innocuous metabolites and/or endogenous substances and in the opinion of the Committee the endogenous levels of these metabolites would not give rise to perturbations outside the physiological range. Accordingly, the combined intake of these substances was considered to be of no safety concern.

Conclusions

On the basis of the results of the Procedure, the Committee concluded that the 66 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids evaluated pose no safety concern when used at the estimated levels of current intake as

flavouring agents. The evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

No toxicity data were required for the application of the Procedure for this group of esters. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.7 Esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids

A safety evaluation of a group of 26 terpenoid esters used as flavouring agents (see Table 6) was conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

One member of the group, geranyl acetate (*trans*-3,7-dimethyl-2,6-octadien-1-yl acetate), was previously evaluated at the twenty-third meeting of the Committee (Annex 1, reference *50*). It was evaluated as part of a group of other terpenoid flavouring substances, citral, citronellol and linalool, which have close chemical, biochemical and toxicological relationships. The Committee allocated a group ADI of 0–0.5 mg/kg of body weight, expressed as citral, to these substances on the basis of their clearly defined metabolism and their low toxicity in short-term toxicity studies.

Intake data

The total annual volume of production of the 26 terpenoid esters used as flavouring agents is approximately 13 tonnes in Europe and 2 tonnes in the USA. In both Europe and the USA approximately 60% of the total annual volume is accounted for by the acetate and butyrate esters of citronellol, geraniol and nerol. On the basis of these annual volumes of production, the total estimated daily per capita intake of the 26 terpenoid esters used as flavouring substances is 1800 μg in Europe and 410 μg in the USA. The total estimated daily per capita intake of the terpenoid alcohols (i.e. citronellol, geraniol, nerol and rhodinol) formed via hydrolysis of these esters is 1400 μg in Europe and 320 μg in the USA.

Terpenoid esters are principal flavour components of citrus fruit and citrus peel oils, and have also been detected in a wide variety of other fruits, spices and vegetables. The terpenoid esters are usually found at concentrations of up to 1 mg/kg in citrus fruit juices, 20 g/kg in citrus

Table 6
Summary of the results of safety evaluations of 26 esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	Conclusion based on current levels of intake
Citronellyl formate	0053	No Europe: 103 USA: 2.5	
Geranyl formate	0054	No Europe: 330 USA: 48	
Neryl formate	0055	No Europe: 0.01 USA: 0.04	
Rhodinyl formate	0056	No Europe: ND USA: 0.10	
Citronellyl acetate	0057	No Europe: 217 USA: 36	
Geranyl acetate[c]	0058	No Europe: 580 USA: 205	
Neryl acetate	0059	No Europe: 180 USA: 63	
Rhodinyl acetate	0060	No Europe: 1.1 USA: 0.8	
Citronellyl propionate	0061	No Europe: 41 USA: 1.5	No safety concern
Geranyl propionate	0062	No Europe: 81 USA: 11	
Neryl propionate	0063	No Europe: 4.3 USA: 1.1	
Rhodinyl propionate	0064	No Europe: ND USA: 0.02	
Citronellyl butyrate	0065	No Europe: 32 USA: 5	
Geranyl butyrate	0066	No Europe: 60 USA: 25	
Neryl butyrate	0067	No Europe: 0.4 USA: 0.02	
Rhodinyl butyrate	0068	No Europe: ND USA: 1.0	

Table 6 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	Conclusion based on current levels of intake
Citronellyl valerate	0069	No Europe: 0.7 USA: 4.0	
Geranyl hexanoate	0070	No Europe: 0.07 USA: 0.5	
Citronellyl isobutyrate	0071	No Europe: 13 USA: 1.3	
Geranyl isobutyrate	0072	No Europe: 124 USA: 3.0	
Neryl isobutyrate	0073	No Europe: 2.0 USA: 0.4	
Rhodinyl isobutyrate	0074	No Europe: 0.03 USA: 0.04	No safety concern
Geranyl isovalerate	0075	No Europe: 43 USA: 1.7	
Neryl isovalerate	0076	No Europe: 0.03 USA: 0.04	
Rhodinyl isovalerate	0077	No Europe: 0.01 USA: 0.02	
Geranyl 2-ethylbutanoate	0078	No Europe: 0.6 USA: 0	

ND, no data reported.
[a] *Step 1*: All of the esters in this group are in structural class I.
Step 2: All of the esters in this group are metabolized to innocuous products.
[b] The threshold for human intake for class I is 1800 µg per day. All intake values are expressed in µg per day.
[c] The ADI for this substance was maintained.

peel oils and 50 g/kg in spices. In the USA terpenoid esters are consumed predominantly as components of traditional foods and the total annual volume of consumption of the most common terpenoid esters as natural components of food is estimated to be approximately 300 tonnes (Stofberg & Grundschober, personal communication, 1987).

Information on absorption, metabolism and elimination

The terpenoid esters are hydrolysed to their corresponding terpenoid alcohols (geraniol, citronellol, nerol and rhodinol) and aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, hexanoic,

isobutyric and isovaleric acids). Both the hydrolysis and the metabolism of aliphatic carboxylic acids are discussed on pages 26–27.

Following hydrolysis, the terpenoid alcohols undergo a complex pattern of alcohol oxidation, ω-oxidation, hydration, selective hydrogenation and subsequent conjugation to form oxygenated polar metabolites which are excreted primarily in the urine. Geraniol, related terpenoid alcohols (citronellol and nerol) and the aldehydes (geranial and neral) follow similar metabolic pathways in animals (Fig. 2).

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned terpenoid esters, the Committee assigned all 26 esters to structural class I.

Step 2. The esters in this group are expected to be readily hydrolysed to their component alcohols and carboxylic acids, which are considered to be innocuous. The terpenoid alcohols are expected to undergo ω-oxidation and functional group oxidation to yield polar metabolites which are excreted as the glucuronic acid conjugate in the urine. Eight of the nine component carboxylic acids are endogenous in humans and are metabolized in the fatty acid β-oxidation pathway, amino acid pathways, the citric acid cycle or the C_1-tetrahydrofolate pathway to eventually yield carbon dioxide and water. The remaining carboxylic acid, 2-ethylbutyric acid, undergoes oxidation to polar metabolites which are conjugated with glucuronic acid and excreted. At current levels of intake these esters and their component terpenoid alcohols and aliphatic carboxylic acids would not be expected to saturate these metabolic pathways.

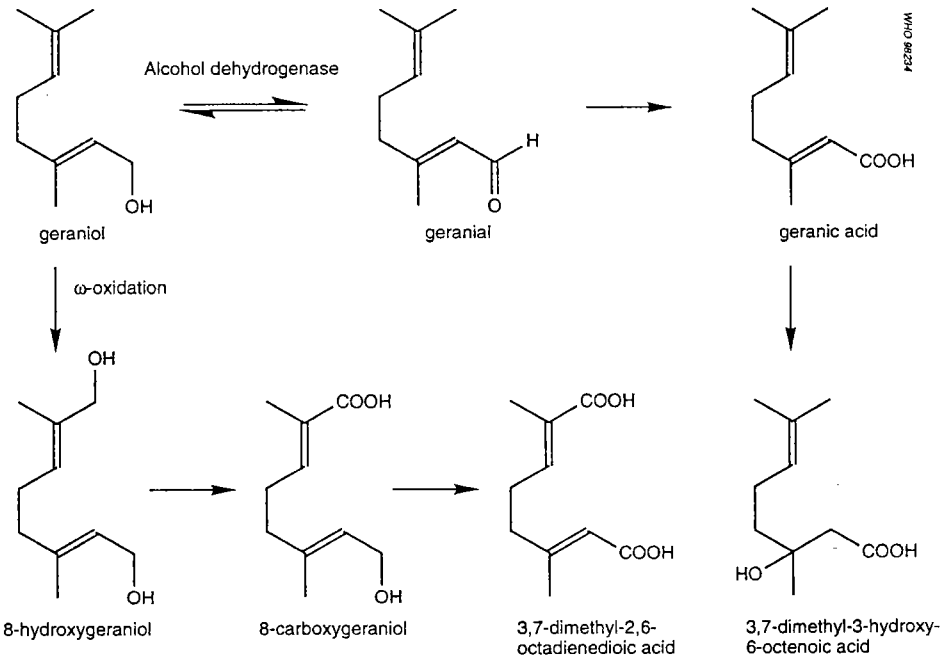
Step A3. The daily per capita intakes of each of the 26 terpenoid esters in both Europe and the USA are below the threshold for class I (1800 μg). Therefore none of the 26 terpenoid esters evaluated were considered to pose a safety concern when used at current levels of intake as flavouring substances.

Table 6 summarizes the evaluation of the 26 terpenoid esters used as flavouring substances.

Consideration of combined intake

In the unlikely event that all foods containing these 26 terpenoid esters used as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake would still be below the threshold for human intake for class I (1800 μg). The

Figure 2
Metabolism of geraniol in rats



Committee noted that the terpene alcohols, geraniol, citronellol and linalool, are used as flavouring agents and that the combined intakes of these alcohols and esters would be less than the group ADI.

Conclusions

The Committee concluded that the esters derived from branched-chain terpenoid alcohols and aliphatic acyclic linear and branched-chain carboxylic acids present no safety concern at the estimated levels of current intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

The Committee noted that some of the esters are metabolized to α,β-unsaturated carbonyl compounds, but concluded that these had been adequately evaluated previously (Annex 1, reference *50*). The Committee maintained the group ADI of 0–0.5 mg/kg of body weight for geranyl acetate, citral, citronellol and linalool.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.8 Specifications for flavouring agents

The specifications for 173 flavouring agents on the modified agenda were reviewed. New specifications were prepared for 153 flavouring agents, 28 of which were designated as "tentative". The existing specifications for 11 flavouring agents reviewed toxicologically and for two flavouring agents reviewed for specifications only (see section 6) were revised. In addition, the existing specifications for the 52 flavouring agents in FAO Food and Nutrition Paper, No. 52, Add. 4 (Annex 1, reference *124*) were given identification numbers.

5. Contaminants: aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 are mycotoxins that may be produced by three species of *Aspergillus*: *A. flavus*, *A. parasiticus* and *A. nomius*, which contaminate plants and plant products. Aflatoxins M_1 and M_2, the hydroxylated metabolites of aflatoxins B_1 and B_2, may be found in milk or milk products obtained from livestock that have ingested contaminated feed. Of these six aflatoxins, aflatoxin B_1 is the most frequently present in contaminated samples and aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of aflatoxin B_1. Most of the toxicological data relate to aflatoxin B_1. Intake of aflatoxins in the diet arises mainly from eating contaminated maize and groundnuts and their products.

The aflatoxins were evaluated at the thirty-first meeting of the Committee (Annex 1, reference *77*). At that time, the Committee considered aflatoxins to be potential human carcinogens. Sufficient information was not available to establish a figure for a tolerable level of intake. The Committee urged that the intake of dietary aflatoxins be reduced to the lowest practicable levels so as to reduce the potential risk as far as possible. A working group convened by the International Agency for Research on Cancer also concluded that naturally occurring aflatoxins are carcinogenic to humans (*12*).

At its forty-sixth meeting (Annex 1, reference *122*), the Committee considered estimates of the carcinogenic potency of aflatoxins and the potential risks associated with their intake. In view of the value of such estimates, the Committee recommended that this task be continued at its next meeting and that a monograph be published summarizing the data and analyses.

At its present meeting, the Committee reviewed a wide range of studies in both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of aflatoxins. The Committee evaluated the potency of these contaminants, linked these potencies to intake estimates and discussed the impact of hypothetical standards on sample populations and their overall risks.

Carcinogenicity

The aflatoxins are among the most potent mutagenic and carcinogenic substances known. Extensive experimental evidence from test species shows that aflatoxins are capable of inducing liver cancer in most animal species studied. In addition, most epidemiological studies show a correlation between exposure to aflatoxin B_1 and an increased incidence of liver cancer. Aflatoxins are metabolized in humans and test species to the corresponding epoxide which is usually considered to be the ultimate reactive intermediate. There is some evidence suggesting that humans are at substantially lower risk from exposure to aflatoxins than test species. The Committee was aware of epidemiological studies which suggest that intake of aflatoxins poses no detectable independent risk and of studies which suggest that they pose risks only in the presence of other risk factors such as hepatitis B infection. Several current studies are likely to allow more accurate estimates of the risks to humans from the intake of aflatoxins, most notably cohort studies in China (Qidong and Shanghai) and Thailand and hepatitis B vaccination trials in China (Qidong and Taiwan) and the Gambia. When these studies are complete, the Committee may wish to re-evaluate the risks of aflatoxins in humans.

A number of factors influence the risk of primary liver cancer, most notably carriage of hepatitis B virus as determined by the presence in serum of the hepatitis B surface antigen (presence denoted $HBsAg^+$ and absence denoted $HbsAg^-$). The potency of aflatoxin B_1 appears to be significantly enhanced in individuals with simultaneous hepatitis B infection. This interaction makes it difficult to interpret the epidemiological studies to determine the extent to which aflatoxins act as independent risk factors. The conclusions of the Committee regarding the carcinogenic potency of aflatoxins are therefore contingent upon the dynamics of hepatitis B infection in a human population.

The identification of hepatitis C virus is an important recent advance in understanding the etiology of liver cancer. Two studies have inves-

tigated interactions between hepatitis C infection, aflatoxins and liver cancer, but the results so far are inconclusive. It is estimated that between 50 and 100% of cases of liver cancer are associated with persistent infection with hepatitis B and/or hepatitis C.

The Committee considered that the weight of scientific evidence, which includes epidemiological data, studies in laboratory animals and *in vivo* and *in vitro* studies of metabolism, supports a conclusion that aflatoxins should be treated as carcinogenic food contaminants, the intake of which should be reduced to levels as low as reasonably achievable.

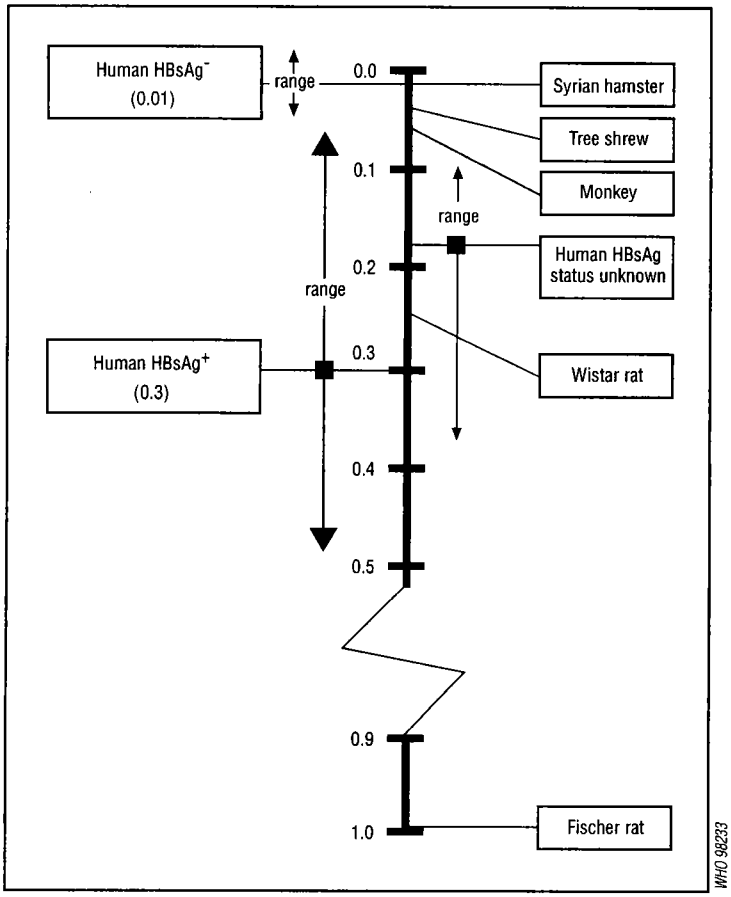
Carcinogenic potency

The Committee reviewed dose–response analyses that have been performed on aflatoxins. All of these analyses have limitations, three of which predominate. First, all of the epidemiological data from which a dose–response relationship can be determined are confounded by concurrent infection with hepatitis B. The epidemiological data are from geographical areas where both the prevalence of $HBsAg^+$ individuals and of contamination with aflatoxins are high; the relationship between these risk factors in areas in which aflatoxin contamination and prevalence of hepatitis B are low is unknown. Second, the reliability and precision of the estimates of exposure to aflatoxins in the relevant study populations are unknown. For example, aflatoxin biomarkers in humans do not reflect long-term intake of aflatoxins; analyses of crops for aflatoxins do not reflect the levels of aflatoxins consumed in foods after selection and processing. Finally, the shape of the dose–response curve is unknown and this introduces an additional element of uncertainty when choosing mathematical models for interpolation.

Observations concerning the interaction between hepatitis B infection and aflatoxins suggest two separate aflatoxin potencies; one is apparent in populations in which chronic hepatitis infections are common, the other in populations in which chronic hepatitis infections are rare. In analyses based on toxicological and epidemiological data, potency estimates for aflatoxins were divided into two basic groups, potencies applicable to individuals without hepatitis B infection and those applicable to individuals with chronic hepatitis B infection. The Committee found these estimates useful even though, through the use of differing mathematical models, they covered a broad range of possible values (Fig. 3). Potencies calculated from epidemiological studies in which hepatitis B infection status was unknown were in the range of potencies found in individuals with and without hepatitis B infection. The review also considered the extrapolation of animal data

Figure 3

Potency estimates for human liver cancer resulting from exposure to aflatoxin B_1 (cases per year/100 000 people per ng of aflatoxin B_1/kg of body weight per day), based on epidemiological and toxicological studies



HBsAg⁺: Hepatitis B surface antigen detected in serum; HBsAg⁻: hepatitis B surface antigen not detected in serum.

to estimate potency in humans; these estimates also generally fell within the range of the potency estimates derived from the epidemiological data.

There are several potential biases in the potency estimates depicted in Fig. 3:

- Only studies showing a positive association between aflatoxins and liver cancer were used as opposed to considering all studies (negative as well as positive); this leads to overestimation of the aflatoxin potency.

- When current levels of intake (i.e. using biomarkers or dietary surveys) are related to current levels of liver cancer (which presumably has a long induction period), historical levels of intake are ignored, and since intakes are likely to have been higher in the past, aflatoxin potency will be overestimated.
- The earliest studies systematically underestimated the prevalence of hepatitis B infection in patients with liver cancer by as much as 20–30% due to limitations in the methodology used to detect hepatitis B virus, which also leads to an overestimate of the relative potency of any other factor, including aflatoxins.
- Histological confirmation of the cases of liver cancer is limited in most epidemiological studies, allowing the possibility that cases of non-primary liver cancer have been included, which could lead to an underestimation or overestimation of the aflatoxin potency.

When these biases are taken into account, the values in Fig. 3 should be viewed as overestimates of the potency of the aflatoxins, leading to the hypothesis that it is possible that humans are in fact less sensitive to aflatoxins than the animal species tested in laboratory experiments.

The Committee reviewed the extensive data available on the metabolism of aflatoxins in various species. It was agreed that differences in the carcinogenic potency of aflatoxins between species can be partially attributed to differences in metabolism. However, there was insufficient quantitative information available about competing aspects of metabolic activation and detoxification of aflatoxin B_1 in various species to identify an adequate animal model for humans and to explain the apparent differences in potency between species.

The intake assessments used in many of the epidemiological studies ignored the contributions to total aflatoxin intake made by milk and milk products. Thus, the potencies shown in Fig. 3 do not generally apply to aflatoxin M_1. The results of a comparative toxicity study in rats suggest that aflatoxin M_1 has a potency approximately one order of magnitude less than that of aflatoxin B_1 in this species.

The Committee reviewed the potency estimates from the epidemiological studies which showed a positive association between aflatoxins and liver cancer and chose separate potency estimates and ranges for $HBsAg^+$ and $HBsAg^-$ individuals. Potency values of 0.3 cancers per year/100 000 population per ng of aflatoxins/kg of body weight per day (range 0.05–0.5) for $HBsAg^+$ individuals and of 0.01 cancers per year/100 000 population per ng of aflatoxins/kg of body weight per day (range 0.002–0.03) in $HBsAg^-$ individuals were chosen.

Population risks

The fraction of the incidence of liver cancer in a population attributable to intake of aflatoxins was derived by combining estimates of aflatoxin potency (risk per unit dose) and estimates of aflatoxin intake (dose per person). The Committee reviewed the frequency and amount of aflatoxin contamination in a variety of products (e.g. groundnuts, cereals and maize) in numerous countries. Many of the data on levels of aflatoxin contamination were derived from non-random samples which appeared to be biased upwards because monitoring studies focus on products that are thought to be contaminated. Some of the data on levels of contamination are not likely to be based on current Codex Alimentarius Commission sampling recommendations for aflatoxins. Accordingly, data on levels of contamination should be interpreted with caution and used only to infer patterns of importance in setting standards and not to provide exact contamination estimates.

The Committee considered the possible impact of applying hypothetical standards to aflatoxin contamination. It noted that the magnitude of the difference between two hypothetical standards is substantially larger than the magnitude of the difference in the mean contamination levels resulting from application of the separate standards. For example, in the case of maize from the USA, which has a mean level of aflatoxin contamination of 4.7 μg/kg, application of a hypothetical standard of 20 μg/kg would result in rejection of 3.9% of the crop and a mean level of aflatoxin contamination of 0.9 μg/kg. Imposition of a stricter hypothetical standard of 10 μg/kg would result in rejection of 6.2% of the crop and reduce the mean level of aflatoxin contamination by 0.3 μg/kg to 0.6 μg/kg (Table 7). Similar results would be obtained if aflatoxin B_1 levels in maize and total aflatoxins or aflatoxin B levels in groundnuts were examined.

Using the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme regional diets combined with data on levels of aflatoxin contamination, the Committee was able to provide relative estimates of the mean dietary intake of aflatoxins for various regions. If these intakes are linked to the potency estimates shown in Fig. 3, it is possible to calculate the overall population risks on the basis of the prevalence of hepatitis B infection in various regions.

From its analysis the Committee noted that the application of a standard would prevent human consumption of the most highly contaminated samples, thus greatly reducing average estimated intakes of aflatoxins. The use of standards by all countries should be encour-

Table 7
Distribution of total aflatoxin contamination in maize from the USA and comparison of the impact of two different hypothetical standards on the percentage of samples rejected and the mean contamination level

Concentration of total aflatoxins in maize (µg/kg)	Percentile	Impact of hypothetical standard on percentage of samples rejected and mean contamination level[a]
0.1	10.0	
0.2	30.0	
0.3	50.0	
0.4	70.0	
0.5	80.0	
1.0	88.0	
5.0	90.6	
10	93.8	6.2% of samples rejected at a standard of 10 µg/kg (mean contamination level = 0.6 µg/kg)
15	95.0	
20	96.1	3.9% of samples rejected at a standard of 20 µg/kg (mean contamination level = 0.9 µg/kg)
30	96.8	
40	97.6	
50	98.0	

[a] The mean level of aflatoxin contamination with no standard was 4.7 µg/kg.

aged. The Committee considered the effect of modifying a given standard through the use of several hypothetical calculations. Two illustrations are given below.

The first example pertains to areas in which the level of contamination of food by aflatoxins is low and the proportion of the population who are $HBsAg^+$ is small. For this purpose, monitoring data from Europe on aflatoxin B_1 levels in groundnuts, maize[1] and their products were used. In this example, 1% of the population were assumed to be $HBsAg^+$. From the potency estimates given earlier (see Fig. 3), this yields an estimated potency for this population of $0.01 \times 99\% + 0.3 \times 1\% = 0.013$ cancers per year/100 000 people per ng of aflatoxins/kg of body weight per day (range 0.002–0.035). If it is assumed that all samples with levels of contamination above 20 µg/kg are removed and that these foods are ingested according to the "European diet", the mean estimated intake of aflatoxins is 19 ng per person per day. Assuming that an adult human weighs 60 kg, the estimated population

[1] The Committee noted that the aflatoxin data for Europe were for "all cereals". However, in these calculations, it was assumed that the aflatoxin level for "all cereals" applied to maize only.

risk (potency × intake) is 0.0041 cancers per year per 100000 people (range 0.0006–0.01). In contrast, if the same assumptions are used but a hypothetical standard of 10 μg/kg is applied, the average aflatoxin intake is 18 ng per person per day, resulting in an estimated population risk of 0.0039 cancers per year per 100000 people (range 0.0006–0.01). Thus, reducing the hypothetical standard from 20 μg/kg to 10 μg/kg yields a reduction in the estimated population risk of approximately two cancers per year per 1000 million people.

The second example pertains to areas with higher levels of aflatoxin contamination (for these purposes monitoring data from China on aflatoxin B_1 levels in groundnuts, maize and their products were used) and where a larger percentage of the population are carriers (in this case, 25% were assumed to be $HBsAg^+$). The estimated potency for this population is $0.01 \times 75\% + 0.3 \times 25\% = 0.083$ cancers per year per ng of aflatoxins ingested/kg of body weight per day (range 0.014–0.15). If a hypothetical standard of 20 μg/kg and the "Far Eastern diet" are used, the average estimated intake is 125 ng per person per day, yielding an average population risk of 0.17 cancers per year per 100000 people (range 0.03–0.3). If a hypothetical standard of 10 μg/kg is used, the average estimated intake falls to 103 ng per person per day, yielding an estimated population risk of 0.14 cancers per year per 100000 people (range 0.02–0.3). Thus, reducing the hypothetical standard for this population from 20 μg/kg to 10 μg/kg yields a reduction in the estimated population risk of 300 cancers per year per 1000 million people.

Conclusions

1. Aflatoxins are considered to be human liver carcinogens. Aflatoxin B_1 is the most potent carcinogen of the aflatoxins and most of the toxicological data available are related to aflatoxin B_1. Aflatoxin M_1, the hydroxylated metabolite of B_1, has a potency approximately one order of magnitude less than that of B_1.
2. The potency of aflatoxins in $HBsAg^+$ individuals is substantially higher than in $HBsAg^-$ individuals. Thus, reduction of the intake of aflatoxins in populations with a high prevalence of $HBsAg^+$ individuals will result in a greater reduction in liver cancer rates than reduction of the intake of aflatoxins in populations with a low prevalence of $HBsAg^+$ individuals.
3. Vaccination against hepatitis B will reduce the number of carriers of the virus. The present analysis suggests that reducing the number of carriers would reduce the potency of the aflatoxins in vaccinated populations and consequently reduce the risk of liver cancer.

4. Analyses of the application of hypothetical standards for aflatoxin contamination in food (10 μg/kg or 20 μg/kg) to model populations indicates that:
   (a) populations in which the prevalence of $HBsAg^+$ individuals is low and/or in which the mean intake of aflatoxins is low (less than 1 ng/kg of body weight per day) are unlikely to exhibit detectable[1] differences in population risks;
   (b) populations in which both the prevalence of $HBsAg^+$ and the intake of aflatoxins are high would benefit from reductions in aflatoxin intake.
5. The Committee has previously noted that reductions in the intake of aflatoxins can be achieved through avoidance measures such as improved farming and proper storage practices and/or through enforcing standards for levels of contamination in food or feed within countries and across borders (Annex 1, reference 77).
6. When two alternative standards for aflatoxin contamination in food are being considered, if the fraction of the samples excluded under the two standards is similar, the higher standard will yield essentially the same risk of liver cancer as the lower standard. When a substantial fraction of the current food supply is heavily contaminated with aflatoxins, reducing the levels of contamination may result in a detectable[1] reduction in rates of liver cancer. Conversely, when only a small fraction of the current food supply is heavily contaminated, reducing the standard by an apparently substantial amount may have little appreciable effect on health.

6. Revision of certain specifications

A total of 40 substances were examined for specifications only (see Annex 2), and the specifications for 30 were revised.

The specifications for agar, carthamus yellow, microcrystalline wax, propylene glycol, propylene glycol alginate and gellan gum were revised, with minor changes.

The specifications for alginic acid, ammonium alginate, calcium alginate, potassium alginate and sodium alginate were revised and the requirements for relative molecular mass range were changed in

[1] In the context of this statement "detectable" refers to an aflatoxin-induced change in liver cancer rates which exceeds the year-to-year variability around the current incidence and mortality rates. Hence "detectable" refers to the ability to observe a significant effect on the occurrence of liver cancer following intervention and will depend on the quality of the data available on historical trends in incidence and mortality.

order to reflect more precisely the products on the market. Some minor changes were also made.

The specifications for aluminium powder, mixed carotenoids, ethyl hydroxyethyl cellulose, propylene glycol esters of fatty acids and talc were revised and the "tentative" qualifications were deleted.

The specifications for citric acid, calcium propionate (calcium propanoate), potassium propionate (potassium propanoate) and sodium propionate (sodium propanoate) were revised, with minor changes. Citric acid was also added to the section on flavouring agents in the specifications monograph.

The specifications for propionic acid used as a preservative and an antimould and antirope agent were revised and designated as "tentative". The Committee requested information on the method of analysis and levels of readily oxidizable substances.

The Committee agreed to revise the specifications for modified starches to include enzyme treatment as an alternative method for modifying food starches. In addition, the Committee agreed to delete the minimum and maximum levels of reagents used in the processing of the starches since the end-product specifications were deemed sufficient to assure the quality and safety of the various modified starches. As a result of these recommendations, the existing tentative monograph for enzyme-treated starches was withdrawn.

The specifications for allyl cyclohexane propionate, ethyl octanoate, ethyl nonanoate, isoamyl acetate, isoamyl butyrate, isoamyl isobutyrate and isoamyl isovalerate used as flavouring agents were reviewed and given identification numbers. The specifications for isoamyl acetate used as a carrier solvent were revised in order to reflect that the substance is a mixture.

The existing tentative specifications for carbon dioxide and sulfur dioxide were revised in order to define purer products and the "tentative" designation was deleted.

The specifications for gum arabic obtained from certain species of acacia trees were last revised at the forty-fourth meeting of the Committee (Annex 1, reference *116*). In revising the specifications for gum arabic at its present meeting, the Committee considered two new extensive project reports from FAO (*13*, *14*) on the production, marketing and physicochemical characterization of this substance. The reports demonstrated that chemometric analysis of analytical compositional data is a suitable procedure for evaluating the chemical rela-

tionship of gums obtained from various acacia species. In the light of these reports, the Committee reiterated that gum from other acacia species closely related to *Acacia senegal* and meeting the newly revised specifications would adequately reflect the materials that had been toxicologically evaluated. The specifications were revised to make a clear distinction between gum arabic obtained from *A. seyal* and from *A. senegal.*

New specifications for enzyme-hydrolysed sodium carboxymethyl cellulose were prepared and designated as "tentative". The Committee requested information on the physical state of the substance as manufactured, the nature and proportion of material of low relative molecular mass present in the substance, and tests that distinguish the substance from sodium carboxymethyl cellulose.

The existing tentative specifications for carthamus red were revised and the "tentative" designation was maintained. The Committee requested information on the content of carthamin and method of assay.

The existing tentative specifications for petroleum jelly were revised, with minor corrections, and the "tentative" designation was maintained. The Committee requested information on the method of analysis and levels of viscosity at 100 °C, carbon number at 5% distillation point, average relative molecular mass and oil content. Unless this information was received by 31 March 1998, the specifications would be withdrawn.

The Committee compared the specifications for "diacetyltartaric and fatty acid esters of glycerol" (DATEM) with the existing tentative specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and noted that the substances appear very similar. Both substances were toxicologically evaluated at the seventeenth meeting of the Committee in 1973 (Annex 1, reference *32*). At that meeting, DATEM was assigned an ADI of 0–50 mg/kg of body weight, whereas the mixed tartaric, acetic and fatty acids of glycerol were assigned an ADI "not limited". The Committee has not received information which would allow these substances to be distinguished analytically. The Committee decided to maintain the tentative status of the existing specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and requested data that would distinguish this substance from DATEM. Unless such data were provided by 31 March 1998, the Committee would consider combining the two specifications. The existing specifications of DATEM were revised.

The Committee withdrew the tentative specifications for anoxomer since none of the information requested at its twenty-sixth meeting (Annex 1, reference *59*) has been submitted. The Committee was informed that anoxomer has been neither produced nor used for many years.

The specifications for turmeric as a food colour were on the agenda for revision. However, as the use of this substance was considered to be as a spice and not as a food colour, the Committee decided to withdraw the specifications.

7. Recommendations

1. In view of the large number of food additives, food ingredients and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining 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.
2. One of the substances referred to the present meeting for evaluation is an example of materials that have been defined as "novel foods" by some countries and organizations. The Committee recommended that FAO and WHO arrange at future meetings of the Committee for the review of procedures outlined in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*) for the safety evaluation of these types of products and for the development of guidelines for assessing their safety and wholesomeness, taking into account guidelines developed by other organizations.

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References

1. *Joint FAO/WHO Conference on Food Additives.* Rome, Food and Agriculture Organization of the United Nations, 1956 (FAO Nutrition Meetings Report Series, No. 11); Geneva, World Health Organization, 1956 (WHO Technical Report Series, No. 107).

2. Codex Alimentarius Commission. *Report of the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants, The Hague, 17–21 March 1997.* Rome, Food and Agriculture Organization of the United Nations, 1997 (unpublished FAO document ALINORM 97/12A; available from FAO or WHO).

3. *Safety evaluation of certain food additives and contaminants.* Geneva, World Health Organization, 1998 (WHO Food Additives Series, No. 40).

4. *Application of risk analysis to food standards issues. Report of the Joint FAO/WHO Expert Consultation, Geneva, Switzerland, 13–17 March 1995.* Geneva, World Health Organization, 1995 (unpublished document WHO/FNU/FOS/95.3; available on request from Programme of Food Safety and Food Aid, World Health Organization, 1211 Geneva 27, Switzerland).

5. *Food consumption and exposure assessment to chemicals. Report of a FAO/WHO Consultation, Geneva, Switzerland, 10–14 February 1997.* Geneva, World Health Organization, 1997 (unpublished document WHO/FNU/FOS/97.5; available on request from Programme of Food Safety and Food Aid, World Health Organization, 1211 Geneva 27, Switzerland).

6. Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme. *Guidelines for predicting dietary intake of pesticide residues.* Geneva, World Health Organization, 1997 (unpublished document WHO/FSF/FOS/97.7; available on request from Programme of Food Safety and Food Aid, World Health Organization, 1211 Geneva 27, Switzerland).

7. Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme. *Guidelines for establishing or strengthening national food contamination monitoring programmes.* Geneva, World Health Organization, 1979 (unpublished document WHO/HCS/FCM/78.1; available on request from Programme of Food Safety and Food Aid, World Health Organization, 1211 Geneva 27, Switzerland).

8. *Guidelines for the study of dietary intakes of chemical contaminants.* Geneva, World Health Organization, 1985 (WHO Offset Publication, No. 87).

9. Kari FW et al. Toxicity and carcinogenicity of hydroquinone in F344/N rats and B6C3F1 mice. *Food and chemical toxicology.* 1992, 30:737–747.

10. *Evaluating the safety of food chemicals.* Washington, DC. National Academy of Sciences, 1987.

11. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard — a decision-tree approach. *Food and cosmetics toxicology*, 1978, 16:255–276.

12. *Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins.* Lyon, International Agency for Research on Cancer, 1993 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 56):245–395.

13. Chikamai BN, Philips GO, Casadei E. *The characterization and specification of gum arabic.* Rome, Food and Agriculture Organization of the United Nations, 1996 (Project No. TCP/RAF/4557).

14. Chikamai BN. *A review of production, markets and quality control of gum arabic in Africa.* Rome, Food and Agriculture Organization of the United Nations, 1996 (Project No. TCP/RAF/4557).

Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. I. *Antimicrobial preservatives and antioxidants.* Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. II. *Food colours.* Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants.* FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours.* FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25 (out of print).
11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C: WHO/Food Add/67.29 (out of print).
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967 (out of print).
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968 (out of print).
15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33 (out of print).
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31 (out of print).
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969 (out of print).
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Report Series, No. 45A, 1969; WHO/Food Add/69.34 (out of print).
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970 (out of print).
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36 (out of print).
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37 (out of print).
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971 (out of print).
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39 (out of print).
24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40 (out of print).
25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41 (out of print).
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972 (out of print).
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972 (out of print).
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum (out of print).
31. *Evaluation of mercury, lead, cadmium, and the food additives amaranth, diethylpyrocarbonate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974 (out of print).
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum (out of print).
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents, and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975 (out of print).
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others.* FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978 (out of print).
48. *Summary of toxicological data of certain food additives and contaminants.* WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives.* FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.
59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda (out of print).
63. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications — General notices, general methods, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of

the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710. 1984. and corrigendum.
67. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours.* FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives.* FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 20).
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 21).
75. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 37, 1986.
76. *Principles for the safety assessment of food additives and contaminants in food.* Geneva, World Health Organization, 1987 (WHO Environmental Health Criteria, No. 70).
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. *Toxicological evaluation of certain food additives.* Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 22).
79. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. *Toxicological evaluation of certain veterinary drug residues in food.* Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 23).
82. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41, 1988 (out of print).
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 766, 1989.
84. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1989 (WHO Food Additives Series, No. 24).
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 25, 1990.
87. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 26, 1990.

90. *Specifications for identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 49, 1990.
91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA). Combined specifications from 1st through the 37th Meetings, 1956–1990).* Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. *Toxicological evaluation of certain veterinary residues in food.* WHO Food Additives Series, No. 29, 1991.
99. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/4, 1991.
100. *Guide to specifications — General notices, general analytical techniques, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.
102. *Toxicological evaluation of certain food additives and naturally occurring toxicants.* WHO Food Additives Series, No. 30, 1993.
103. *Compendium of food additive specifications, addendum 1.* FAO Food and Nutrition Paper, No. 52, Add. 1, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 32, 1993.
109. *Compendium of food additive specifications, addendum 2.* FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the

Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/7, 1995.
116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 35, 1996.
118. *Compendium of food additive specifications, addendum 3.* FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/8, 1996.
122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 37, 1996.
124. *Compendium of food additives specifications, addendum 4.* FAO Food and Nutrition Paper, No. 52, Add. 4, 1996 (out of print).
125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/10, 1998.

Annex 2

Acceptable Daily Intakes, other toxicological information, and information on specifications

Substance	Specifications[a]	Acceptable Daily Intake (ADI) in mg/kg of body weight and other toxicological recommendations
Antioxidant		
tert-Butylhydroquinone (TBHQ)	R	0–0.7
Emulsifiers		
Microcrystalline cellulose	R	Not specified[b, c]
Sucrose esters of fatty acids and sucroglycerides	R	0–30 (group ADI)
Enzyme preparations		
α-Acetolactate decarboxylase	N, T	Not specified (temporary)[b, d]
Maltogenic amylase	N, T	Not specified (temporary)[b, d]
Flavouring agent		
trans-Anethole	R	0–0.6 (temporary)[e]
Glazing agent		
Hydrogenated poly-1-decene	N	No ADI allocated because insufficient data were available
Sweetening agent		
Maltitol syrup	R	Not specified[b, c]
Miscellaneous substance		
Salatrim (short- and long-chain acyltriglyceride molecules)	N	Adequate information was not available to evaluate its safety and nutritional effects

[a] N, new specifications prepared; R, existing specifications revised; T, the existing, new or revised specifications are tentative and comments are invited.

[b] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[c] This ADI applies to the substance conforming to the revised specifications.

[d] Temporary ADI, pending consideration of the "tentative" qualification of the specifications. The "tentative" qualification of Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Rome, Food and Agriculture Organization of the United Nations, 1992) will be reviewed in 1998.

[e] Temporary ADI extended to 1998, pending the submission of the results of studies currently underway that were requested at earlier meetings of the Committee.

Flavouring agents

The substances listed here were evaluated using the Procedure for the Evaluation of Flavouring Agents. For further details, see section 2.2.1 of the main report.

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Allyl ester			
Allyl 2-furoate (2-propenyl furan-2-carboxylate)	0021	S, T	No safety concern
Saturated aliphatic acyclic linear primary alcohols, aldehydes and acids			
Formic acid[c]	0079	R	
Acetaldehyde (ethanal)	0080	N	
Acetic acid[c]	0081	R	
Propyl alcohol (1-propanol)	0082	R	
Propionaldehyde (propanal)	0083	N	
Propionic acid[c] (propanoic acid)	0084	N	
Butyl alcohol (1-butanol)	0085	R	
Butyraldehyde (butanal)	0086	N	
Butyric acid (butanoic acid)	0087	N	
Amyl alcohol (1-pentanol)	0088	N	
Valeraldehyde (pentanal)	0089	N	
Valeric acid (pentanoic acid)	0090	N	
Hexyl alcohol (1-hexanol)	0091	N	
Hexanal	0092	N	
Hexanoic acid	0093	N	
Heptyl alcohol (1-heptanol)	0094	N	
Heptanal	0095	N	
Heptanoic acid	0096	N	
1-Octanol	0097	N	No safety concern
Octanal[c]	0098	R	
Octanoic acid	0099	N	
Nonyl alcohol (1-nonanol)	0100	N	
Nonanal[c]	0101	R	
Nonanoic acid	0102	N	
1-Decanol	0103	N	
Decanal	0104	R	
Decanoic acid	0105	N	
Undecyl alcohol (1-undecanol)	0106	N	
Undecanal	0107	N	
Undecanoic acid	0108	N	
Lauryl alcohol (1-dodecanol)	0109	N	
Lauric aldehyde (dodecanal)	0110	N	
Lauric acid (dodecanoic acid)	0111	N	
Myristaldehyde (tetradecanal)	0112	N	
Myristic acid (tetradecanoic acid)	0113	N	
1-Hexadecanol	0114	N	
Palmitic acid (hexadecanoic acid)	0115	N	
Stearic acid (octadecanoic acid)	0116	N	
Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Structural class I: methyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Isobutyl alcohol (2-methyl-1-propanol)	0251	NR	No safety concern
Isobutyraldehyde (2-methylpropanal)	0252	NR	
Isobutyric acid (2-methylpropanoic acid)	0253	NR	
2-Methylbutyraldehyde (2-methylbutanal)	0254	NR	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
2-Methylbutyric acid (2-methylbutanoic acid)	0255	NR	No safety concern
3-Methylbutyraldehyde (3-methylbutanal)	0258	NR	
Isovaleric acid (3-methylbutanoic acid)	0259	NR	
2-Methylpentanal	0260	NR	
2-Methylvaleric acid (2-methylpentanoic acid)	0261	NR	
3-Methylpentanoic acid	0262	NR	
3-Methyl-1-pentanol	0263	NR	
4-Methylpentanoic acid	0264	NR	
2-Methylhexanoic acid	0265	NR	
5-Methylhexanoic acid	0266	NR	
3,5,5-Trimethyl-1-hexanol	0268	NR	
3,5,5-Trimethylhexanal	0269	NR	
2-Methyloctanal	0270	NR	
4-Methyloctanoic acid	0271	NR	
3,7-Dimethyl-1-octanol	0272	NR	
2,6-Dimethyloctanal	0273	NR	
4-Methylnonanoic acid	0274	NR	
2-Methylundecanal	0275	NR	
Structural class II: ethyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
2-Ethylbutyraldehyde (2-ethylbutanal)	0256	NR	No safety concern
2-Ethylbutyric acid (2-ethylbutanoic acid)	0257	NR	
2-Ethyl-1-hexanol[c]	0267	NR	
Aliphatic lactones			
Structural class I			
4-Hydroxybutyric acid lactone (γ-butyrolactone)	0219	NR	No safety concern
γ-Valerolactone	0220	NR	
γ-Hexalactone	0223	NR	
δ-Hexalactone	0224	NR	
γ-Heptalactone	0225	NR	
γ-Octalactone	0226	NR	
δ-Octalactone	0228	NR	
γ-Nonalactone[c]	0229	NR	
Hydroxynonanoic acid δ-lactone	0230	NR	
γ-Decalactone	0231	NR	
δ-Decalactone	0232	NR	
ε-Decalactone	0241	NR	
γ-Undecalactone[c]	0233	NR	
5-Hydroxyundecanoic acid δ-lactone	0234	NR	
γ-Dodecalactone	0235	NR	
δ-Dodecalactone	0236	NR	
ε-Dodecalactone	0242	NR	
δ-Tetradecalactone	0238	NR	
ω-Pentadecalactone	0239	NR	
4-Hydroxy-3-pentenoic acid lactone	0221	NR	
5-Hydroxy-7-decenoic acid δ-lactone	0247	NR	
5-Hydroxy-8-undecenoic acid δ-lactone	0248	NR	
1,4-Dodec-6-enolactone	0249	NR	
ω-6-Hexadecenlactone	0240	NR	
4,4-Dibutyl-γ-butyrolactone	0227	NR	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	0244	NR	No safety concern
4-Hydroxy-3-methyloctanoic acid γ-lactone	0437	NR	
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	NR	
γ-Methyldecalactone	0250	NR	
Structural class III			
5-Hydroxy-2-decenoic acid δ-lactone	0246	NR	
5-Hydroxy-2,4-decadienoic acid δ-lactone	0245	NR	
Mixture of 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone and 5-hydroxy-2-tetradecenoic acid δ-lactone	0276	NR	Not evaluated[d]
5-Hydroxy-2-dodecenoic acid δ-lactone	0438	NR	
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	0222	NR	No safety concern
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	NR	
Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids			
Methyl isobutyrate (methyl 2-methylpropanoate)	0185	N	No safety concern
Ethyl isobutyrate (ethyl 2-methylpropanoate)	0186	N	
Propyl isobutyrate (*n*-propyl 2-methylpropanoate)	0187	N	
Butyl isobutyrate (butyl 2-methylpropanoate)	0188	N	
Hexyl isobutyrate (hexyl 2-methylpropanoate)	0189	N	
Heptyl isobutyrate (heptyl 2-methylpropanoate)	0190	N	
trans-3-Heptenyl 2-methylpropanoate	0191	N	
Octyl isobutyrate (octyl 2-methylpropanoate)	0192	N	
Dodecyl isobutyrate (dodecyl 2-methylpropanoate)	0193	N	
Isobutyl isobutyrate (2-methylpropyl 2-methylpropanoate)	0194	N	
Methyl isovalerate (methyl 3-methylbutanoate)	0195	N	
Ethyl isovalerate (ethyl 3-methylbutanoate)	0196	R	
Propyl isovalerate (propyl 3-methylbutanoate)	0197	N	
Butyl isovalerate (butyl 3-methylbutanoate)	0198	N	
Hexyl 3-methylbutanoate	0199	N	
Octyl isovalerate (octyl 3-methylbutanoate)	0200	N	
Nonyl isovalerate (nonyl 3-methylbutanoate)	0201	N	
3-Hexenyl 3-methylbutanoate	0202	N	
2-Methylpropyl 3-methylbutyrate (2-methylpropyl 3-methylbutanoate)	0203	N	
2-Methylbutyl 3-methylbutanoate	0204	N, T	
Methyl 2-methylbutyrate (methyl 2-methylbutanoate)	0205	N	
Ethyl 2-methylbutyrate (ethyl 2-methylbutanoate)	0206	N	
n-Butyl 2-methylbutyrate (butyl 2-methylbutanoate)	0207	N	
Hexyl 2-methylbutanoate	0208	N	
Octyl 2-methylbutyrate (octyl 2-methylbutanoate)	0209	N	
Isopropyl 2-methylbutyrate (1-methylethyl 2-methylbutanoate)	0210	N, T	
3-Hexenyl 2-methylbutanoate	0211	N, T	
2-Methylbutyl 2-methylbutyrate (2-methylbutyl 2-methylbutanoate)	0212	N	
Methyl 2-methylpentanoate	0213	N, T	
Ethyl 2-methylpentanoate	0214	N	
Ethyl 3-methylpentanoate	0215	N, T	
Methyl 4-methylvalerate	0216	N	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids			
Propyl formate	0117	N	No safety concern
Butyl formate	0118	N	
n-Amyl formate (pentyl formate)	0119	N	
Hexyl formate	0120	N	
Heptyl formate	0121	N, T	
Octyl formate	0122	N	
cis-3-Hexenyl formate (*cis*-3-hexenyl-1-yl formate)	0123	N	
Isobutyl formate (2-methylpropyl formate)	0124	N	
Methyl acetate	0125	N	
Propyl acetate	0126	N	
Butyl acetate	0127	R	
Hexyl acetate	0128	N	
Heptyl acetate	0129	N	
Octyl acetate	0130	N	
Nonyl acetate	0131	N	
Decyl acetate	0132	N	
Lauryl acetate (dodecyl acetate)	0133	N	
cis-3-Hexenyl acetate (*cis*-3-hexenyl-1-yl acetate)	0134	N	
trans-3-Heptenyl acetate (*trans*-3-heptenyl-1-yl acetate)	0135	N	
10-Undecen-1-yl acetate	0136	N	
Isobutyl acetate (2-methylpropyl acetate)	0137	N	
2-Methylbutyl acetate (2-methyl-1-butyl acetate)	0138	N	
2-Ethylbutyl acetate	0140	N, T	
Methyl propionate (methyl propanoate)	0141	N	
Propyl propionate (*n*-propyl propanoate)	0142	N	
Butyl propionate (butyl propanoate)	0143	N	
Hexyl propionate (*n*-hexyl propanoate)	0144	N	
Octyl propionate (octyl propanoate)	0145	N	
Decyl propionate (decyl propanoate)	0146	N	
cis-3- and *trans*-2-Hexenyl propionate (*cis*-3- and *trans*-2-hexenyl propanoate)	0147	N	Not evaluated[e]
Isobutyl propionate (2-methylpropyl propanoate)	0148	N	No safety concern
Methyl butyrate (methyl butanoate)	0149	N	
Propyl butyrate (*n*-propyl butanoate)	0150	N	
Butyl butyrate (*n*-butyl *n*-butanoate)	0151	N	
n-Amyl butyrate (pentyl butanoate)	0152	N	
Hexyl butyrate (hexyl butanoate)	0153	N	
Heptyl butyrate (heptyl butanoate)	0154	N, T	
Octyl butyrate (octyl butanoate)	0155	N, T	
Decyl butyrate (decyl butanoate)	0156	N, T	
cis-3-Hexenyl butyrate (*cis*-3-hexen-1-yl butanoate)	0157	N	
Isobutyl butyrate (2-methylpropyl butanoate)	0158	N	
Methyl valerate (methyl pentanoate)	0159	N	
Butyl valerate (butyl pentanoate)	0160	N	
Propyl hexanoate	0161	N	
Butyl hexanoate	0162	N	
n-Amyl hexanoate (pentyl hexanoate)	0163	N	
Hexyl hexanoate	0164	N	
cis-3-Hexenyl hexanoate (*cis*-3-hexen-1-yl hexanoate)	0165	N, T	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Isobutyl hexanoate (2-methylpropyl hexanoate)	0166	N	No safety concern
Methyl heptanoate	0167	N	
Propyl heptanoate	0168	N, T	
Butyl heptanoate	0169	N, T	
n-Amyl heptanoate (pentyl heptanoate)	0170	N	
Octyl heptanoate	0171	N, T	
Isobutyl heptanoate (2-methylpropyl heptanoate)	0172	N, T	
Methyl octanoate	0173	N	
n-Amyl octanoate (pentyl octanoate)	0174	N	
Hexyl octanoate	0175	N	
Heptyl octanoate	0176	N, T	
Octyl octanoate	0177	N, T	
Nonyl octanoate	0178	N, T	
Methyl nonanoate	0179	N	
Methyl laurate (methyl dodecanoate)	0180	N	
Butyl laurate (butyl dodecanoate)	0181	N	
Isoamyl laurate (3-methylbutyl dodecanoate)	0182	N, T	
Methyl myristate (methyl *n*-tetradecanoate)	0183	N	
Butyl stearate (butyl octadecanoate)	0184	N, T	
Esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids			
Citronellyl formate (3,7-dimethyl-6-octen 1-yl formate)	0053	N	No safety concern
Geranyl formate (*trans*-3,7-dimethyl-2,6-octadien-1-yl formate)	0054	N	
Neryl formate (*cis*-3,7-dimethyl-2,6-octadien-1-yl formate)	0055	N	
Rhodinyl formate (3,7-dimethyl-7-octen-1-yl formate)	0056	N	
Citronellyl acetate (3,7-dimethyl-6-octen-1-yl acetate)	0057	N	
Geranyl acetate[c] (*trans*-3,7-dimethyl-2,6-octadien-1-yl acetate)	0058	R	
Neryl acetate (*cis*-3,7-dimethyl-2,6-octadien-1-yl acetate)	0059	N	
Rhodinyl acetate (3,7-dimethyl-7-octen-1-yl acetate)	0060	N	
Citronellyl propionate (3,7-dimethyl-6-octen-1-yl propanoate)	0061	N	
Geranyl propionate (*trans*-3,7-dimethyl-2,6-octadien-1-yl propanoate)	0062	N	
Neryl propionate (*cis*-3,7-dimethyl-2,6-octadien-1-yl propanoate)	0063	N	
Rhodinyl propionate (3,7-dimethyl-7-octen-1-yl propanoate)	0064	N, T	
Citronellyl butyrate (3,7-dimethyl-6-octen-1-yl butanoate)	0065	N	
Geranyl butyrate (*trans*-3,7-dimethyl-2,6-octadien-1-yl butanoate)	0066	N	
Neryl butyrate (*cis*-3,7-dimethyl-2,6-octadien-1-yl butanoate)	0067	N	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Rhodinyl butyrate (3,7-dimethyl-7-octen-1-yl butanoate)	0068	N	No safety concern
Citronellyl valerate (3,7-dimethyl-6-octen-1-yl pentanoate)	0069	N, T	
Geranyl hexanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl hexanoate)	0070	N, T	
Citronellyl isobutyrate (3,7-dimethyl-6-octen-1-yl 2-methyl propanoate)	0071	N	
Geranyl isobutyrate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl propanoate)	0072	N, T	
Neryl isobutyrate (*cis*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl propanoate)	0073	N	
Rhodinyl isobutyrate (3,7-dimethyl-7-octen-1-yl 2-methyl propanoate)	0074	N, T	
Geranyl isovalerate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 3-methyl butanoate)	0075	N, T	
Neryl isovalerate (*cis*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl butanoate)	0076	N	
Rhodinyl isovalerate (3,7-dimethyl-7-octen-1-yl 3-methylbutanoate)	0077	N, T	
Geranyl 2-ethylbutanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-ethylbutanoate)	0078	N, T	

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.
[b] N, new specifications prepared; NR, specifications not reviewed; R, existing specifications revised; S, specifications exist, revision not considered or not required; T, the existing, new or revised specifications are tentative and comments are invited.
[c] The ADI for this substance was maintained.
[d] Evaluation postponed, pending consideration of other α,β-unsaturated compounds.
[e] Evaluation postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

Contaminant	Toxicological recommendations
Aflatoxins	Potencies were estimated

Substance[a] (considered for specifications only)	No.	Specifications[b]
Flavouring agents		
Allyl cyclohexane propionate (2-propenyl cyclohexane propanoate)	0013	S
Ethyl octanoate	0033	S
Ethyl nonanoate	0034	S
Isoamyl acetate (3-methylbutyl acetate)	0043	R
Isoamyl butyrate (3-methylbutyl butanoate)	0045	S
Isoamyl isobutyrate (3-methylbutyl 2-methylpropanoate)	0049	S
Isoamyl isovalerate (3-methylbutyl 3-methylbutanoate)	0050	S
Food additives		
Agar	—	R
Alginic acid	—	R
Aluminium powder	—	R
Ammonium alginate	—	R

Substance[a] (considered for specifications only)	No.	Specifications[b]
Anoxomer	—	W
Calcium alginate	—	R
Calcium propionate (calcium propanoate)	—	R
Carbon dioxide	—	R
Carthamus red	—	R, T[c]
Carthamus yellow	—	R
Citric acid	—	R
Diacetyltartaric and fatty acid esters of glycerol (DATEM)	—	R
Enzyme-hydrolysed sodium carboxymethyl cellulose	—	N, T[d]
Enzyme-treated starches	—	W
Ethyl hydroxyethyl cellulose	—	R
Gellan gum	—	R
Gum arabic	—	R
Microcrystalline wax	—	R
Mixed carotenoids	—	R
Modified starches	—	R
Petroleum jelly	—	R, T[e]
Potassium alginate	—	R
Potassium propionate (potassium propanoate)	—	R
Propionic acid	—	R, T[f]
Propylene glycol	—	R
Propylene glycol alginate	—	R
Propylene glycol esters of fatty acids	—	R
Sodium alginate	—	R
Sodium propionate (sodium propanoate)	—	R
Sulfur dioxide	—	R
Talc	—	R
Tartaric, acetic and fatty acid esters of glycerol, mixed	—	R, T[g]
Turmeric	—	W

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

[b] N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not required; T, the existing, new or revised specifications are tentative and comments are invited; W, existing specifications withdrawn.

[c] The existing tentative specifications for carthamus red were revised and the "tentative" designation was maintained. The Committee requested information on the content of carthamin and method of assay.

[d] New specifications for enzyme-hydrolysed sodium carboxymethyl cellulose were prepared and designated as "tentative". The Committee requested information on the physical state of the substance as manufactured, the nature and proportion of material of low relative molecular mass present, and tests that distinguish it from sodium carboxymethyl cellulose.

[e] The existing tentative specifications for petroleum jelly were revised and the "tentative" designation was maintained The Committee requested information on the method of analysis and levels of viscosity at 100°C, carbon number at 5% distillation point, average relative molecular mass and oil content. Unless this information was received by 31 March 1998, the specifications would be withdrawn.

[f] The specifications for propionic acid used as a preservative and an antimould and antirope agent were revised and designated as "tentative". The Committee requested information on the method of analysis and levels of readily oxidizable substances.

[g] The Committee compared the specifications for "diacetyltartaric and fatty acid esters of glycerol" (DATEM) with the existing tentative specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and noted that the two substances appear very similar. The Committee has not received information which would allow these substances to be distinguished analytically. It therefore decided to maintain the tentative status of the existing specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and requested data that would distinguish this substance from DATEM. Unless such data were provided by 31 March 1998, the Committee would consider combining the specifications for the two substances.

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Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats

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A series of in vitro and in vivo studies evaluated the potential effects of tobacco flavoring and casing ingredients. Study 1 utilized as a reference control cigarette a typical commercial tobacco blend without flavoring ingredients, and a test cigarette containing a mixture of 165 low-use flavoring ingredients. Study 2 utilized the same reference control cigarette as used in study 1 and a test cigarette containing eight high-use ingredients. The in vitro Ames *Salmonella typhimurium* assay did not show any increase in mutagenicity of smoke condensate from test cigarettes designed for studies 1 and 2 as compared to the reference. Sprague-Dawley rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 wk to smoke from the test or reference cigarettes already described, or to air only, and necropsied after 13 wk of exposure or following 13 wk of recovery from smoke exposure. Exposure to smoke from reference or test cigarettes in both studies induced increases in blood carboxyhemoglobin (COHb) and plasma nicotine, decreases in minute volume, differences in body or organ weights compared to air controls, and a concentration-related hyperplasia, squamous metaplasia, and inflammation in the respiratory tract. All these effects were greatly decreased or absent following the recovery period. Comparison of rats exposed to similar concentrations of test and reference cigarette smoke indicated no difference at any concentration. In summary, the results did not indicate any consistent differences in toxicologic effects between smoke from cigarettes containing the flavoring or casing ingredients and reference cigarettes.

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Flavoring ingredients are added to tobacco during the manufacture of many types of commercial cigarettes, and humectants such as glycerol are added to increase the moisture-holding capacity of the tobacco. There has been much speculation about the effect of these added ingredients on the toxicity of the resultant smoke. Wynder and Hoffman (1967) hypothesized that adding

nontobacco ingredients might increase or decrease the toxic effects of inhaled tobacco smoke, and later publications (LaVoie et al., 1980; Hoffman and Hoffman, 1997, 2001; World Health Organization, 2001) supported that hypothesis. Recently published research results (Gaworski et al., 1998; Paschke et al., 2002; Rodgman, 2002a, 2002b; Rodgman and Green, 2002; Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Baker et al., 2004) have presented data from in vitro, and in vivo toxicity studies that indicate the addition of ingredients to tobacco does not increase the toxicity of the smoke. Baker et al. (2004), using a pyrolysis technique that mimics closely the combustion conditions inside burning cigarettes (Baker and Bishop, 2004), studied the effects of pyrolysis on the chemistry, in vitro genotoxicity and cytotoxicity, and inhalation toxicity in rodents of 291 single ingredients added to cigarettes.

The studies described herein were designed to evaluate the potential influence of low-use flavoring ingredients and high-use mixed casing or flavoring ingredients on the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against an identical reference cigarette respectively produced without flavors or casings.

MATERIALS AND METHODS

Cigarette Design

In study 1, 165 low-use flavoring ingredients were added to a single test cigarette and compared to a reference cigarette without these ingredients. In study 2, eight high-use flavoring or casing ingredients were added to a single test cigarette and compared to the same reference cigarette that was used in study 1. Thus, the design covered these ingredients as well as possible interactions between them and/or their combustion or pyrolysis products. The prototype cigarettes were designed to be representative of commercial, full flavor filter cigarettes. Test and reference cigarettes were constructed with conventional commercial equipment.

The ingredients selected for evaluation in these studies comprise low-use and high-use ingredients normally utilized in the manufacture of commercial cigarettes. The point of addition was chosen to mimic actual process conditions. Study 1 and study 2 ingredients were incorporated into a flavoring or casing system at levels exceeding their normal use. Table 1 outlines the tobacco components of the blend used to construct the cigarettes in both study 1 and study 2. The blends were cased with a mixture of glycerin and water (at a ratio of 2:1) to provide the necessary moisture for standard processing. In preparation of study 1 cigarettes, the ingredients were applied at a rate of 10 kg/1000 kg leaf blend, that is, at 1% on the test cigarettes, and the casing was applied at a rate of 30 kg/1000 kg leaf blend. The study 2 ingredient system was applied at a rate of 31 kg/1000 kg leaf blend (3.1%). The 165 ingredients included in the study 1 mixture appear listed in order of descending application rate in Table 2,

TABLE 1
Blend composition of prototype cigarettes

	Percent of blend component in cigarettes	
Blend components	Tobacco wet weight	Tobacco dry weight
Burley	24	22.9
Virginia	28	25.7
Oriental	14.8	13.6
Reconstituted sheet	23.4	20.1
Expanded tobacco	9.7	8.8

along with the corresponding CAS-Number, regulatory identifiers (where applicable) and application rate. The seven casings and one flavoring included in the study 2 mixture appear listed in order of descending application rate in Table 3. Cellulose acetate filters with 32% average air dilution were used in all cigarettes. Monogram inks were not subject to these studies.

Cigarette Performance

A preliminary cigarette performance evaluation was carried out prior to the toxicology studies. Prior to characterization, the cigarettes were conditioned for a minimum of 48 h at a temperature of $22 \pm 1°C$ and a relative humidity (RH) of $60 \pm 2\%$, in accordance with ISO Standard 3402. Subsequently, the cigarettes were smoked on a 20-port Borgwaldt smoking machine under the conditions stipulated in ISO Standard 3308. Therefore, the puffing regime for mainstream smoke used a 35 ± 0.3 ml puff volume, with 2.0 ± 0.05 s puff duration once every 60 ± 0.5 s. Smoke samples were respectively collected in accordance with the analytical method.

In Vitro Study Design

The mutagenicity of total particulate matter (TPM) in study 1 and 2 cigarettes was investigated using an Ames assay protocol that conformed to OECD Guideline 471. For this purpose, prototype cigarettes containing a mixture of ingredients, reference cigarettes without these ingredients, and 2R4F cigarettes (a standard reference cigarette developed and validated by the University of Kentucky) were smoked on a Borgwaldt RM200 rotary smoking machine under the ISO standard 3308 condition. TPM was collected in a standard fiberglass (Cambridge) trap with dimethyl sulfoxide (DMSO), and the DMSO solution was stored in the dark at $-80°C$ prior to performance of the Ames assay. Each sample was tested with and without S9 metabolic activation in five strains of *Salmonella typhimurium*: TA98, TA100, TA102, TA1535, and TA1537. Evaluation of the Ames assay data was carried out in terms of the mutagenic response, taking into consideration the reproducibly dose-related increase in number of revertants, even if the increase was less than twofold. The mutagenic response to TPM from the reference and test cigarettes was compared using the linear portion of the slope (revertants/mg TPM).

TABLE 2
Ingredients added to test cigarettes in study 1

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Benzyl alcohol	100-51-6	2137	172.515	58c	260
2	Immortelle extract	8023-95-8	2592	182.20	225n	156
3	Coriander oil	8008-52-4	2334	182.20	154n	65
4	Balsam peru resinoid	8007-00-9	2117	182.20	298n	65
5	Anise star oil	8007-70-3	2096	N.A.	238n	65
6	Celery seed oil	89997-35-3	2271	182.20	52n	65
7	Vanillin	121-33-5	3107	182.60	107c	65
8	Potassium sorbate	24634-61-5	2921	182.3640	N.A.	39
9	Propyl *para*-hydroxybenzoate	94-13-3	2951	172.515	N.A.	39
10	Benzoin resinoid	9000-05-9	2133	172.510	439n	26
11	Cedarwood oil	8000-27-9	N.A.	N.A.	252n	26
12	Clary extract	8016-63-5	2321	182.20	415n	26
13	Methylcyclopentenolone	80-71-7	2700	172.515	758c	26
14	Phenethyl alcohol	60-12-8	2858	172.515	68c	26
15	Piperonal	120-57-0	2911	182.60	104c	26
16	Tea extract	84650-60-2	N.A.	182.20	451n	26
17	Vanilla oleoresin	8024-06-4	3106	182.20	474n	26
18	Brandy	N.A.	N.A.	N.A.	N.A.	26
19	*trans*-Anethole	4180-23-8	2086	182.60	183c	19.5
20	Coffee extract	84650-00-0	N.A.	182.20	452n	19.5
21	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	698-10-2	3153	N.A.	2300c	19.5
22	Propionic acid	79-09-4	2924	184.1081	3c	13
23	Acetic acid	64-19-7	2006	184.1005	2c	13
24	Amyl formate	638-49-3	2068	172.515	497c	13
25	Angelica root oil	8015-64-3	2088	182.20	56n	13
26	Beeswax absolute	8012-89-3	2126	184.1973	N.A.	13
27	Benzyl benzoate	120-51-4	2138	172.515	262c	13
28	Benzyl propionate	122-63-4	2150	172.515	413c	13
29	Cardamom oil	8000-66-6	2241	182.20	180n	13
30	beta-Carotene	7235-40-7	N.A.	184.1245	N.A.	13
31	Ethyl acetate	141-78-6	2414	182.60	191c	13
32	Ethyl butyrate	105-54-4	2427	182.60	264c	13
33	Ethyl levulinate	539-88-8	2442	172.515	373c	13
34	Eucalyptol	470-82-6	2465	172.515	182c	13
35	Geranium oil	8000-46-2	2508	182.20	324n	13
36	Labdanum resinoid	8016-26-0	2610	172.510	134n	13
37	Lavandin oil	8022-15-9	2618	182.20	257n	13
38	Maltol	118-71-8	2656	172.515	148c	13
39	Spearmint oil	8008-79-5	3032	182.20	285n	13
40	Ethyl hexanoate	123-66-0	2439	172.515	310c	10.4
41	Acetylpyrazine	22047-25-2	3126	N.A.	2286c	9.1
42	Ethylmaltol	4940-11-8	3487	172.515	692c	9.1
43	Chamomile oil, Roman	8015-92-7	2275	182.20	48n	6.5
44	Citronella oil	8000-29-1	2308	182.20	39n	6.5
45	delta-Decalactone	705-86-2	2361	172.515	621c	6.5
46	gamma-Decalactone	706-14-9	2360	172.515	2230c	6.5
47	Ethyl phenylacetate	101-97-3	2452	172.515	2156c	6.5

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
48	Ethyl valerate	539-82-2	2462	172.515	465c	6.5
49	Ethyl vanillin	121-32-4	2464	182.60	108c	6.5
50	Fennel sweet oil	8006-84-6	2485	182.20	200n	6.5
51	Glycyrrhizin ammoniated	53956-04-0	N.A.	184.1408	N.A.	6.5
52	gamma-Heptalactone	105-21-5	2539	172.515	2253c	6.5
53	3-Hexen-1-ol	928-96-1	2563	172.515	750c	6.5
54	3-Hexenoic acid	1577-18-0	3170	N.A.	2256c	6.5
55	Hexyl alcohol	111-27-3	2567	172.515	53c	6.5
56	Isoamyl phenylacetate	102-19-2	2081	172.515	2161c	6.5
57	Methyl phenylacetate	101-41-7	2733	172.515	2155c	6.5
58	Nerol	106-25-2	2770	172.515	2018c	6.5
59	Nerolidol	142-50-7	2272	172.515	67c	6.5
60	Peruvian (bois de rose) oil	8015-77-8	2156	182.20	44n	6.5
61	Phenylacetic acid	103-82-2	2878	172.515	672c	6.5
62	Pyruvic acid	127-17-3	2970	172.515	19c	6.5
63	Rose absolute	8007-01-0	2988	182.20	405n	6.5
64	Sandalwood oil	8006-87-9	3005	172.510	420n	6.5
65	Sclareolide	564-20-5	3794	N.A.	N.A.	6.5
66	Triethyl citrate	77-93-0	3083	184.1911	N.A.	6.5
67	2,3 5-Trimethylpyrazine	14667-55-1	3244	N.A.	735c	6.5
68	Olibanum absolute	8016-36-2	2816	172.510	93n	6.5
69	delta-Octalactone	698-76-0	3214	N.A.	2195c	6.5
70	2-Hexenal	6728-26-3	2560	172.515	748c	5.2
71	Ethyl octadecanoate	111-61-5	3490	N.A.	N.A.	5.2
72	4-Hydroxy-3-pentenoic acid lactone	591-12-8	3293	N.A.	731c	3.9
73	Methyl 2-pyrrolyl ketone	1072-83-9	3202	N.A.	N.A.	3.9
74	Methyl linoleate (48%) methyl linolenate (52%) mixture	112-63-0 301-00-8	3411	N.A.	713c	3.9
75	Petitgrain mandarin oil	8014-17-3	2854	182.20	142n	3.9
76	Propenylguaethol	94-86-0	2922	172.515	170c	3.9
77	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-2-en-4-one	23696-85-7	3420	N.A.	N.A.	3.9
78	2-Propionyl pyrrole	1073-26-3	3614	N.A.	N.A.	3.9
79	Orange essence oil	8008-57-9	2825	182.20	143n	2.6
80	Benzyl phenylacetate	102-16-9	2419	172.515	232c	2.6
81	2,3-Butanedione	431-03-8	2370	184.1278	752c	1.95
82	2,3,5,6-Tetramethylpyrazine	1124-11-4	3237	N.A.	734c	1.95
83	Hexanoic acid	142-62-1	2559	172.515	9c	1.56
84	Cinnamaldehyde	104-55-2	2286	182.60	102c	1.3
85	Acetophenone	98-86-2	2009	172.515	138c	1.3
86	2-Acetylthiazole	24295-03-2	3328	N.A.	N.A.	1.3
87	Amyl alcohol	71-41-0	2056	172.515	514c	1.3
88	Amyl butyrate	540-18-1	2059	172.515	270c	1.3
89	Benzaldehyde	100-52-7	2127	182.60	101c	1.3
90	Butyl butyrate	109-21-7	2186	172.515	268c	1.3
91	Butyric acid	107-92-6	2221	182.60	5c	1.3
92	Cinnamyl alcohol	104-54-1	2294	172.515	65c	1.3

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
93	DL-Citronellol	106-22-9	2309	172.515	59c	1.3
94	Decanoic acid	334-48-5	2364	172.860	11c	1.3
95	para-Dimethoxybenzene	150-78-7	2386	172.515	2059c	1.3
96	3,4-Dimethyl-1,2-cyclopentanedione	13494-06-9	3268	N.A.	2234c	1.3
97	Ethylbenzoate	93-89-0	2422	172.515	261c	1.3
98	Ethyl heptanoate	106-30-9	2437	172.515	365c	1.3
99	Ethyl isovalerate	108-64-5	2463	172.515	442c	1.3
100	Ethyl myristate	124-06-1	2445	172.515	385c	1.3
101	Ethyl octanoate	106-32-1	2449	172.515	392c	1.3
102	Ethyl palmitate	628-97-7	2451	N.A.	634c	1.3
103	Ethyl propionate	105-37-3	2456	172.515	402c	1.3
104	2-Ethyl-3-methylpyrazine	15707-23-0	3155	N.A.	548c	1.3
105	Genet absolute	8023-80-1	2504	172.510	436n	1.3
106	Geraniol	106-24-1	2507	182.60	60c	1.3
107	Geranyl acetate	105-87-3	2509	182.60	201c	1.3
108	gamma-Hexalactone	695-06-7	2556	172.515	2254c	1.3
109	Hexyl acetate	142-92-7	2565	172.515	196c	1.3
110	Isoamyl acetate	123-92-2	2055	172.515	214c	1.3
111	Isoamyl butyrate	106-27-4	2060	172.515	282c	1.3
112	3,7-Dimethyl-1,6-octadiene-3-ol	78-70-6	2635	182.60	61c	1.3
113	Menthyl acetate	89-48-5	2668	172.515	206c	1.3
114	Methyl isovalerate	556-24-1	2753	172.515	457c	1.3
115	Methyl salicylate	119-36-8	2745	175.105	433c	1.3
116	3-Methylpentanoic acid	105-43-1	3437	N.A.	N.A.	1.3
117	gamma-Nonalactone	104-61-0	2781	172.515	178c	1.3
118	Oakmoss absolute	9000-50-4	2795	172.510	194n	1.3
119	Orris absolute	8002-73-1	N.A.	172.510	241n	1.3
120	Palmitic acid	57-10-3	2832	172.860	14c	1.3
121	Phenethyl phenylacetate	102-20-5	2866	172.515	234c	1.3
122	3-Propylidenephthalide	17369-59-4	2952	172.515	494c	1.3
123	Sage oil	8022-56-8	3001	182.20	61n	1.3
124	alpha-Terpineol	98-55-5	3045	172.515	62c	1.3
125	Terpinyl acetate	80-26-2	3047	172.515	205c	1.3
126	gamma-Undecalactone	104-67-6	3091	172.515	179c	1.3
127	gamma-Valerolactone	108-29-2	3103	N.A.	757c	1.3
128	3-Butylidenphthalide	551-08-6	3333	N.A.	N.A.	1.04
129	Davana oil	8016-03-3	2359	172.510	69n	0.65
130	3,5-Dimethyl-1, 2-cyclopentanedione	13494-07-0	3269	N.A.	2235c	0.65
131	Ethyl cinnamate	103-36-6	2430	172.515	323c	0.65
132	Farnesol	4602-84-0	2478	172.515	78c	0.65
133	Geranyl phenylacetate	102-22-7	2516	172.515	231c	0.65
134	alpha-Irone	79-69-6	2597	172.515	145c	0.65
135	Jasmine absolute	8022-96-6	2598	182.20	245n	0.65
136	Kola nut tincture	68916-19-8	2607	182.20	149n	0.65
137	Linalool oxide	1365-19-1	3746	172.515	N.A.	0.65
138	Linalyl acetate	115-95-7	2636	182.60	203c	0.65
139	*para*-Methoxybenzaldehyde	123-11-5	2670	172.515	103c	0.65

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
140	2-Methylbutyric acid	116-53-0	2695	172.515	2002c	0.65
141	Myristic acid	544-63-8	2764	172.860	16c	0.65
142	gamma-Octalactone	104-50-7	2796	172.515	2274c	0.65
143	Opoponax oil	8021-36-1	N.A.	172.510	313n	0.65
144	Tagetes oil	8016-84-0	3040	172.510	443n	0.65
145	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	3152	N.A.	759c	0.52
146	4-Methylacetophenone	122-00-9	2677	172.515	156c	0.26
147	Isobutyraldehyde	78-84-2	2220	172.515	92c	0.13
148	3-Methylbutyraldehyde	590-86-3	2692	172.515	94c	0.13
149	2,3-Dimethylpyrazine	5910-89-4	3271	N.A.	N.A.	0.13
150	2,5-Dimethylpyrazine	123-32-0	3272	N.A.	2210c	0.13
151	2,6-Dimethylpyrazine	108-50-9	3273	N.A.	2211c	0.13
152	Dimethyltetrahydrobenzofuranone	13341-72-5	3764	N.A.	N.A.	0.13
153	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	3658-77-3	3174	N.A.	536c	0.13
154	4-(*para*-Hydroxyphenyl)-2-butanone	5471-51-2	2588	172.515	755c	0.13
155	alpha-Ionone	127-41-3	2594	172.515	141c	0.13
156	beta-Ionone	8013-90-9	2595	172.515	142c	0.13
157	Isovaleric acid	503-74-2	3102	172.515	8c	0.13
158	Lime oil	8008-26-2	2631	182.20	141n	0.13
159	Mace absolute	8007-12-3	N.A.	182.20	296n	0.13
160	Nutmeg oil	8008-45-5	2793	182.20	296n	0.13
161	Caprylic acid	124-07-2	2799	184.1025	10c	0.13
162	Phenylacetaldehyde	122-78-1	2874	172.515	116c	0.13
163	5,6,7,8-Tetrahydroquinoxaline	34413-35-9	N.A.	N.A.	721c	0.13
164	Thyme oil	8007-46-3	3064	182.20	456n	0.13
165	Valeraldehyde	110-62-3	3098	172.515	93c	0.13

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturers Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

Inhalation Toxicity Study Design

Groups of 30 Sprague-Dawley rats of each sex were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 consecutive weeks to concentrations of 0.06, 0.2, or 0.8 mg/L WTPM of smoke from test cigarettes containing flavoring (study 1) or to flavoring or casing ingredients (study 2). Additional groups of 30 rats/sex were exposed to the same concentrations of smoke from reference cigarettes, similar to the test cigarettes but without the flavoring or casing ingredients (as described above), or to filtered air only (sham controls). This exposure regimen (1 h/day, 5 days/wk) reflects current laboratory practices for animal inhalation studies comparing the effects of smoke from test and reference cigarettes, and does not simulate human usage patterns. However, this difference should not influence the validity of the results.

Each group of 30 rats/sex was subdivided into 2 groups: 20 rats/sex scheduled for necropsy immediately after 13 wk of exposure (interim sacrifice) and up to 10 rats/sex scheduled for necropsy following 13 wk of recovery from smoke exposure (final sacrifice). Target smoke concentrations were 0.06, 0.2, or 0.8 mg WTPM/L for the test and reference cigarettes. An additional group of 30 rats/sex served as sham controls.

Biological endpoints for the 13-wk exposure and 13-wk recovery groups included clinical appearance, body weight, organ weights, and gross and microscopic lesions. Plasma nicotine, COHb, and respiratory parameters were measured periodically during the 13-wk exposure period and clinical pathology parameters were measured at the end of the 13-wk exposure period.

Smoke Generation and Exposure System

Animal exposures were conducted in AMESA exposure units (C. H. Technologies, Westwood, NJ). The smoke exposure machines were designed to contain 30 cigarettes on a smoking head that rotated 1 revolution per minute (Baumgartner and Coggins,

TABLE 3
Ingredients added to study 2 test cigarettes

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Invert sugar	8013-17-0	N.A.	184-1859	N.A.	20,000
2	Block chocolate	N.A.	N.A.	N.A.	N.A.	2,500
3	Plum extract	90082-87-4	N.A.	N.A.	371n	2,200
4	Fig extract	90028-74-3	N.A.	N.A.	198n	2,000
5	Molasse extract and tincture	68476-78-8	N.A.	N.A.	371n	2,000
6	Gentian root extract	97676-22-7	2506	172-510	214n	1,000
7	Lovage extract	8016-31-7	2650	172-510	261n	1,000
8	Peppermint oil	8006-90-4	2848	182-20	282n	250

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturer's Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

1980; Ayres et al., 1990). A vacuum port aligned with, and drew a puff from, one test or reference cigarette at a time as the head rotated. Air was drawn through the vacuum port by a peristaltic pump operating at a flow rate of ~1.05 L/min, creating a 2-s, 35-ml puff through each cigarette once each minute. The smoke vacuum flow rate was regulated by a concentration control unit consisting of a real-time aerosol monitor [(RAM)-1; MIE, Inc., Bedford, MA], a computer, and an electronic flow controller (Emerson Electric Co., Brooks Instrument Division, Hatfield, PA). The computer monitored analog voltage output of the RAM and adjusted the amount of smoke that was drawn from the glass mixing bowl by the flow controller until RAM voltage matched the calculated target voltage. The exposure units contained 3 tiers, each with 24 animal exposure ports. The exposure ports were connected to a delivery manifold, which transferred smoke to the animal breathing zone, and to an outer concentric manifold that drew the exhaled and excess smoke to an exhaust duct. Each cigarette was retained for seven puffs.

Exposure Atmosphere Characterization

The protocol-prescribed limits for the smoke concentration (WTPM/L) were target ±10% coefficient of variation (%CV). Smoke exposure concentrations were continuously monitored with a RAM at a representative exposure port. Mean exposure concentration was calculated from the mass collected on the filter and the total volume of air drawn through the filter, which was determined by the sample time and flow rate. RAM voltage readings were recorded during filter sample collection and were used to calculate a RAM response factor for subsequent exposures.

Two filters per exposure group per week were chemically analyzed for total nicotine. Nicotine standard reference material (98%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The WTPM:nicotine and CO:nicotine ratios were calculated for the exposure atmospheres. The concentration of CO in the test and reference atmospheres was determined using Horiba PIR-2000 CO analyzers (Horiba Instruments, Inc., Irvine, CA), monitored by DOS-based computers.

Particle size distribution of the smoke was measured using Mercer-style cascade impactors designed specifically for the size range of particles found in cigarette smoke. The mass collected on each impactor stage was analyzed gravimetrically for WTPM and the resulting data were interpreted by probit analysis (NEWCAS; Hill et al., 1977) to obtain the particle size distribution, mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD). Temperature and RH of the exposure atmospheres were measured from a representative animal exposure port once every 2 wk for each exposure group.

Animals and Animal Care

Sprague-Dawley (Crl:CD) rats 4–5 wk of age were purchased from Charles River Laboratories (Raleigh, NC), held for 13 days in quarantine status prior to initial smoke exposure. Health screens were performed following group assignment and at 24 days after arrival. These health evaluations included necropsy, microscopic examination of selected tissues and examination for parasites. The 24 days after arrival screening included serological testing for antibodies to common viral pathogens. Viral antibody testing was also performed on sera collected from 10 sentinel rats at the end of the 13-wk exposure period and from another 10 at the end of the recovery period. All sera were tested for antibodies to Sendai virus, Kilham's rat virus (KRV)/Toolan's H-1 virus, pneumonia virus of mice (PVM), rat corona virus/sialodacryoadenitis virus, and *Mycoplasma pulmonis*. During the 13-wk exposure period, the animals were housed in individual stainless-steel cages on open racks. During the recovery period, the animals were housed in individual polycarbonate cages (Lab Products, Maywood, NJ) bedded with

ALPHA-dri alpha cellulose bedding (Sheperd Specialty Papers, Kalamazoo, MI). The cage space met the requirements stated in the current *Guide for Care and Use of Laboratory Animals* (National Academy of Sciences, 1996).

Body Weight and Clinical Observations

All rats were observed twice daily for mortality and moribundity. Each rat was examined every 4 wk for clinical signs. Individual body weights were measured during the randomization procedure, on exposure day 1, biweekly thereafter, and at necropsy.

Respiratory Function Measurements

Tidal volume (TV), respiratory rate (RR), and minute volume (MV), derived from flow signals from spontaneously breathing animals, were measured in 4 rats/sex/group during wk 2, 8, and 13 using whole-body phethysmography (Coggins et al., 1981). Each animal was monitored once during a single exposure period. MV and the actual WTPM were used to estimate the average total inhaled mass for the 1-h exposure period for each animal.

Carboxyhemoglobin and Plasma Nicotine Determinations

During wk 2 and 10, blood was collected from designated animals at the end of the 1-h smoke exposure. Animals were removed from the exposure unit and bleeding was initiated within ~5 min. The blood samples were obtained from the retro-orbital plexus of carbon dioxide (CO_2)-anesthetized animals into tubes containing potassium ethylenediaminete traacetic acid (K^+-EDTA). The sample tubes were immediately placed into an ice bath and maintained under these conditions until analyzed for blood carboxyhemoglobin (COHb). Plasma nicotine was quantitatively determined using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring.

Clinical Pathology

On the day of the 13-wk interim sacrifice, the rats were anesthetized with ~70% CO_2 in room air and blood samples were obtained from the retro-orbital plexus. One sample was collected in a tube (Monoject, Sherwood Medical, St. Louis, MO) containing K^+-EDTA for hematologic determinations. Another sample was collected in a tube devoid of anticoagulant but containing a separator gel (Vacutainer, Franklin Lakes, NJ) for serum chemistry analysis. The following parameters were determined using an Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) multiparameter hematology instrument: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, volume of packed red cells (VPRC), the red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), platelet count, and WBC differential counts. Results of the differential cell counts were reported as both relative and absolute values. Reticulocytes were stained supravitally with new methylene blue and enumerated as reticulocytes per 1000 enthrocytes using the Miller disc method (Brecher and Schneiderman, 1950).

A Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN) chemistry analyzer was used to determine the following serum analytes: urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), sodium, potassium, chloride, calcium, phosphorus, total bilirubin, cholesterol, and triglycerides.

Necropsy and Tissue Collection

A complete necropsy was done on all 13-wk exposure groups and 13-wk recovery group animals. Rats designated for scheduled sacrifices or sacrificed due to moribund condition were weighed and anesthetized with 70% CO_2 in air, followed by exsanguination before cessation of heartbeat. All abnormalities were recorded on the individual animal necropsy forms. Lungs, liver, kidneys, testes, adrenals, spleen, brain, and heart from all scheduled sacrifice animals were weighed. These organ weights and the body weights at necropsy were used to calculate organ:body weight ratios. In addition, organ:brain weight ratios were calculated. The time from removal of the organ until weighing was minimized to keep tissues moist.

A complete set of over 40 tissues was removed from each animal at necropsy and examined. All tissues were fixed in 10% neutral buffered formalin (NBF) except for the eyes, which were fixed in Karnovsky's fixative. After the lungs were weighed, they were perfused with 10% NBF at 25 cm hydrostatic pressure.

Histopathology

All tissues were fixed in 10% NBF for a minimum of 48 h before being trimmed. Paraffin blocks were microtomed at 5 μm. All sections were stained with hematoxylin and eosin (H&E) stains for standard histopathologic evaluation of morphologic changes. Duplicate slides of nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS/AB) stains for evaluation of goblet cell populations. The lungs, nasal cavity (four sections), nasopharynx, larynx (three cross sections), trachea (three transverse sections), tracheobronchial lymph nodes, mediastinal (thymic) lymph nodes, heart, and all gross lesions were examined microscopically. The lungs were sectioned to present a maximal section of the mainstem bronchi. The nasal cavity was prepared in four sections using the landmarks described by Young (1981). Three transverse laryngeal sections were prepared from the base of the epiglottis, the ventral pouch, and through the caudal larynx at the level of the vocal folds (Renne et al., 1992). In addition, sections of brain, adrenals, spleen, liver, kidneys, and gonads from animals in the sham control and the groups exposed to 0.8 mg/L of smoke from the test or reference cigarettes were examined microscopically. Exposure-related microscopic lesions were observed in the tissues from the rats exposed to 0.8 mg/L; target organs were examined microscopically in the lower concentration groups to ascertain a no-effect concentration.

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

Cell proliferation rates were measured on respiratory tract tissues collected from 10 rats of each sex from each exposure group and the sham controls necropsied immediately after 13 wk of exposure, using a monoclonal antibody to 5-bromo-2′-deoxyuridine (BrdU). Tissues evaluated using the BrdU assay included the respiratory epithelium lining the median nasal septum and distal portions of maxillary and nasal turbinates, the transitional epithelium at the base of the epiglottis, the luminal epithelium dorsolateral to the ventral pouch, the luminal epithelium lining the cranial trachea, the luminal epithelium of the mainstem bronchi and adjacent bronchioles, and selected areas of alveolar epithelium. Data from both sides of bilaterally symmetrical tissues (nose, ventral pouch, mainstem bronchi) were combined for tabulation of results.

Statistical Methods

Body weight, body weight gain, organ:body weight, and organ:brain weight ratios were statistically analyzed for each sex by exposure concentration group using the Xybion PATH/TOX system. Data homogeneity was determined by Bartlett's test. Dunnett's t-test was performed on homogeneous data to identify differences between each concentration group and the sham control group, and between corresponding concentrations of test and reference cigarette smoke-exposed groups. Nonhomogeneous data were analyzed using a modified t-test. Respiratory physiology, clinical pathology, COHb, and plasma nicotine data parameters were statistically evaluated using SAS software (Statistical Analysis System, SAS, Inc., Cary, NC). One-way analysis of variance (ANOVA) between exposure groups was first conducted, followed by Bartlett's test for homogeneity of variance. A two-sided Dunnett's multiple comparison test was employed to determine which exposure groups were different from the controls. An unpaired two-sided t-test was used to compare equivalent exposure groups between cigarette types. Differences were considered significant at $p \leq .05$. The statistical evaluation of incidence and severity of lesions was made using the Kolmogorov–Smirnov two-sample test (Siegel, 1956). All treatment group means were compared to the sham control mean, and means of groups exposed to the test cigarette smoke were compared to the corresponding reference cigarette smoke-exposed group means. Cell proliferation data were compared statistically using Tukey's studentized range test with SAS software.

RESULTS

Cigarette Performance

The results of characterization of the test and reference cigarettes for study 1 and study 2 are presented in Tables 4 and 5. These results show that the filler weight and the number of puffs per cigarette, nicotine yield, and nicotine-free dry particulate matter (NFDPM) were comparable for test and reference cigarettes in both studies. The yields of nicotine and NFDPM and the puff count were also comparable. These results are consistent with the negligible differences in the configuration of both prototype cigarettes, which basically consist of the total relative amount of flavor ingredient contained in the test cigarettes (1% or 3% of the filler weight). A comparison of the burning rates in study 1 illustrates that the addition of the ingredients had little, if any effect on the burning characteristics of the test cigarettes.

TABLE 4
Key parameters for laboratory control of prototype study 1 cigarettes

Parameter	Target	Run average: Test cigarette	Run average: Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	0.963	0.965
Standard deviation	—	0.019	0.018
Non tobacco weight	0.212	0.212	0.215
Net tobacco	0.800	0.751	0.750
Air dilution (%)	32	35	34.1
Standard deviation	—	3.0	3.1
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	49	49
Expanded tobacco (%)	9.7	10.1	9.1
Nicotine (mg/cig)	0.9	0.92	0.97
Nicotine (mg/puff)	n.a.	0.118	0.123
NFDPM (mg/cig)	12.0	11.3	11.5
NFDPM (mg/puff)	n.a.	1.45	1.46
CO (mg/cig)	n.a.	12.4	13.1
CO (mg/puff)	n.a.	1.59	1.66
Puffs/cigarette	n.a.	7.8	7.9
Burning rate (mg tobacco/min)	n.a.	68.1	64.4

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

Figures 1, 2, 3, and 4 summarize the results of Ames assays on test cigarettes from study 1 and 2 with and without metabolic activation. TA100, TA98, and TA1537 strains showed a positive response only with metabolic activation. No response was observed in TA 102 or TA1535. No sporadic responses in revertants were recorded. The highest sensitivity and specificity of the mutagenic response were observed using TA98 with metabolic activation. From the comparison of the data obtained for the test and reference cigarettes, it was concluded that the addition of ingredients did not result in a positive mutagenic response in any of the strains under the conditions already described. Hence, the use of the tested ingredients had no influence on the mutagenic activity of the cigarettes.

TABLE 5
Key parameters for laboratory control of prototype study 2 cigarettes

Parameter	Target	Run average	
		Test cigarette	Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	1.002	1.025
Standard deviation	—	0.0208	0.0173
Nontobacco weight	0.212	0.212	0.212
Net tobacco	0.800	0.790	0.813
Air dilution (%)	32	33.2	36.6
Standard deviation	—	1.6	1.4
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	50	47
Expanded tobacco (%)	9.5	9.6	9.3
Nicotine (mg/cig)	0.9	0.93	0.93
Nicotine (mg/puff)	n.a.	0.112	0.107
NFDPM (mg/cig)	12.0	11.4	11.0
NFDPM (mg/puff)	n.a.	1.37	1.26
CO (mg/cig)	n.a.	12.9	12.8
CO (mg/puff)	n.a.	1.55	1.47
Puffs/cigarette	n.a.	8.3	8.7

Note. Cig, cigarette.

Exposure Atmosphere Characterization

Tables 6 and 7 summarize the exposure data for the inhalation exposure periods for study 1 and study 2. The mean exposure concentrations (WTPM) were all within 3% of the target concentration, with CVs of 6.6%, or less. Nicotine and CO concentrations correlated well with WTPM in reference and test cigarette smoke atmospheres in both study 1 and study 2. Particle sizes were slightly larger in the study 1 test and reference cigarette smokes. All concentrations of the smoke from each cigarette were highly respirable for the rat model under investigation.

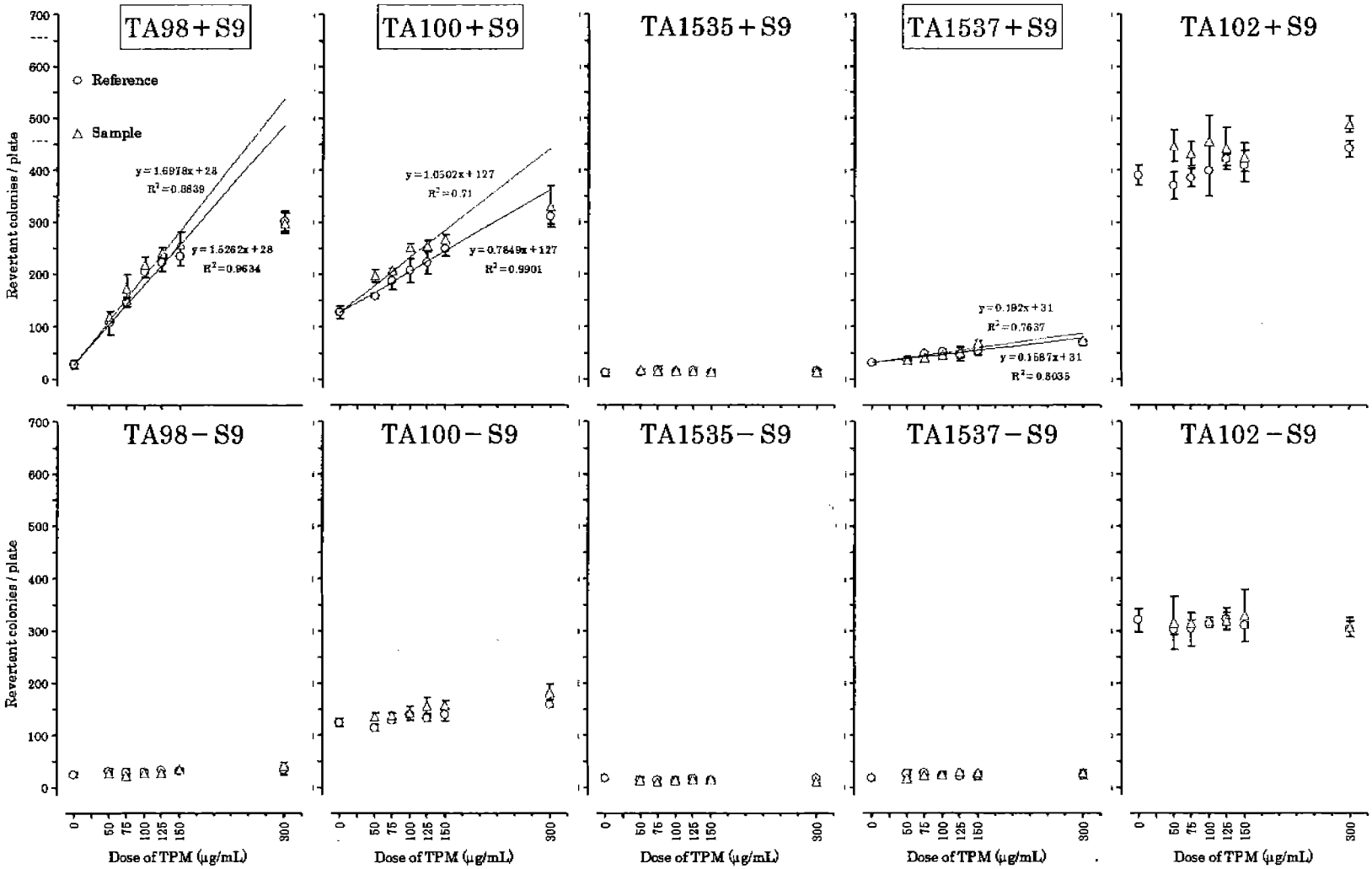
Body Weights and Clinical Observations

No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

Mean body weight data for all groups on study throughout the exposure and recovery periods are illustrated in Figure 5. In study 1, mean body weights were consistently decreased compared to sham controls during the exposure period in male rats exposed to 0.8 mg/L of reference cigarette smoke and in males exposed to all 3 concentrations of test cigarette smoke. With the exception of day 71 (0.8 mg/L test), all female smoke-exposed groups in study 1 were comparable to sham control females throughout the study. In study 2, mean body weights were consistently decreased compared to sham controls in males exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.8 mg/L of reference cigarette smoke. Mean body weights of smoke-exposed groups were similar to sham control weights during the recovery period of both study 1 and study 2. The only consistent statistical difference in body weight changes between the test and reference cigarette smoke-exposed groups in either study was the decreased mean body weight in males exposed to 0.8 mg/L of reference cigarette smoke during the exposure period of study 1.

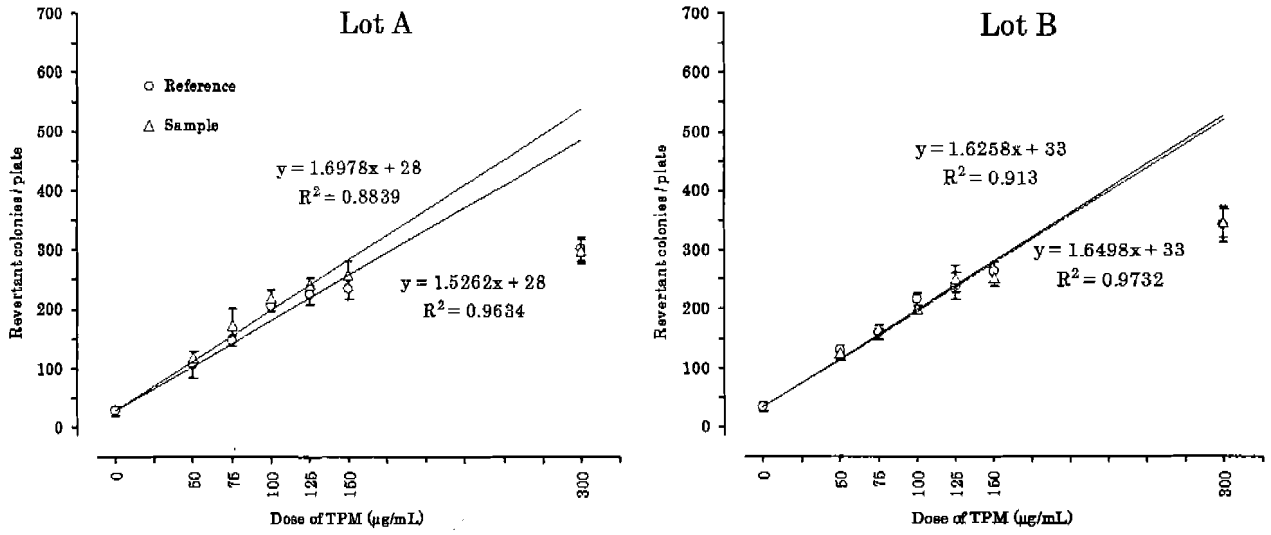
Organ Weights

Comparisons of selected group mean organ weights between smoke-exposed and sham controls in study 1 are presented in Table 8. Statistically significant differences in organ weights in groups of smoke-exposed rats were primarily low mean organ weights compared to their respective sham controls. There was no clear pattern of differences in any absolute or relative organ weight in smoke-exposed groups compared to sham controls, or in groups exposed to test versus reference cigarette smoke at either the interim sacrifice or the recovery sacrifices. Sham controls for the interim sacrifice of study 2 were inadvertently not fasted overnight prior to necropsy, which made comparison of absolute and relative organ weights of smoke-exposed and sham control groups from the interim sacrifice of questionable scientific value; thus these comparisons were not made for study 2. Statistical comparison of absolute and relative organ weights between groups exposed to test and reference cigarette smoke in study 2 showed very few statistically significant differences, none of which were considered toxicologically



N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot.

FIG. 1. Ames assay results, study 1 cigarettes.



MEAN ± SD of Specific Activity (50 to 150 µg/plate)

Reference	1576 ± 141.9	Reference.....	1734 ± 170.9
Sample.....	1783 ± 167.3	Sample.....	1703 ± 151.2

FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

significant. Comparison of organ weights in rats necropsied following the 13-wk recovery of study 2 indicated no consistent differences between sham control and smoke-exposed groups, or between groups exposed to similar concentrations of test and reference cigarette smoke.

Respiratory Physiology

Reductions in RR and/or TV resulted in consistently lower MV in rats exposed to test or reference cigarette smoke compared to sham controls in both study 1 and study 2. There was no consistent difference in MV between groups of rats exposed to test and reference cigarette smoke in either study. Because the overall MV in study 1 was similar among groups exposed to smoke, total inhaled mass was proportional to increasing smoke concentration in this study. In study 2, decreases in MV in groups exposed to 0.8 or 0.2 mg/L compared to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to the exposure concentration of inhaled smoke.

Clinical Pathology

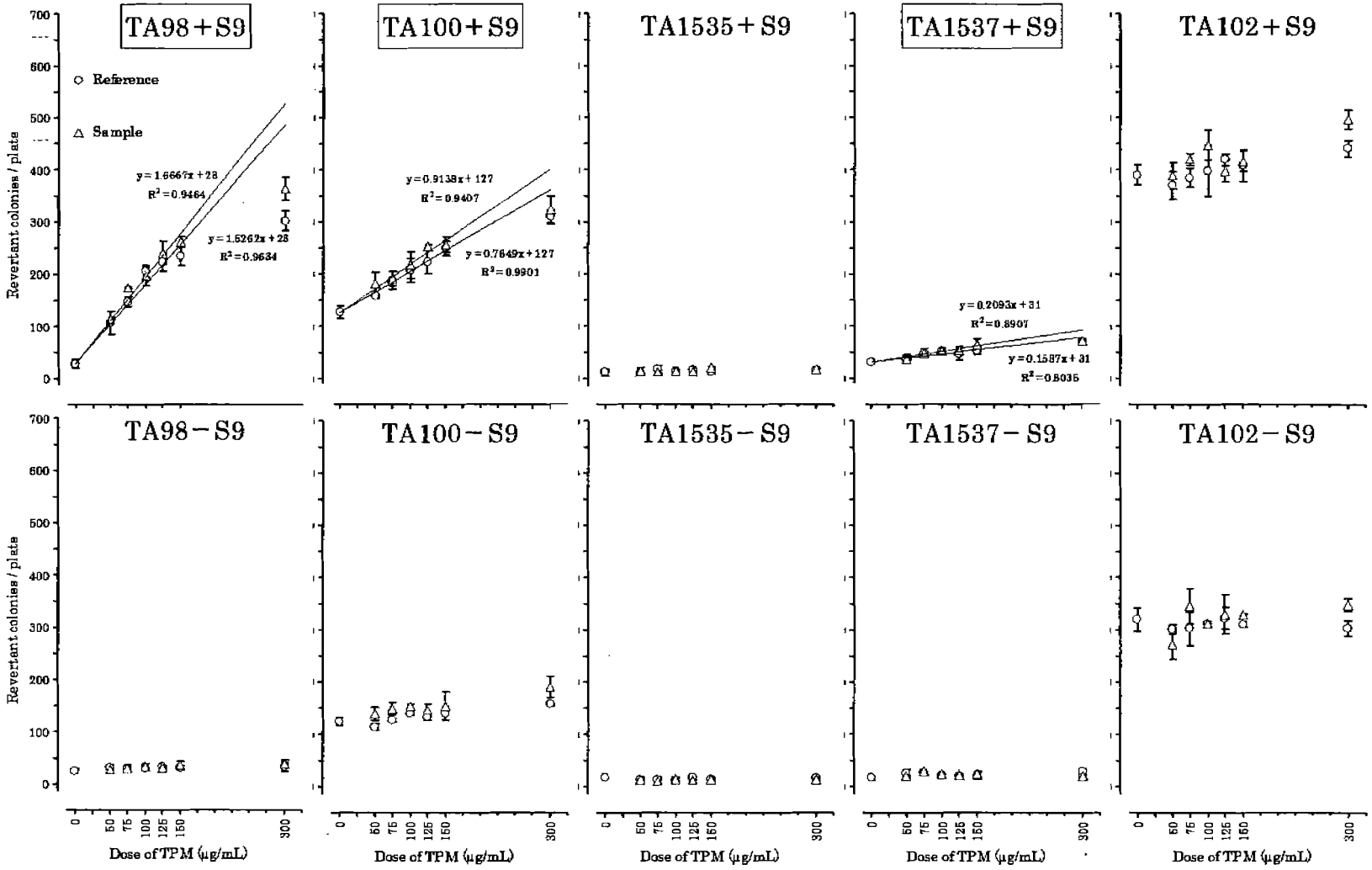
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 standard deviations of historic values for control Sprague-Dawley rats of comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure related.

Whole-blood COHb levels were increased in a graded dose-response fashion as a function of exposure concentration for all test and reference cigarette smoke-exposed groups in both studies. In study 2 rats bled during exposure wk 2, there was a statistically significant decrease in COHb levels in both sexes exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.2 mg/L of test cigarette smoke, compared to groups exposed to reference cigarette smoke. There were no other clear differences in whole blood COHb levels between the test and reference cigarette groups at equivalent exposure levels in either study.

Plasma nicotine levels increased in a graded dose-response fashion for test and reference males and female groups in both studies. In study 2, test female groups exposed to 0.8 mg/L had significantly lower plasma nicotine levels than the 0.8 mg/L reference females at both 2- and 10-wk sampling. Comparing males to females at all exposure levels for test and reference cigarettes, the females consistently had higher plasma nicotine levels in both studies.

Pathology

Few gross lesions were observed in either study, with no evidence of changes attributable to exposure to smoke from the test



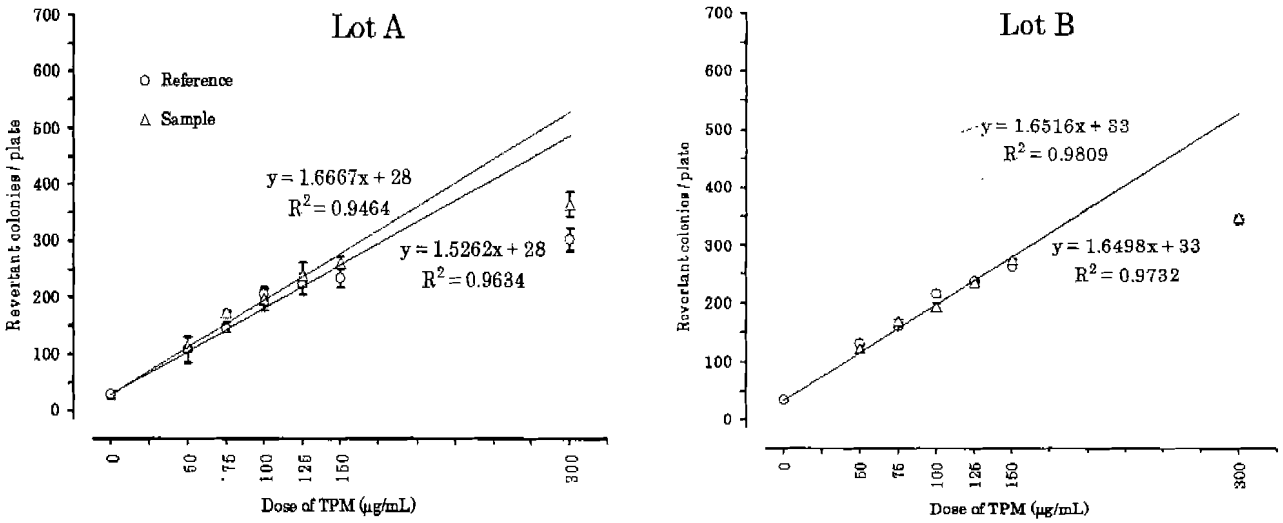
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

FIG. 3. Ames assay results, study 2 cigarettes.

TABLE 6
Study 1, exposure concentration data for rats exposed to mainstream smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 126$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 63$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.800	0.787 ± 0.035 (4.4)	68.2 ± 2.5 (3.7)	584 ± 27 (4.6)	98.4 ± 4.3	0.73 ± 0.08
0.200	0.199 ± 0.009 (4.5)	15.5 ± 1.0 (6.5)	144 ± 6 (4.2)	99.3 ± 4.3	0.74 ± 0.12
0.060	0.061 ± 0.004 (6.6)	4.4 ± 0.5 (11.4)	47 ± 3 (6.4)	101 ± 6	0.69 ± 0.09
Reference target exposure concentration (mg WTPM/L)					
0.800	0.795 ± 0.023 (2.9)	70.1 ± 2.1 (2.9)	608 ± 20 (3.3)	99.4 ± 2.7	0.74 ± 0.08
0.200	0.202 ± 0.004 (2.0)	15.8 ± 0.7 (4.5)	147 ± 4 (2.7)	101 ± 2	0.72 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.4 ± 0.4 (9.8)	50 ± 2 (4.8)	100 ± 4	0.74 ± 0.10

Note. CO, carbon monoxide; WTPM, wet total particulate matter.



MEAN±SD of Specific Activity (50 to 150 µg/plate)

Reference.....	1576±141.9	Reference.....	1734±170.9
Sample.....	1726±138.6	Sample-1.....	1701±107.9

FIG. 4. Ames assay results, study 2 cigarettes with TA98 metabolic activation.

TABLE 7
Study 2, exposure concentration data for rats exposed to smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 134$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 67$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.8	0.798 ± 0.040 (5.0)	56.8 ± 2.6 (4.6)	646 ± 34 (5.3)	100 ± 5	0.65 ± 0.01
0.2	0.194 ± 0.007 (3.6)	12.9 ± 0.6 (4.7)	158 ± 9 (5.7)	97 ± 4	0.62 ± 0.04
0.060	0.060 ± 0.002 (3.3)	4.0 ± 0.2 (5.0)	54 ± 3 (5.6)	100 ± 3	0.66 ± 0.03
Reference target exposure concentration (mg WTPM/L)					
0.8	0.784 ± 0.031 (4.0)	55.1 ± 2.3 (4.2)	676 ± 31 (4.6)	98 ± 4	0.57 ± 0.03
0.2	0.201 ± 0.004 (1.8)	13.0 ± 0.4 (3.4)	170 ± 15 (8.7)	100 ± 2	0.64 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.1 ± 0.2 (4.4)	57 ± 3 (5.8)	99 ± 3	0.66 ± 0.06

Note. CO, carbon monoxide; WTPM, wet total particulate matter.

or the reference cigarettes. Exposure to smoke from reference or test cigarettes in both studies induced concentration-related proliferative, metaplastic, and inflammatory microscopic lesions in the respiratory tract after 13 wk of exposure. The incidence of exposure-related respiratory-tract lesions observed at microscopic examination of tissues from rats necropsied at the interim sacrifice immediately following 13 wk of exposure is summarized in Table 9 for study 1 and Table 10 for study 2.

Hyperplasia of respiratory epithelium lining the anterior nasal cavity was present in all rats exposed to 0.8 mg/L in both studies, a few rats exposed to 0.2 mg/L in both studies, and in 3/40 rats exposed to 0.06 mg/L in study 1. Areas most severely and most frequently affected were the distal portions of the nasal and maxillary turbinates in sections of nose just caudal to the incisor teeth. In affected rats, the epithelium in the distal turbinates was up to six cells thick. There was also a clear dose response in the severity of nasal respiratory epithelial hyperplasia, with severity ranging from minimal to moderate. Comparison of incidence and severity data for nasal respiratory epithelial hyperplasia in rats exposed to similar concentrations of smoke from the test and reference cigarettes did not indicate any statistically significant differences in either study. Minimal goblet-cell hyperplasia was observed in the mucosal epithelium lining the median nasal septum in some smoke-exposed and sham control rats. Although not statistically significant compared to concurrent sham controls, the incidence of nasal goblet cell hyperplasia in male rats exposed to the 0.8-mg/L concentration of smoke from the reference cigarette or test cigarette in study 1 were considered to be toxicologically significant. There was no clear difference in the incidence of goblet cell hyperplasia between groups exposed to similar concentrations of reference and test cigarette smoke in either study.

Exposure to smoke from the reference or test cigarette in both study 1 and study 2 induced squamous metaplasia, hyperplasia, and hyperkeratosis of the transitional epithelium lining the base of the epiglottis and the epithelium lining the dorsal border of the ventral pouch and the adjacent laryngeal lumen. In control rats, the epithelium lining the base of the epiglottis was a mixture of ciliated columnar epithelium and slightly flattened, oval, rounded, or cuboidal cells one or two cells thick over a poorly defined basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, the base of the epiglottis was covered by a stratified squamous epithelium up to eight cells thick with a variably keratinized surface layer and a distinct basal cell layer. There was a concentration-related increase in severity of squamous metaplasia and hyperplasia of epiglottis epithelium in rats exposed to test or reference cigarette smoke. Statistical analysis did not indicate any significant differences in incidence or severity of these lesions between test and reference cigarette smoke-exposed groups in either study. Hyperkeratosis (accumulation of keratinized squamous cells on the surface) was observed in association with squamous metaplasia of the epithelium lining the base of the epiglottis in most rats exposed to smoke from reference or test cigarettes. Comparison of incidence/severity of hyperkeratosis in the epiglottis between test and reference cigarette smoke-exposed groups indicated a statistically

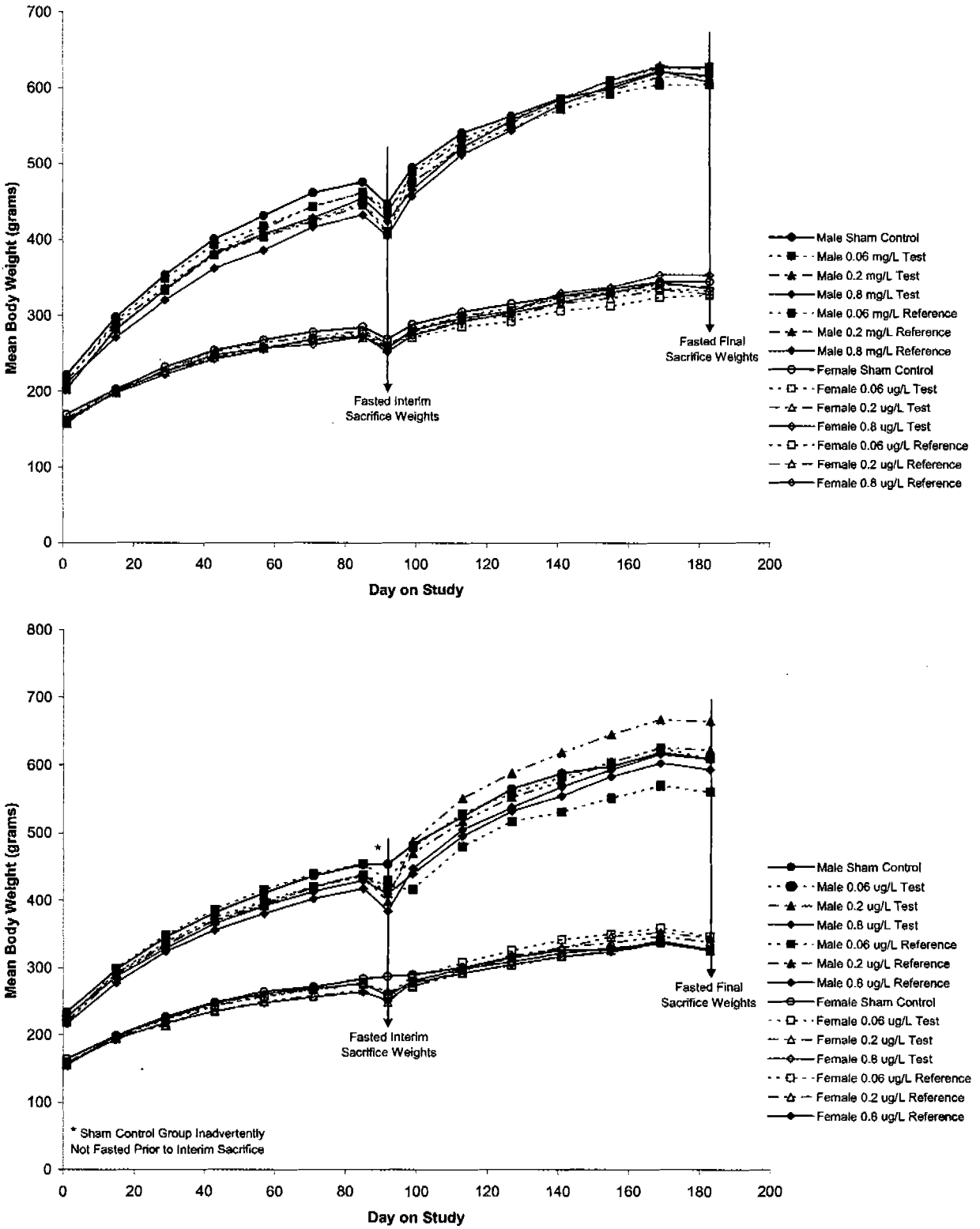


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

TABLE 8
Organ weights for rats exposed to smoke from study 1 cigarettes ($n = 20$, g ± SD)

		Test			Reference		
	Sham control	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L
Males							
Heart	1.60 ± 0.16	1.48 ± 0.15[a,b]	1.43 ± 0.16[a,c]	1.55 ± 0.15	1.60 ± 0.13	1.57 ± 0.16	1.52 ± 0.15
Kidneys	3.39 ± 0.33	3.17 ± 0.39	2.92 ± 0.30[a,c]	3.05 ± 0.33[a]	3.38 ± 0.33	3.20 ± 0.31	3.02 ± 0.27[a]
Lungs	1.95 ± 0.22	1.89 ± 0.17	1.82 ± 0.23[c]	1.93 ± 0.14	2.02 ± 0.28	1.98 ± 0.26	1.89 ± 0.15
Adrenals	0.066 ± 0.010	0.066 ± 0.012	0.059 ± 0.010	0.064 ± 0.012	0.062 ± 0.007	0.064 ± 0.008	0.063 ± 0.008
Females							
Heart	1.06 ± 0.09	1.02 ± 0.10	1.00 ± 0.10[c]	1.05 ± 0.12	1.03 ± 0.09	1.07 ± 0.09	1.09 ± 0.12
Kidneys	2.18 ± 0.21	2.02 ± 0.24	1.90 ± 0.19[a]	1.93 ± 0.18[a]	2.04 ± 0.21	1.99 ± 0.19[a]	1.95 ± 0.19[a]
Lungs	153 ± 0.13	1.50 ± 0.13	1.52 ± 0.17[c]	1.52 ± 0.15	1.55 ± 0.14	1.50 ± 0.17	1.60 ± 0.19
Adrenals	0.080 ± 0.010	0.081 ± 0.011	0.078 ± 0.008	0.082 ± 0.012	0.078 ± 0.008	0.080 ± 0.010	0.081 ± 0.013

[a] $p < .05$, Dunnett's t-test of significance, compared to sham control.
[b] $p < .05$, Dunnett's t-test of significance, compared to 0.06 reference group.
[c] $p < .05$, Dunnett's t-test of significance, compared to 0.2 reference group.

significant difference only in the 0.06-mg/L groups from study 1, in which females exposed to test cigarette smoke had a higher incidence/severity than females exposed to reference cigarette smoke. Chronic inflammation was present in the submucosa of the epiglottis in some rats exposed to reference or test cigarette smoke in study 1, most frequently in rats exposed to the 0.8 mg/L smoke concentration. Squamous metaplasia, hyperplasia, and hyperkeratosis were also present in the epithelium lining the opening of the ventral pouch and the adjacent laryngeal lumen in most rats exposed to smoke from the test or reference cigarette in both studies. In control rats, the epithelium lining the opening of the ventral pouch and adjacent laryngeal lumen was slightly flattened, oval, rounded, or cuboidal cells one or two cells thick with no discernible basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, this area was covered by a stratified squamous epithelium from three to six cells thick with a variably keratinized surface layer and a distinct basal cell layer. Comparison of incidence/severity of lesions at this site between test and reference cigarette smoke-exposed groups did not indicate any statistically significant differences in either study. Minimal or mild squamous metaplasia of the mucosal epithelium lining the caudal larynx was observed in 2/20 rats exposed to the 0.8 mg/L concentration of smoke from the test cigarette and 1/20 rats exposed to the 0.8 mg/L concentration of smoke from the reference cigarette in study 1.

Exposure to smoke from reference or test cigarettes induced a dose-related increase in minimal hyperplasia of the mucosal epithelium lining the tracheal lumen in both sexes of rats in study 1 and in males in study 2. Comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences in either study.

There were increased numbers of macrophages diffusely scattered through the pulmonary alveoli of rats exposed to smoke from reference or test cigarettes in both studies, compared to concurrent controls. There was some evidence of a dose response in the incidence and severity of macrophage accumulation in alveoli of smoke-exposed rats. This increase was graded as minimal in the vast majority of affected rats. Comparison of incidence and severity data for macrophages in alveoli of rats exposed to smoke from the test and reference cigarettes did not indicate any statistically significant differences. Minimal goblet-cell hyperplasia was observed in AB/PAS-stained sections of the mainstem bronchi of some rats exposed to smoke from reference or test cigarettes in both studies. There was some evidence of a dose response in the incidence of this lesion. Analysis of data indicated a statistically significant increase compared to controls in rats of both sexes exposed to the 0.8 mg/L concentration of smoke from reference cigarettes and in female rats exposed to the 0.8-mg/L concentration of smoke from the test cigarette in study 1, and in both sexes exposed to 0.8 mg/L of reference cigarette smoke in study 2. The incidence (7/20) of goblet-cell hyperplasia in males exposed to the 0.8-mg/L concentration of smoke from the test cigarette in both studies, although not statistically significant, was considered to be toxicologically significant. The incidence of bronchial goblet-cell hyperplasia was slightly higher in male rats exposed to smoke from reference cigarettes compared to similar concentrations of smoke from test cigarettes, but comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences. There was a very low incidence of a variety of microscopic lesions in other tissues examined in both studies, with no evidence of an effect of exposure to smoke from the reference or test cigarette on these tissues.

TABLE 9
Study 1, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	2 (0.2)	4 (0.3)	20 (2.2)	1 (0.1)	8 (0.4)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	6 (0.3)	3 (0.2)	9 (0.5)	5 (0.3)	5 (0.3)	10 (0.5)
Suppurative inflammation	2 (0.2)	2 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	9 (0.5)	20 (1.4)	19 (1.9)	16 (0.9)	20 (1.8)	20 (1.9)
Ventral pouch, squamous metaplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	9 (0.6)	19 (1.4)	1 (0.2)	17 (1.4)	18 (1.5)
Chronic inflammation	0 (0.0)	2 (0.1)	8 (0.4)	16 (0.9)	0 (0.0)	4 (0.2)	13 (0.7)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	6 (0.3)	6 (0.3)	18 (0.9)	5 (0.3)	12 (0.6)	16 (0.8)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	15 (0.8)	14 (0.7)	20 (1.4)	8 (0.4)	11 (0.6)	20 (1.1)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	7 (0.4)	3 (0.2)	4 (0.2)	11 (0.6)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	7 (0.4)	20 (2.0)	0 (0.0)	3 (0.2)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	2 (0.1)	2 (0.1)	7 (0.4)	2 (0.1)	2 (0.1)	4 (0.2)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	19 (1.4)[c]	20 (2.2)	20 (2.2)	13 (0.7)	20 (2.0)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	15 (1.3)	20 (1.8)	1 (0.1)	18 (1.5)	18 (1.5)
Chronic inflammation	0 (0.0)	3 (0.2)	2 (0.2)	10 (0.6)	0 (0.0)	4 (0.2)	17 (1.0)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	2 (0.1)	8 (0.4)	12 (0.6)	3 (0.2)	7 (0.4)	18 (0.9)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	10 (0.5)	13 (0.7)	20 (1.2)	12 (0.6)	17 (0.9)	20 (1.3)
Bronchi, goblet-cell hyperplasia	0 (0.0)	2 (0.1)	3 (0.2)	10 (0.5)	1 (0.1)	4 (0.2)	13 (0.7)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.
[c]$p < .05$, Kolmogorov–Smirnov test, compared to 0.06-mg/L reference group.

TABLE 10
Study 2, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	2 (0.1)	20 (2.0)	0 (0.0)	4 (0.2)	20 (1.9)
Goblet-cell hyperplasia	2 (0.1)	3 (0.2)	3 (0.2)	3 (0.2)	3 (0.2)	4 (0.2)	3 (0.2)
Suppurative inflammation	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	6 (0.4)	15 (1.2)	20 (2.0)	13 (1.0)	20 (1.8)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.4)	16 (1.2)	0 (0.0)	5 (0.4)	16 (1.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	2 (0.1)	8 (0.4)	9 (0.5)	11 (0.6)	6 (0.3)	8 (0.4)	10 (0.5)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	4 (0.2)	11 (0.6)	16 (0.9)	20 (1.4)	11 (0.6)	14 (0.7)	20 (1.4)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	4 (0.2)	0 (0.0)	1 (0.1)	9 (0.5)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	4 (0.2)	20 (1.5)	0 (0.0)	4 (0.2)	20 (1.6)
Goblet-cell hyperplasia	3 (0.2)	3 (0.2)	5 (0.3)	5 (0.3)	5 (0.3)	2 (0.1)	8 (0.4)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, hyperkeratosis	0 (0.0)	16 (1.0)	20 (2.0)	20 (2.2)	15 (0.9)	20 (1.6)	20 (2.4)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	15 (1.2)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	14 (1.1)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.5)	18 (1.4)	0 (0.0)	9 (0.6)	20 (1.7)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	0 (0.0)	1 (0.1)	2 (0.1)	2 (0.1)	1 (0.1)	2 (0.1)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	9 (0.5)	10 (0.5)	19 (1.1)	10 (0.5)	10 (0.5)	17 (1.0)
Perivascular lymphoid infiltrate	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)	3 (0.2)	4 (0.2)	10 (0.5)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.

Examination of tissue sections from rats necropsied at the end of the recovery period demonstrated nearly complete regression of nasal and tracheal lesions and a substantial decrease in the incidence and severity of smoke-induced lesions in the larynx and lungs in rats exposed to smoke from test or reference cigarettes in both studies. Macrophages observed in alveoli of smoke-exposed and control recovery group rats were in small focal aggregates, as opposed to the diffuse distribution of macrophages in lungs of rats necropsied at the interim sacrifice. There was no statistically significant difference in the incidence or severity of respiratory-tract lesions between recovery group rats previously exposed to similar concentrations of test and reference cigarette smoke in either study.

Evaluation of Cell Proliferation Rates

There was a dose-related trend toward higher mean nuclear labeling rates in the epithelium lining the median nasal septum in groups exposed to progressively higher concentrations of test or reference cigarette smoke compared to sham controls, but the increases were statistically significant only in females exposed to 0.8 mg/L of test cigarette smoke in study 1 and males exposed to 0.8 mg/L of reference cigarette smoke in study 2. Mean nuclear labeling rates of nasal epithelium lining the distal portions of the nasal and maxillary turbinates were statistically increased compared to control rates in both sexes of rats exposed to 0.8 mg/L of smoke from the test or reference cigarettes in both studies. Mean labeling rates in nasal and maxillary turbinates of study 1 males exposed to 0.8 mg/L of test cigarette smoke were statistically increased compared to labeling rates at these sites in males exposed to the same concentration of reference cigarette smoke.

Mean nuclear labeling rates in laryngeal epithelium were increased compared to sham control groups at all dose levels in both studies. Labeling rates in laryngeal epithelium were statistically different between several test and reference cigarette smoke-exposed groups in both studies, with no clear trend. The histopathology findings of laryngeal epithelial hyperplasia in smoke-exposed rats confirmed the relative sensitivity of these laryngeal sites to smoke-induced hyperplastic changes.

Mean nuclear labeling rates in the tracheal epithelium of rats exposed to smoke from test or reference cigarettes were not clearly different from those of sham controls of the same sex in either study. Labeling rates of bronchial, bronchiolar, and alveolar epithelium in both studies were difficult to evaluate due to wide standard deviations, low labeling rates, and variable sample sizes, and therefore labeling data from these sites were not used in evaluating effects of smoke exposure.

DISCUSSION

The studies described here were designed to evaluate the potential influence of ingredients on the chemical composition and the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against reference cigarettes identical except produced without flavors or casings. The configuration and ISO-condition tar, nicotine, and CO yields of all cigarettes investigated are representative of American blend cigarettes. Both test and reference cigarettes had the same tobacco blend and humectant composition (glycerine plus water) and were prepared by the same manufacturing process. Similarly, identical nontobacco materials (NTM) were used throughout. The weight of the filler remained constant between test and reference cigarettes. These studies illustrate that the application of 165 low-use flavoring or 8 high-use flavoring or casing ingredients had little, if any, observable effect on the deliveries or physical parameters of the cigarettes.

From comparison of the mutagenicity data obtained in Ames assays of studies 1 and 2 test and reference cigarettes, it was concluded that the addition of these ingredients did not increase the mutagenic response of any of the strains of *Salmonella typhimurium* under the conditions described, and the results did not suggest any mutagenic activity of the added ingredients.

The objectives of the two inhalation toxicity studies were to compare the biologic activity of mainstream smoke from the two test cigarettes with reference cigarettes in a series of two 13-wk inhalation exposures, each followed by a 13-wk recovery period. Data collected during the 13-wk exposures confirmed that both the particulate (WTPM, nicotine) and vapor (CO) phases of the inhalation atmospheres presented to the rats were well controlled and provided appropriate data for comparison of the responses of the study animals to smoke from the two cigarettes under investigation in each of the two studies. WTPM was used as the basis for exposure concentration in these studies, since the predominant known toxicologic effects of cigarette smoke are associated with the mainstream particulate phase (Coggins et al., 1980).

Blood COHb concentrations demonstrated that exposure of rats to smoke from either the test or reference cigarette resulted in reproducible biomarkers of exposure consistent with the concentration of CO in the smoke. Samples taken for plasma nicotine analysis confirmed exposure to nicotine in test or reference smoke, which resulted in exposure-related increases in plasma nicotine concentrations.

The only occurrence during either study that affected the utility of the data was the failure to fast the sham control rats prior to necropsy at the interim sacrifice immediately following the exposure period in study 2. This error did not allow direct comparison of the body and organ weights of controls with smoke-exposed groups sacrificed at that time point.

Other investigations have noted effects similar to those we observed of cigarette smoke exposure on body weight, including the relative resistance of females to this change (Coggins et al., 1989; Baker et al., 2004). We concluded that the decreased body weights in smoke-exposed groups in both studies compared to sham controls were the result of smoke exposure. However, we do not consider these effects on body weight to be toxicologically significant due to their recovery after smoke exposure was terminated, and due to the lack of any concurrent clinical observations that would indicate any significant dysfunction.

In study 1 there were a number of statistically significant differences in absolute or relative organ weights between test or reference cigarette smoke-exposed groups and sham controls necropsied immediately following 13 wk of smoke exposure. However, these statistical differences showed no clear dose-response pattern, and no exposure-related histopathologic effects were observed in any weighed organ except the lungs. It is possible that the increased lung/body weight ratios in study 1 rats exposed to 0.8-mg/L of smoke from test or reference cigarettes were related to the minimal increase in numbers of macrophages in alveoli of these rats. These increases in lung/body weight ratio more likely reflect the decreased body weight in these groups at the interim sacrifice. In any case, these and the other statistical differences in absolute or relative organ weights in smoke-exposed rats compared to sham controls are not considered toxicologically significant. There was no consistent difference in organ weights between groups of rats exposed to similar concentrations of test and reference cigarette smoke in either study. Increases in total inhaled mass were proportional to increasing exposure concentration in study 1, but in study 2 decreases in MV in groups exposed to 0.8- or 0.2-mg/L relative to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to exposure concentration of smoke.

Inhalation exposure to smoke from test or reference cigarettes in both studies clearly induced microscopic changes in the nasal cavity, larynx, trachea, and lungs of exposed rats. Results of histopathologic examination of the recovery groups illustrated that these respiratory-tract lesions were either completely resolved or in the process of resolving by 13 wk after cessation of smoke exposure, and thus represent an adaptive response to the inhaled smoke. The nasal cavity and larynx were much more affected by inhaled smoke than the lungs in our studies, and the mucosal epithelium lining the base of the epiglottis and adjacent ventral pouch was the most affected site. The extreme susceptibility of the rodent laryngeal mucosa to inhaled smoke and other xenobiotics has been described in detail (Lewis, 1980, 1991; Gopinath et al., 1987; Burger et al., 1989). Since the most notable cellular changes observed in the respiratory tract of rodents in response to inhaled smoke involve cellular proliferation and metaplasia, a quantitative measure of cell turnover in affected tissue is a useful tool to measure the effect of exposure. Cell proliferation rate measurements in nasal turbinates and laryngeal epithelium using nuclear labeling with BrdU correlated well with histopathology data, reinforcing the conclusion that exposure to smoke from test or reference cigarette smoke for 13 wk clearly induced epithelial hyperplasia at these sites. Results of BrdU labeling in the trachea and lungs were less clear, and probably reflect the more subtle effects of inhaled smoke on the epithelium at these sites.

The effects of inhaled cigarette smoke on the respiratory tract of rats in both the studies described herein are similar to those described in a number of previously reported cigarette smoke inhalation studies in rats (Dalbey et al., 1980; Gaworski et al., 1997; Coggins et al., 1989; Ayres et al., 2001; Vanscheeuwijck et al., 2002) and hamsters (Lewis, 1980; Wehner et al., 1990). Four recently published papers have described studies similar to those presented here, in which smokes from cigarettes with and without flavoring or casing ingredients were compared on the basis of chemical composition and biologic effects on rodents (Gaworski et al., 1998; Paschke et al., 2002; Carmines, 2002; Baker et al., 2004). Results of the studies presented here are consistent with the conclusions of these authors that the presence of flavoring and casing ingredients studied to date did not significantly change the type or extent of toxicologic effects observed in rodents inhaling cigarette smoke.

REFERENCES

Ayres, P., Mosberg, A. T., and Coggins, C. R. 1990. Modernization of nose-only smoking machines for use in animal studies. *J. Am. Coll. Toxicol.* 9:441–446.

Ayres, P. H., Hayes, J. R., Higuchi, M. A., Mosberg, A. T., and Sagartz, J. W. 2001. Subchronic inhalation by rats of mainstream smoke from a cigarette that primarily heats tobacco compared to a cigarette that burns tobacco. *Inhal. Toxicol.* 13:149–186.

Baker, R. R., and Bishop, L. J. 2004. The pyrolysis of tobacco ingredients. *J. Anal. Appl. Pyrol.* 71:223–311.

Baker, R. R., Massey, E. H., and Smith, G. 2004. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chem. Toxicol.* 42:S53–S83.

Baumgartner, H., and Coggins, C. R. E. 1980. Description of a continuous-smoking inhalation machine for exposing small animals to tobacco smoke. *Beitr. Tabakforsch. Int.* 10:169–174.

Brecher, G., and Schneiderman, M. 1950. A time-saving device for the counting of reticulocytes. *Am. J. Clin. Pathol.* 20:1079.

Burger, G. T., Renne, R. A., Sagartz, J. W., Ayres, P. H., Coggins, C. R. E., Mosberg, A. T., and Hayes, A. W. 1989. Histologic changes in the respiratory tract induced by inhalation of xenobiotics: Physiologic adaptation or toxicity? *Toxicol. Appl. Pharmacol.* 101:521–542.

Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Food Chem. Toxicol.* 40:77–91.

Coggins, C. R. E., Fouillet, X. L., Lam, R., and Morgan, K. T. 1980. Cigarette smoke induced pathology of the rat respiratory tract. A comparison of the effects of the particulate and vapor phases. *Toxicology* 16:83–101.

Coggins, C. R. E., Duchosal, F., Musy, C., and Ventrone, R. 1981. The measurement of respiratory patterns in rodents, using whole body plethysmography and pneumotachography. *Lab. Anim.* 15:137–140.

Coggins, C. R. E., Ayres, P. H., Mosberg, A. T., and Burger, G. T. 1989. Comparative inhalation study in rats, using a second prototype of a cigarette that heats rather than burns tobacco. *Inhal. Toxicol.* 1:197–226.

Dalbey, W. E., Nettesheim, P., Griesemer, R., Caton, J. E., and Guerin, M. R. 1980. Chronic inhalation of cigarette smoke by F344 rats. *J. NCI.* 64:383–390.

Gaworski, C. L., Dozier, M. M., Gerhart, J. M., Rajendran, N., Brennecke, L. H., Aranyi, C., and Heck, J. D. 1997. 13-wk inhalation study of menthol cigarette smoke. *Food Chem. Toxicol.* 35:683–692.

Gaworski, C. L., Dozier, M. M., Heck, J. D., Gerhart, J. M., Rajendran, N., David, R. M., Brennecke, L. H., and Morrisey, R. 1998. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-wk inhalation exposures in rats. *Inhal. Toxicol.* 10:357–381.

Gopinath, C., Prentice, D. E., and Lewis, D. J. 1987. *Atlas of experimental toxicologic pathology*. Lancaster, PA: MTP Press.

Hill, M. A., Watson, C. R., and Moss, O. R. 1977. *NEWCAS—An interactive computer program for particle size analysis*. PNL-2405. Richland, WA: Battelle Pacific Northwest Laboratories.

Hoffman, D., and Hoffman, I. 1997. The changing cigarette, 1950–1995. *J. Toxicol. Environ. Health* 50:307–364.

Hoffman, D., and Hoffman, I. 2001. The changing cigarette: chemical studies and bioassays. In *National Cancer Institute (NCI) Monograph 13, Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine*, pp. 159–191. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Cancer Institute, Bethesda, MD, USA.

LaVoie, E. J., Hecht, S. S., Hoffman, D., and Wynder, E. L. 1980. The less harmful cigarettes and tobacco smoke flavours. In *Banbury Report 3, A Safe Cigarette?* eds. G. B. Gori and F. G. Back, pp. 251–260. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Lewis, D. J. 1980. Factors affecting the distribution of tobacco smoke-induced lesions in rodent larynx. *Toxicol. Lett.* 9:189–194.

Lewis, D. J. 1991. Morphologic assessment of pathological changes within the rat larynx. *Toxicol. Pathol*, 19:352–357.

National Academy of Sciences. 1996. *Guide for the care and use of laboratory animals*. Washington, DC: Institute of Laboratory Animal Resources, Commission on Life Sciences, National Reserch Council. National Academy Press.

Paschke, T., Scherer, G., and Heller, W. F. 2002. Effects of ingredients on cigarette smoke composition and biological activity: A literature review. *Beitr. Tabakforsch. Int./Contrib. Tobacco Res.* 20:107–247.

Renne, R. A., Gideon, K. M., Miller, R. A., Mellick, P. W., and Grumbein, S. L. 1992. Histologic methods and interspecies variations in the laryngeal histology of F344/N rats and B6C3F1 mice. *Toxicol. Pathol.* 20:44–51.

Rodgman, A. 2002a. Some studies of the effects of additives on cigarette mainstream smoke properties. I. Flavorants. *Beitr. Tabakforsch. Int.* 20:83–103.

Rodgman, A. 2002b. Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials. *Beitr. Tabakforsch. Int.* 20:279–299.

Rodgman, A., and Green, C. R. 2002. Toxic chemicals in cigarette mainstream smoke—Hazard and hoopla. *Beitr. Tabakforsch. Int.* 20:481–545.

Roemer, E., Tewes, F. J., Mesigen, T. J., Veltel, D. J., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. *Food Chem. Toxicol.* 40:105–111.

Rustemeier, K., Stabbert, R., Haussmann, H. J., Roemer, E., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food Chem. Toxicol.* 40:93–104.

Siegel, S. 1956. *Non-parametric statistics for the behavioral sciences*. New York: McGraw-Hill.

Vanscheeuwijck, P. M., Teredesai, A., Terpstra, P. M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food Chem. Toxicol.* 40:113–131.

Wehner, A. P., Renne, R. A., Greenspan, B. J., DeFord, H. S., Ragan, H. A., Westerberg, R. B., Wright, C. W., Buschbom, R. L., Burger, G. T., Hayes, A. W., Coggins, C. R. E., and Mosberg, A. T. 1990. Comparative subchronic inhalation bioassay in hamsters of a cigarette that only heats tobacco. *Inhal. Toxicol.* 2:255–284.

World Health Organization. 2001. *Advancing knowledge on regulating tobacco products*, pp. 40–46. Geneva: WHO.

Wynder, E. L., and Hoffman, D. 1967. *Tobacco and tobacco smoke. Studies in experimental carcinogenesis*, pp. 526–528. New York: Academic Press.

Young, J. T. 1981. Histopathologic examination of the rat nasal cavity. *Fundam. Appl. Toxicol.* 1:309–312.

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3):

Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 63 flavouring substances in the Flavouring Group Evaluation 10, including additional two substances in this Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. For one substance [FL-no: 10.170] a concern for genotoxicity could not be ruled out. The remaining 62 substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the 62 substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For four substances evaluated through the Procedure, the stereoisomeric composition has not been specified sufficiently.

1 On request from the Commission, Question No EFSA-Q-2011-01010, EFSA-Q-2010-01554, adopted on 2 February 2012.

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KEYWORDS

Flavourings, safety, lactones, saturated, unsaturated, primary, secondary, alcohols, aldehydes, acids, acetals, esters, additional oxygenated functional group, FGE.10.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 63 flavouring substances in the Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These flavouring substances belong to chemical groups 9, 13 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.10, FGE.10Rev3, includes the assessment of two additional candidate substances [FL-no: 09.951 and 10.170].

The flavouring substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-six of the candidate substances possess one or more chiral centres and eight can exist as geometrical isomers due to the presence and the position of a double bond. For five of these substances [FL-no: 10.038, 10.040, 10.059, 10.063 and 10.170] the stereoisomeric composition / composition of mixture has not been specified sufficiently.

Fifty-five candidate substances belong to structural class I, six belong to structural class II, and two belong to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifty of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intakes" (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

The candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the two candidate substances assigned to structural class III have estimated European daily *per capita* intakes of 0.011 and 1.2 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

The combined estimated daily *per capita* intake as flavourings of the 55 candidate substances assigned to structural class I is 1600 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. Likewise, the combined estimated daily *per capita* intake as flavouring of the six candidate substances assigned to structural class II is 1.2

microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

For 5-pentyl-3H-furan-2-one [FL-no: 10.170], the flavour Industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], which is an alpha, beta-unsaturated alcohol (after hydrolysis of the lactone), allocated to subgroup 4.1 of FGE.19 (FGE.217). The Panel concluded that 5-pentyl-3H-furan-2-one [FL-no: 10.170] should not be evaluated through the Procedure until the additional gentoxicity data for [FL-no: 10.054] are available, as stated in FGE 217.

The Panel reconsidered the fact that 1-hydroxypropan-2-one [FL-no: 07.169] is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person, corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore decided that further genotoxicity data are not required and that the substance could be taken through the Procedure.

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern. So, 62 substances are evaluated through the Procedure in the present revision of FGE.10.

It can be anticipated that, at the estimated levels of intake as flavouring substances, 59 of the alcohols, aldehydes, acetals, carboxylic acids and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products, many of which are endogenous in humans. For three of the flavouring substances [FL-no: 02.242, 06.097 and 09.824], it cannot be concluded that they are metabolised to innocuous products. Adequate margins of safety could be established for these three substances in step B4 of the Procedure.

It was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was considered that on the basis of the default MSDI approach that the flavouring substances, to which the Procedure have been applied, would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

The mTAMDI for the flavouring substances, for which use levels information is available, range from 800 to 5100 microgram/person/day. For 58 of these substances the mTAMDI is above the threshold of concern of their structural classes and for three substances the mTAMDI is below the threshold. The three flavouring substances which have mTAMDI intake estimates below the threshold of concern for their structural class are also expected to be metabolised to innocuous products. For two flavouring substances use levels have not been provided and no mTAMDI could be estimated. Thus, for 60 flavouring substances, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

Thus, in conclusion, 62 of the 63 flavouring substances were evaluated through the Procedure (based on the MSDI approach), as one flavouring substance, 5-pentyl-3H-furan-2-one [FL-no: 10.170] could not be evaluated through the Procedure until adequate genotoxicity data become available.

In order to determine whether the conclusion for the candidate substances evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 58 flavouring substances. For four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] information on composition of mixture and / or stereoisomerism has not been specified sufficiently. For one substance [FL-no: 10.063] an identity test is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 10.038, 10.040, 10.059 and 10.063], pending further information.

For the remaining 58 candidate substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.088, 06.090, 06.095, 06.097, 06.102, 06.135, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874, 09.916, 09.951, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.068 and 10.168] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

KEYWORDS

Flavourings, safety, lactones, saturated, unsaturated, primary, secondary, alcohols, aldehydes, acids, acetals, esters, additional oxygenated functional group, FGE.10.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

The first version of the Flavouring Group Evaluation 10 (FGE.10) dealt with 51 alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones.

The first revision of FGE.10, FGE.10Rev1, included the assessment of eight additional candidate substances [FL-no: 06.088, 06.095, 06.102, 06.135, 09.565, 09.916, 10.040 and 10.168] and additional information on 32 substances [FL-no: 02.132, 02.198, 02.242, 06.090, 06.097, 07.169, 08.090, 09.333, 09.349, 09.360, 09.502, 09.580, 09.590, 09.601, 09.629, 09.633, 09.644, 09.683, 09.815, 09.824, 09.832, 09.862, 09.874, 10.038, 10.039, 10.043, 10.045, 10.048, 10.049, 10.052, 10.058 and 10.068] which had become available since the first FGE. Furthermore, substance [FL-no: 10.043], which can be metabolised to an alpha, beta-unsaturated ketone, was withdrawn from FGE.10Rev1 to be evaluated together with other alpha, beta-unsaturated ketones in FGE.217 (EFSA, 2008b).

The second revision of FGE.10 concerned the assessment of three additional candidate substances [FL-no: 08.113, 10.059 and 10.063] as well as additional information submitted by the Industry on the stereoisomeric composition/composition of mixture requested in FGE.10Rev1 for eight substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168], and identity information for [FL-no: 06.088 and 06.095].

FGE	Opinion adopted by EFSA	Link	No. Of candidate substances
FGE.10	28 October 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1232_en.html	51
FGE.10Rev1	30 January 2008	http://www.efsa.europa.eu/en/efsajournal/pub/934.htm	58
FGE.10Rev2	23 March	http://www.efsa.europa.eu/en/efsajournal/pub/2164.htm	61

	2011		
FGE.10Rev3	1 February 2012		63

The present revision of FGE.10, FGE.10Rev3, includes the assessment of two additional candidate substances [FL-no: 09.951 and 10.170]. No toxicity or metabolism data were provided for these two substances. A search in open literature was conducted for metabolism, genotoxicity, repeated dose toxicity as well as reproductive/developmental toxicity for [FL-no: 09.951 and 10.170]. This search did not reveal any pertinent new information on the two substances.

FGE.10Rev3 also include additional information submitted by the Industry on specifications for [FL-no: 06.135 and 08.113] which had been requested in FGE.10Rev2.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letters of the 30th July 2010 and 20th September 2010, the Commission requested EFSA to carry out an evaluation of the flavouring substances 5-pentyl-3H-furan-2-one [FL-no: 10.170] and dioctyl adipate [FL-no: 09.951], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 10, Revision 3

1.1. Description

The present Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 63 alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The flavouring substances (candidate substances) under consideration are listed in Table 1, as well as their chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications.

The outcome of the Safety Evaluation is summarised in Table 2a.

Fifteen candidate substances are aliphatic lactones [FL-no: 10.038, 10.039, 10.040, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068, 10.168 and 10.170]; thirty-two candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874, 09.916 and 09.951]; six candidate substances are acetals [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135]; one candidate substance is an alpha-hydroxyacid

[FL-no: 08.090]; one candidate substance is a ketoalcohol [FL-no: 07.169]; one candidate substance is an alkoxy-alcohol [FL-no: 02.242]; two candidate substances are diols [FL-no: 02.132 and 02.198]; one candidate substance is a dialdehyde [FL-no: 05.149] and four candidate substances are aliphatic dicarboxylic acids [FL-no: 08.053, 08.082, 08.103 and 08.113].

The hydrolysis products of candidate esters, lactones and acetals as well as their evaluation status are listed in Table 2b.

The candidate substances are structurally related to 29 aliphatic lactones (supporting substances) evaluated at the 49th JECFA meeting (JECFA, 1998a) and to 47 aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups evaluated at the 53rd JECFA meeting (JECFA, 2000c). These supporting substances are listed in Table 3, together with their evaluation status.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Thirty-six of the substances possess one or more chiral centres [FL-no: 02.132, 02.198, 06.088, 06.090, 06.095, 06.135, 08.090, 09.333, 09.346, 09.349, 09.360, 09.502, 09.580, 09.590, 09.601, 09.629, 09.633, 09.644, 09.683, 09.815, 09.824, 09.832, 09.862, 09.874, 09.916, 10.038, 10.039 10.040, 10.045, 10.048, 10.049, 10.052, 10.058, 10.068, 10.168 and 10.170]. For thirty-five substances the stereoisomeric composition has been specified. For [FL-no: 10.170] the Industry has informed that the commercial substance is a mixture of two structural isomers. One of these isomers possesses a chiral centre for which the configuration has not been specified.

Due to the presence and the position of a double bond, eight substances can exist as geometrical isomers [FL-no: 09.350, 09.351, 09.565, 10.038, 10.039, 10.040, 10.059 and 10.063]. For four of the substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition / composition of stereoisomeric mixture has not been specified sufficiently. Industry has stated that [FL-no: 10.038 and 10.040] exist as mixtures of (Z)- and (E)-isomers (EFFA, 2010a), however, the composition of the isomeric mixtures have to be provided.

1.3. Natural Occurrence in Food

Fifty of the flavouring substances have been reported to occur in one or more of the following food items: fruits (apple, pineapple, melon, guava, banana, starfruit, papaya, raspberry, mango, plum, citrus), oats, chestnut, juice, butter, meat, cheese, milk and milk products, skimmed milk powder, green tea, coffee, beer, wine and whisky.

Quantitative data on the natural occurrence in food have been reported for thirty-eight of the candidate substances (TNO, 2000; TNO, 2010). These reports include:

1.3.1 Candidate substances reported to occur in food (TNO, 2000; TNO, 2010)

FL-no:	Name:	Quantitative data reported:
02.198	Octane-1,3-diol	Up to 21 mg/kg in apple and up to 95.1 mg/kg in apple juice
02.242	2-Butoxyethan-1-ol	0.02 mg/kg in mozzarella cheese
06.088	2-Ethyl-4-methyl-1,3-	Up to 2 mg/kg in port wine
06.095	4-Methyl-2-propyl-1,3-	Up to 2 mg/kg in port wine
06.097	1,1,3-Triethoxypropane	Up to 3 mg/kg in pear brandy and less than 0.8 mg/kg in whisky
06.135	2-Isobutyl-4-methyl-1,3-dioxolane	Up to 2 mg/kg in port wine
07.169	1-Hydroxypropan-2-one	Up to 4 mg/kg in coffee
08.103	Nonanedioic acid	Up to 1.5 mg/kg in beer
09.590	Isobutyl lactate	20 mg/kg in port wine
09.916	Ethyl 3-hydroxyoctanoate	Up to 0.05 mg/kg in papaya, 0.02 mg/kg in orange juice and 0.03
10.045	Heptano-1,5-lactone	Up to 0.4 mg/kg in green tea
10.047	Hexadecano-1,16-lactone	0,0145 mg/kg in skimmed milk powder
10.048	Hexadecano-1,4-lactone	Up to 16.7 mg/kg in heated butter
10.049	Hexadecano-1,5-lactone	Up to 10.6 mg/kg in butter and up to 1.3 mg/kg in heated lamb and mutton fat

According to TNO, 13 of the substances have not been reported in any food items. These substances are listed in Table 1.3.1 (TNO, 2000; TNO, 2010):

1.3.1 Candidate substances not reported to occur in food (TNO, 2000; TNO, 2010)

FL-no:	Name:
06.102	2-Hexyl-5-hydroxy-1,3-dioxane
08.113	Succinic acid, disodium salt
09.502	Ethyl butyryl lactate
09.633	Methyl 5-hydroxydecanoate
09.644	Methyl lactate
09.824	Ethyl 2-acetylbutyrate
09.832	Ethyl 3-acetohexanoate
09.833	iso-Propyl 4-oxopentanoate
09.874	Di(2-methylbutyl) malate
10.040	Dec-8-eno-1,5-lactone
10.059	Hexadec-7-en-1,16-lactone
10.063	Hexadec-9-en-1,16 lactone
10.068	Pentadecano-1,14-lactone

2. Specifications

Purity criteria for the substances have been provided by the Flavouring Industry (EFFA, 2003c; EFFA, 2004ag; Flavour Industry, 2011a; Flavour Industry, 2010g; Flavour Industry, 2010n; Flavour Industry, 2011g) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for 62 substances. For one substance [FL-no: 10.063] an identity test is missing.

Furthermore, for five substances [FL-no: 10.038, 10.040, 10.059, 10.063 and 10.170], the stereoisomeric composition has not been specified sufficiently (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) (SCF, 1999) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

The total annual volumes of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 13220kg (EFFA, 2000c; EFFA, 2003d; EFFA,

[4] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

2008b; Flavour Industry, 2010g; Flavour Industry, 2010n). For the 60 of the 76 supporting substances the annual volume of production is 357000 kg (JECFA, 1999b; JECFA, 2000b).

On the basis of the annual volumes of production reported for the candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a).

98 % of the total annual volume of production for the candidate substances is accounted for by three substances, succinic acid disodium salt [FL-no: 08.113], hexadec-9-en-1,16-lactone [FL-no: 10.063] and diethyl maleate [FL-no: 09.351]. The estimated daily *per capita* intake of succinic acid disodium salt from use as a flavouring substance is 1500 microgram, that of hexadec-9-en-1,16-lactone is 48 microgram and that of diethyl maleate is 12 microgram. The daily *per capita* intakes for each of the remaining substances are less than 10 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For 61 candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n). For two substances [FL-no: 06.135 and 08.113] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000.

The candidate substances, for which use levels have been provided, are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the candidate substances, for which use levels have been provided, are in the range of 1 - 101 mg/kg food, and the maximum use levels are in the range of 5 - 1005 mg/kg (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n).

[5] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[7] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances in Various Food Categories for 61 Candidate Substances for which Data on Use have been provided.

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	All except [FL-no: 09.951]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 09.951]
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 09.951]
04.1	Processed fruits	All except [FL-no: 09.951]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 10.170]
05.0	Confectionery	All except [FL-no: 09.951]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 09.951]
07.0	Bakery wares	All except [FL-no: 09.951]
08.0	Meat and meat products, including poultry and game	All except [FL-no: 10.170]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 08.090, 09.551 and 10.170]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 06.095, 09.551 and 09.644]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 06.095, 09.551, 09.644 and 10.170]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 09.951]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 09.951]
15.0	Ready-to-eat savouries	All except [FL-no: 09.951]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All

* Information on use levels has not been provided for [FL-no: 06.135 and 08.113]

The mTAMDI values for the 54 candidate substances from structural class I, for which use levels have been reported, range from 800 to 5100 microgram/person/day, for the five candidate substances from structural class II, for which use levels are available, the mTAMDI range from 3800 to 3900 microgram/person/day for each. For the two candidate substances from structural class III the mTAMDIs are 3800 and 4100 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain

hydroxycarboxylate anion and the lactone ring. In basic and neutral media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured. Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclication are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones, with low molecular weight, being uncharged may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte. The hydroxycarboxylic acid obtained from lactone hydrolysis enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by 2-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then metabolised in the citric acid cycle. The Panel anticipated that the two unsaturated omega-lactones ([FL-no: 10.059], hexadec-7-en-1,16-lactone and [FL-no: 10.063], hexadec-9-en-1,16-lactone) are metabolised like the structurally related saturated lactones, namely through ring opening followed by fatty acid degradation.

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma-, delta- and omega-lactones and lactones fused to alicyclic rings such as 2-(2-hydroxycyclopent-4-enyl)ethanoic acid gamma-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993).

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (linear or branched-chain aliphatic alcohols) and acid components (i.e. alpha-, beta- or gamma-keto or hydroxy acids, or simple aliphatic acids, diacids or triacids), which would be further metabolised and excreted. It has to be noted that the 2-acetyl butyric acid, formed as one of the hydrolysis products of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], has some structural similarities to valproic acid, which, together with a number of its derivatives, has been recognised as teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Although it can be predicted that 2-acetylbutyric acid is further metabolised through the usual pathways of detoxication for carboxylic acids (i.e. mainly *via* glucuronidation reaction), the structural similarity with valproic acid does not allow the prediction that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised only to innocuous products.

The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters. The most probable metabolic reactions of the hydrolysis products are, oxidation of alcohols to aldehydes and acids, conjugation of alcohols and acids to glucuronides and sulphates and beta- and omega-oxidation of carboxylic acids.

Beta-keto acids and derivatives like acetoacetic acid undergo ready decarboxylation. Along with alpha-keto and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways. The gamma-keto acids and related substances may undergo complete or partial beta-oxidation to yield metabolites that are eliminated in the urine. Omega-substituted derivatives are readily oxidised and/or excreted in the urine. Simple aliphatic di- and tricarboxylic acids participate in the tricarboxylic acid cycle. For instance, succinic acid is a normal intermediary metabolite and a constituent of the citric acid cycle; it occurs normally in human urine (1.9 - 8.8 mg/L). Succinic acid is readily metabolized when administered to animals, but may be partly excreted unchanged in the urine if large doses are given (Patty, 1993, Vol. II, p. 3579).

One of the candidate substances, 1-hydroxypropan-2-one [FL-no: 07.169] (acetol), is a metabolite of acetone, which is an endogenous substance formed from the degradation of body fat / fatty acids. The major metabolic pathway in mammals of acetone at low blood concentrations (i.e. in healthy humans not exposed to external sources, acetone occurs in amounts of approximately 4 - 12 mg per person,

corresponding to approximately 0.7 to 2 mg/l blood (Ashley et al., 1994; Dick el al., 1988; Wang et al, 1994c), is via the methylglyoxal route, where acetone is first oxidised to 1-hydroxypropan-2-one, which is then oxidised to 2-oxopropanal (methylglyoxal [FL-no: 07.001]). 2-Oxopropanal will after further metabolism give rise to glucose (Morgott, 1993; WHO, 1998a; NAS/COT, 2005).

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may be expected to undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption. Once hydrolysed, the component alcohols and aldehydes are expected to be metabolised primarily through the above mentioned common routes of biotransformations and excreted.

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation. It may be anticipated that glutaraldehyde [FL-no: 05.149] is biotransformed through the common pathways of detoxication of aldehydes to innocuous products.

Among the candidate substances, an alkoxy-alcohol, 2-butoxyethanol [FL-no: 02.242], is mainly metabolised to butoxyacetic acid, which has been identified as the metabolite responsible for the haemolysis of red blood cells induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with a second oxygenated functional group and aliphatic lactones included in the present FGE are generally metabolised to innocuous products (many of which are endogenous in humans), at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethan-1-ol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound.

A more detailed description of the metabolism of the candidate substances in this FGE is given in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For 5-pentyl-3H-furan-2-one [FL-no: 10.170] flavour industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], –

which is an alpha,beta-unsaturated alcohol (after hydrolysis of the lactone) allocated FGE.19 subgroup 4.1. This subgroup was evaluated in FGE.217 with the conclusion – additional genotoxicity data required. Therefore, the Panel concluded that [FL-no.10.170] should not be evaluated through the Procedure until these data are available.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], should not be evaluated through the Procedure until new data became available because it was found to be genotoxic *in vitro* in bacterial assays. However, in the first revision of FGE.10 (FGE.10Rev1) the Panel reconsidered this compound and concluded that it is an endogenous metabolite of acetone which is formed from the degradation of body fat/fatty acids and that it would be further metabolised to innocuous compounds, and thus not be of concern at the exposure levels resulting from its use as a flavouring substance (see Section 8.4, conclusion on the genotoxicity). The Panel therefore decided that 1-hydroxypropan-2-one [FL-no: 07.169] could be evaluated along the A side of the Procedure in FGE.10Rev1.

For the safety evaluation of the 62 candidate substances in the present revision of FGE.10 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

Fifty-five of the candidate substances are classified according to the decision tree approach by Cramer *et al.* (1978) into structural class I, six are classified into structural class II [FL-no: 02.242, 06.088, 06.090, 06.095, 06.097 and 06.135] and one into structural class III [FL-no: 06.102].

Step 2

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with some structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Therefore, these substances are evaluated via the B-side of the Procedure. The evaluation of the remaining 59 candidate substances proceeds via the A-side of the Procedure.

Step A3

Step A3 applies to 54 candidate substances from structural class I [FL-no: 02.132, 02.198, 05.149, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.832, 09.833, 09.862, 09.874, 09.916, 09.951, 10.038, 10.039, 10.040, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068 and 10.168], four candidate substances from structural class II [FL-no: 06.088, 06.090, 06.095 and 06.135] and one candidate substance from structural class III [FL-no: 06.102].

The 54 candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The four candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the one candidate substance assigned to structural class III has an estimated European daily *per capita* intake of 0.011 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

Accordingly, these 59 candidate substances do not pose a safety concern when used at estimated levels of intake as flavouring substances, based on the MSDI approach.

Step B3

The MSDIs of the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097] and ethyl 2-acetylbutyrate [FL-no: 09.824], were estimated to be 0.0012 microgram/*capita*/day for each. Thus, the MSDI-values of all three candidate substances are below the threshold of concern for their structural classes of 540 microgram/person/day (class II) for [FL-no: 02.242 and 06.097] and of 1800 microgram/person/day (class I) for [FL-no: 09.824]. Accordingly, the three substances proceed to step B4 of the Procedure.

Step B4

The candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824] is expected to be hydrolysed to the corresponding alpha-ethylated carboxylic acid, 2-acetylbutyric acid and ethanol. No toxicity studies that would permit establishing a No Observed Adverse Effect Level (NOAEL) are available for ethyl 2-acetylbutyrate or its hydrolysis product 2-acetylbutyric acid. 2-Acetylbutyric acid is structurally related to 2-ethylhexanol [FL-no: 02.082] for which the JECFA has established an ADI of 0.5 mg/kg bw/day (JECFA, 1993b). The estimated daily *per capita* intake, based on the MSDI approach and expressed in microgram/kg bw/day for the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate (and 2-acetylbutyric acid) is approximately 25 x 10^6 fold below the acceptable daily intake (ADI) value of the structurally related 2-ethylhexanol. Furthermore, the hydrolysis product, 2-acetylbutyric acid, shows some structural similarities to valproic acid, a known teratogenic compound. If 2-acetylbutyric acid is considered to be as potent as valproic acid (NOAEL = 600 mg/kg bw/day) the margin of safety would be 3 x 10^9, based on the MSDI of 0.0012 microgram/*capita*/day. Accordingly, it is concluded that ethyl 2-acetylbutyrate [FL-no: 09.824] does not pose a safety concern at the estimated level of intake, based on the MSDI approach.

For the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-triethoxypropane [FL no: 06.097], the hydrolysis product of which has some structural similarities to 2-butoxyethan-1-ol, a NOAEL could not be established in sub-chronic/chronic toxicity studies with respect to haemotoxicity. Thus, strictly according to the Procedure additional toxicity data would be needed to finalise the evaluation of these two substances in step B4 of the Procedure. However, reconsidering and updating the previous version of this FGE, the Panel noted that at least for 2-butoxyethan-1-ol [FL-no: 02.242] a wealth of toxicity data is available, so that this substance can be evaluated on a broader basis than only the Procedure for the Evaluation of Flavouring substances, which in principle has been designed for the evaluation of data-poor substances.

Considering the data available, especially those on kinetics and mechanism of action (see US-EPA, 1999 and draft EU-RAR 2007, human health part) it becomes clear that there are major differences in sensitivity between humans and rats regarding the prime toxic effect (haemotoxicity) of this substance, with humans (together with dog, guinea pig, pig, cat and rabbit) being considerably less sensitive than rats (together with mouse, hamster and baboon). For that reason it seems inappropriate to ask for further toxicity data in animals, as the available data already cover the most sensitive species. In this case an alternative approach is needed and possible for this data-rich substance (EPA, 1999; EU-RAR, 2007).

In their evaluation, US-EPA, using a Bench Mark Dose approach, combined with physiologically-based kinetic modelling arrived at an oral Reference dose (RfD) for chronic exposure of 0.5 mg/kg body weight (bw)/day (EPA, 1999).

In the EU-RAR (2007) a Human equivalent Lowest Observed Adverse Effect Level (LOAEL) of 9.5 mg/kg bw/day is used, which was derived from the LOAEL in the rat using the same kinetic models as applied by US-EPA. A Margin of Safety of 3 between the Human equivalent LOAEL and estimates

for chronic exposure of "Consumers" or "Humans, exposed via the Environment" was considered sufficient to reach a conclusion of no concern.

For each of the two candidate flavouring substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-tri-ethoxypropane [FL no: 06.097] an MSDI of 0.0012 microgram/*capita*/day (see Table 6.1) can be calculated. The RfD from US-EPA and the LOAEL from the draft EU-RAR are factors of 2.5×10^7 or 4.75×10^8 above the MSDI, respectively. The Panel concluded that these margins are sufficiently large to decide that based on the MSDI exposure estimates, these substances are of no concern when used as flavouring substances.

In conclusion the Panel considered that all candidate substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The mTAMDI for the 54 candidate substances in structural class I and for which use levels information is available, range from 800 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day.

The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day.

For the two substances from structural class III the mTAMDI is 3800 and 4100 microgram/person/day, which is above the threshold of 90 microgram/person/day.

Thus, for the 58 candidate substances further information is required as the mTAMDIs are above the threshold for the structural class. This would include more reliable intake data and then, if required, additional toxicological data. For two substances [FL-no: 06.135 and 08.113] use levels are required for the food categories as listen in Commission Regulation (EC) No 1565/2000 (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n).

For comparison of the MSDI- and mTAMDI-values see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	0.0061	3900	Class I	1800
02.198	Octane-1,3-diol	0.0012	3900	Class I	1800
05.149	Glutaraldehyde	0.055	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	0.22	1600	Class I	1800
08.053	Malonic acid	0.0012	3200	Class I	1800
08.082	Glutaric acid	0.0012	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	0.0012	3800	Class I	1800
08.103	Nonanedioic acid	0.0012	3200	Class I	1800
08.113	Succinic acid, disodium salt	1500		Class I	1800
09.333	sec-Butyl lactate	3.7	3900	Class I	1800
09.345	Di-isopentyl succinate	0.037	3900	Class I	1800
09.346	Dibutyl malate	0.0012	3900	Class I	1800
09.347	Dibutyl succinate	0.12	3900	Class I	1800
09.348	Diethyl adipate	0.027	3900	Class I	1800
09.349	Diethyl citrate	0.12	3900	Class I	1800
09.350	Diethyl fumarate	0.0012	3900	Class I	1800
09.351	Diethyl maleate	12	3900	Class I	1800
09.352	Diethyl nonanedioate	0.0012	3900	Class I	1800
09.353	Diethyl oxalate	0.0012	3900	Class I	1800
09.354	Diethyl pentanedioate	0.0012	3900	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.360	Ethyl 2-acetoxypropionate	4.9	3900	Class I	1800
09.502	Ethyl butyryl lactate	0.5	3900	Class I	1800
09.558	Dimethyl malonate	0.097	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	0.74	3900	Class I	1800
09.580	Hexyl lactate	0.49	3900	Class I	1800
09.590	Isobutyl lactate	3.7	3900	Class I	1800
09.601	Isopentyl lactate	7.2	5100	Class I	1800
09.626	Methyl 2-oxopropionate	0.024	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	0.0012	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	0.24	3900	Class I	1800
09.634	Methyl acetoacetate	0.012	3900	Class I	1800
09.644	Methyl lactate	0.34	3600	Class I	1800
09.683	Pentyl lactate	0.61	3900	Class I	1800
09.815	Propyl lactate	0.62	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	0.33	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	0.24	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	0.0012	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	0.015	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	0.011	3900	Class I	1800
09.951	Dioctyl adipate	6.1	800	Class I	1800
10.038	Dec-7-eno-1,4-lactone	0.37	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	1.2	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	0.011	3900	Class I	1800
10.045	Heptano-1,5-lactone	0.012	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	0.024	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	0.0061	3900	Class I	1800
10.049	Hexadecano-1,5-lactone	0.024	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	0.61	3900	Class I	1800
10.055	Pentano-1,5-lactone	0.012	3900	Class I	1800
10.058	Tridecano-1,5-lactone	0.61	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	1.9	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	48	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	0.9	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	1.2	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	0.0012	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	0.0061	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	0.012	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	0.012	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane	1.2		Class II	540
02.242	2-Butoxyethan-1-ol	0.0012	3900	Class II	540
06.097	1,1,3-Triethoxypropane	0.0012	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	0.011	4100	Class III	90
10.170	5-Pentyl-3H-furan-2-one	1.2	3800	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

As one of the candidate substances, 5-pentyl-3H-furan-2-one [FL-no: 10.170] show possible genotoxic potential *in vitro*, the substance is not taken through the Procedure. This substance is therefore not included in the calculation of the combined intake of the candidate substances evaluated in FGE.10Rev3.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b; Flavour Industry, 2010n), the combined estimated daily *per capita* intake as flavourings of the 55 candidate flavouring substances assigned to structural class I is 1600 microgram, of the six candidate flavouring substances assigned to structural class II is 1.2 microgram and of the one candidate substance assigned to structural class III, 0.01 microgram. These estimates do not exceed the thresholds of concern for the corresponding structural classes of 1800, 540 and 90 microgram/person/day, respectively.

The candidate lactones are structurally related to 27[8] supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by the JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about 55 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 41 candidate substances are structurally related to 33[9] supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 24000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

8. Toxicity

8.1. Acute Toxicity

Data are available for 16 of the candidate substances (Annex IV, Table IV.1). For the majority of candidate substances, oral LD_{50} values, in mice or rats, varied from 100 mg/kg up to more than 5000 mg/kg body weight (bw). For butane-1,3-diol [FL-no: 02.132] and octane-1,3-diol [FL-no: 02.198] LD_{50} values between 20 g/kg bw and approximately 30 g/kg bw are reported (Annex IV, Table IV.1).

Forty-three supporting substances were tested for acute toxicity in mice and/or rats (Annex IV, Table IV.1). For the majority of the supporting substances, oral LD_{50} values, in mice or rats, varied from 1300 mg/kg up to 18500 mg/kg bw. For diethyl sebacate [FL-no: 09.475] and tributyl acetylcitrate [FL-no: 09.511] LD_{50} values larger than 30 g/kg bw are reported.

The acute toxicity data are summarised in Annex IV, Table IV.1.

[8] European production volumes are only available for 27 of the 29 JECFA evaluated lactones – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.

[9] European production volumes are only available for 33 of the 47 JECFA evaluated alcohols and related substances – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute/subchronic/chronic toxicity data are available for five candidate substances, 2-butoxyethan-1-ol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], glutaraldehyde [FL-no: 05.149], nonanedioic acid [FL-no: 08.103] and for 20 supporting substances of the present Flavouring Group Evaluation (JECFA, 1998a; JECFA, 2000c). Additionally, data are available for two to succinic acid, disodium salt [FL-no: 08.113] structurally related substances, succinate monosodium and disodium hexahydrate.

Available data on repeated dose toxicity show that haemolysis is the primary and critical response elicited in the main animal test models (rats and mice) following oral exposure to 2-butoxyethan-1-ol, in which the haematotoxic action is produced by the metabolite butoxyacetic acid (this effect is also seen following other exposure routes such as inhalation or dermal exposure. These exposure routes are not considered relevant for this evaluation as data from oral exposure are available). Notably, the haematotoxic effect exhibits a pronounced species difference. In sensitive species (rat, mouse, hamster, baboon), 2-butoxyethan-1-ol produces a characteristic toxicity that is revealed clinically by the appearance of haemoglobinuria and pathologically by changes in a variety of blood parameters (EPA, 1999; EU-RAR, 2004a). Slight decrease in body weight gain, haematological and liver effects have been reported for male and female rats, respectively (NTP, 1993a). Human erythrocytes are about 100-times less sensitive than rat erythrocytes as judged by prehaemolytic changes *in vitro* (increase in mean erythrocyte volume, erythrocyte deformability) consistently observed in both species. Studies have also shown that potentially sensitive human sub-populations, including children, the elderly and those with sickle cell anemia, do not show increased sensitivity to the haemolytic action of 2-butoxyethan-1-ol. Furthermore, the *in vivo* blood concentrations producing haemolysis in the animal experiments are considered unlikely to occur under normal conditions of human exposure to 2-butoxyethan-1-ol (EU-RAR, 2004a).

Carcinogenicity:

In a two year inhalation study, F344/N rats were exposed to 0, 0.031, 0.0625 and 0.125 mg/m^3 and B6C3F$_1$ mice were exposed to 0, 0.0625, 0.125 and 0.250 mg/m^3 2-butoxyethan-1-ol (NTP, 2000b). The exposure caused a low incidence of haemangiosarcoma in male mice at the highest exposure concentration; haemangiosarcoma did not occur in female mice or in rats. In female mice, 2-butoxyethan-1-ol caused an increased incidence of forestomach tumours. It was not carcinogenic in rats. The occurrence of haemangiosarcoma in male mice only at highest exposure concentration is suggestive of a threshold phenomenon, related to the induction of haemolysis in rodent species. With regard to human relevance, the mechanism proposed for the induction of haemangiosarcomas strongly supports the conclusion that 2-butoxyethan-1-ol is unlikely to be a carcinogenic hazard at the estimated level of intake as flavouring substance, because human erythrocytes are demonstrably more resistant to haemolysis than are rodent erythrocytes.

Glutaraldehyde[10] [FL-no: 05.149] (50, 250, 1000 mg/l in drinking water, resulting in doses of 2.9-6.9, 14.5-31.8 and 54.7-104.6 mg/kg/day, respectively) was not tumorigenic in a two year carcinogenicity

[10] Glutaraldehyde is also used in food contact material (FCM). It was evaluated by the former Scientific Committee on Food (SCF List 7, http://europa.eu.int/comm/food/fs/sc/scf/out50_en.pdf), however, this is not a final evaluation. According to German recommendations, glutardialdehyde (synonym: glutaraldehyde) may be used for the production of artificial sausage skin (maximum use level 0.1 %). The maximum residual amount of glutardialdehyde is 50 mg per kg artificial sausage skin (ready for use). Furthermore, glutardialdehyde may be used as anti slime agent for the production of paper as FCM (maximum use level 2.5 % based on dry fibre material). The maximum residual amount of glutardialdehyde is 2 mg per kg paper (ready for use). The Panel noted that maximum residual amounts of glutaraldehyde in food contact material (as set e.g. in German recommendations) could apparently conflict with reported use levels of glutaraldehyde as flavouring. However, in the German recommendations, the maximum residual amounts were set considering the technologically needed use levels (limited data submitted) rather than on toxicological data, and the Panel therefore did not find the low maximum residual amounts for glutaraldehyde as such in conflict with higher use levels for glutaraldehyde as flavouring, which could therefore go through the Procedure.

study on male and female rats (Van Miller et al., 2002). Furthermore, malonic acid [FL-no: 08.053] was negative in a liver foci tumour promotion assay.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental toxicity and reproductive toxicity are available for the following five candidate substances: 2-butoxyethan-1-ol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], glutaric acid [FL-no: 08.082], glutaraldehyde [FL-no: 05.149] and nonanedioic acid [FL-no: 08.103]. Studies for supporting substances comprise butyro-1,4-lactone [FL-no: 10.006] and adipic acid [FL-no: 08.026] (JECFA, 1998a; JECFA, 2000c) and one structurally related substance, succinate disodium hexahydrate (Annex IV, Table IV.3).

For 2-butoxyethan-1-ol [FL-no: 02.242] no effects on fertility were observed in female and male mice given 2-butoxyethan-1-ol in the drinking water in a continuous breeding study in which a NOAEL of 720 mg/kg was derived (EU-RAR, 2004a). As to developmental toxicity, studies performed on animals via various administration routes did not demonstrate any teratogenic potential, and foetotoxicity and embryotoxicity (lethality and resorptions) were only observed in the presence of maternal toxicity (regenerative haemolytic anaemia). Other effects seen on foetuses were an increase in the incidence of skeletal variations, which are generally described as ossification delays. The effects seen in developmental toxicity studies with 2-butoxyethan-1-ol are considered to result from haemolysis and subsequent maternal anemia (EU-RAR, 2004a). Overall, 2-butoxyethan-1-ol is not considered to pose a safety concern with respect to reproduction and development at the estimated level of intake as flavouring substance.

No information is available on ethyl 2-acetyl butyrate [FL-no: 09.824], the hydrolysis product of which, 2-acetyl butyric acid, has some structural similarities to valproic acid, which, together with a number of its derivatives, has been recognised as teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Offspring of mothers using > 1000 mg/kg bw/day valproic acid per day were at a significantly increased risk of major congenital malformations especially neural tube defects, compared to offspring exposed < or 600 mg valproic acid/day (RR 6.8; 95 % CI: 1.4 - 32.7). No difference in risk of major congenital malformations was found between the offspring exposed to 601 - 1000 mg/day and < or = 600 mg/kg bw/day. Thus, 600 mg/day is considered as NOAEL for the teratogenic effects of valproic acid in humans.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 12 of the candidate substances. These 12 substances are pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168], glutaraldehyde [FL-no: 05.149], 1-hydroxypropan-2-one [FL-no: 07.169], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], diethyl maleate [FL-no: 09.351], diethyl adipate [FL-no: 09.348], methyl acetoacetate [FL-no: 09.634], 2-butoxyethan-1-ol [FL-no: 02.242], glutaric acid [FL-no: 08.082] and succinic acid, disodium salt [FL-no: 08.113]. There were genotoxicity data on 22 supporting substances and for one structurally related substance (Annex IV, Table IV.4 and IV.5).

For 5-pentyl-3H-furan-2-one [FL-no: 10.170] flavour industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], which is an alpha,beta-unsaturated alcohol (after hydrolysis of the lactone) allocated to FGE.19 subgroup 4.1. This subgroup was evaluated in FGE.217 with the conclusion that additional

genotoxicity data required. Therefore, the Panel concluded that [FL-no: 10.170] should not be evaluated through the Procedure until these data are available.

In vitro

Pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168] methyl acetoacetate [FL-no: 09.634] and succinic acid [FL-no: 08.113] were reported to be negative in microbial mutagenicity assays.

1-Hydroxypropan-2-one [FL-no: 07.169] was positive in Ames tests using strains TA 100 and TA 104 in the presence and absence of S-9 metabolic activation (Garst et al., 1983; Marnett et al., 1985a; Yamaguchi, 1982; Yamaguchi and Nakagawa, 1983);. These results are consistent across the four reported studies which, despite limitations in study design and reporting, suggest that 1-hydroxypropan-2-one should be considered an *in vitro* mutagen in bacteria. There are no data provided on either *in vitro* endpoints nor on *in vivo* studies.

Diethyl maleate [FL-no: 09.351] was reported to produce mutations in the TK +/- locus of L5178Y mouse lymphoma cells. However, the concentration required for a two-fold increase of mutations results in 70 % growth reduction (Wangenheim and Bolcsfoldi, 1988), rendering this effect questionable. Diethyl maleate was positive in an aneuploidy test using V79 Chinese hamster lung cells at 8.7 $x10^{-6}$ M but not at 5.2 $x10^{-6}$ M (Önfelt, 1987); generally aneuploidy is considered as a threshold phenomenon.

In vitro and/or in vivo

Glutaric acid [FL-no: 08.082] was reported to be negative in the Ames and Rec test as well as in an *in vivo* test for rat bone marrow aberrations.

2-Butoxyethan-1-ol [FL no: 02.242] was negative in the Ames test and in *in vitro* tests in mammalian cells for induction of forward mutations, chromosomal aberrations and sister chromatid exchanges (SCE). Positive results were only reported in one study in V79 cells (for induction of forward mutations, SCE and micronuclei) at doses above the maximum level recommended by current OECD Guidelines. Equivocal positive results were reported in an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes. *In vivo*, negative results were obtained in an adequate micronucleus tests in rats and mice following oral or intraperitoneal administration. No evidence of DNA binding or alteration of DNA methylation was obtained in a study in rats and mice. The overall experimental evidence indicated that 2-butoxyethan-1-ol is not genotoxic (see Table IV.5).

Glutaraldehyde [FL-no: 05.149] exhibits genotoxic effects in *in vitro* tests, most consistently in the bacterial mutagenicity assays. Forward gene mutation tests *in vitro* in mammalian cells have given variable results depending on the locus: negative with HGPRT and positive with TK. Also, SCE, chromosome aberration and UDS tests have shown no effect to a weakly positive effect, depending on the laboratory, protocol, dosages and sampling times. However, that any *in vitro* potential for genotoxic effects will not be expressed *in vivo* is indicated by the *in vivo* study results, which include chromosomal aberrations, mammalian erythrocyte micronucleus test, UDS and recessive lethal mutations. The only study suggesting an *in vivo* effect was an increase in micronuclei in mouse blood cells up to 15 mg/kg bw. However, the data are insufficiently reported. The negative results from the well-conducted *in vivo* studies may be related to the rapid metabolism and protein binding characteristics of glutaraldehyde, and the related observation that although 14C-labelled glutaraldehyde may be detected in cell cytoplasm there is no nuclear fraction radioactivity (Vergnes and Ballantyne, 2002).

Butane-1,3-diol [FL-no: 02.132] was reported as not inducing chromosomal aberration in bone marrow and was negative in a rat dominant lethal assay. Butane-1,3-diol [FL-no: 02.132] was checked for cytogenetic effects over a period of three generations at doses of 5 % (5000 mg/kg/day), 10 % and

24 %. None of the doses produced abnormal rates of bone marrow metaphase cells as compared to controls (Hess et al., 1981).

Malonic acid [FL-no: 08.053] was found negative in a rat liver foci assay, diethyl adipate [FL-no: 09.348] was reported to be negative in a mouse dominant lethal assay.

Genotoxicity tests are available for 22 supporting substances. Some positive test results from *in vitro* studies are reported for 4-hydroxybutyric acid lactone [FL-no: 10.006], which, however, was found negative in a reliable *Drosophila in vivo* sex-linked recessive lethal mutation assay (Table IV 4 and 5). Results of *in vivo* bone marrow micronucleus assays in mice available for 4-hydroxybutyric acid lactone were also negative, however, since the PCE/NCE ratio was not reported it is not clear if the test substance reached the bone marrow (Table IV.5). Positive *in vitro* data that cannot be evaluated are reported for hexano-1,5-lactone [FL-no: 10.010], nonano-1,4-lactone [FL-no: 10.001], undecano-1,4-lactone [FL-no: 10.002], undecano-1,5-lactone [FL-no: 10.011] and ethyl acetoacetate [FL-no: 09.402] (Annex IV, Table IV.4).

Conclusions on genotoxicity

Genotoxicity data are only available on a very limited number of the candidate substances in this Flavouring Group Evaluation and none has a complete package of mutagenicity endpoints.

One of the candidate substances (1-hydroxypropan-2-one [FL-no: 07.169]) induced gene mutations in bacteria but has not been studied *in vivo* or in other *in vitro* assays.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that for the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], it was necessary to request additional *in vitro* data from studies in mammalian cells. However, in the first revision of FGE.10 (FGE.10Rev1) the Panel reconsidered the fact that 1-hydroxypropan-2-one is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore concluded that 1-hydroxypropan-2-one [FL-no: 07.169] would not be of safety concern at the exposure level resulting from its use as a flavouring substance. Consequently, the Panel decided that further studies on the *in vitro* genotoxicity of 1-hydroxypropan-2-one [FL-no: 07.169] would not be required.

Glutaraldehyde was tested *in vitro* and *in vivo*, with positive findings *in vitro*. However, based upon the negative results of *in vivo* genotoxicity assays, along with the lack of tumorigenicity in mice and rats, the *in vitro* genotoxicity data are not considered relevant for the safety evaluation of glutaraldehyde.

Disodium succinate [FL-no: 08.113] did not induce mutations in bacterial reverse mutation assays using *S.typhimurium* strains TA97, TA94, TA98, TA100, TA1535, and TA1537 at 5 mg/plate (with metabolic activation) and in TA97 and TA102 at 15 mg/plate (with or without metabolic activation). A chromosomal test with Chinese hamster lung (CHL) cells revealed equivocal effects on polyploidy at 15 mg/mL (Ishidate et al., 1984; Fujita et al., 1994; OECD, 2003). These results are supported by studies on disodium succinate hexahydrate.

5-pentyl-3H-furan-2-one [FL-no: 10.170] should not be evaluated through the Procedure until the additional gentoxicity data for FL-no: 10.054 are available, as stated in FGE 217.

The available experimental data indicate that 2-butoxyethan-1-ol is not genotoxic.

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The candidate substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

The present revision of FGE.10, FGE.10Rev3, includes the assessment of two additional candidate substances [FL-no: 09.951 and 10.170].

Thirty-six of the candidate substances possess one or more chiral centres and eight can exist as geometrical isomers due to the presence and the position of a double bond. For four of these eight substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition has not been specified sufficiently. For [FL-no: 10.170] the Industry has informed that the commercial substance is a mixture of two structural isomers. One of these isomers posses a chiral centre for which the configuration has not been specified.

Fifty-five of the candidate substances belong to structural class I, six of the candidate substances belong to structural class II, and two belong to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifty of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

The candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the two candidate substances assigned to structural class III have estimated European daily *per capita* intakes of 0.011 and 1.2 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I, II and III, respectively.

The combined estimated daily *per capita* intake as flavourings of the 55 candidate substances assigned to structural class I is 1600 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. Likewise, the combined estimated daily *per capita* intake as flavouring of the six candidate substances assigned to structural class II is 1.2 microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The candidate lactones are structurally related to 27 supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about 55 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 41 candidate substances are structurally related to 33 supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 24000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

For 5-pentyl-3H-furan-2-one [FL-no: 10.170], the flavour Industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], which is an alpha, beta-unsaturated alcohol (after hydrolysis of the lactone), allocated to subgroup 4.1 of FGE.19 (FGE.217).The Panel concluded that 5-pentyl-3H-furan-2-one [FL-no: 10.170] should not be evaluated through the Procedure until the additional gentoxicity data for [FL-no: 10.054] are available, as stated in FGE 217.

The Panel reconsidered the fact that 1-hydroxypropan-2-one [FL-no: 07.169] is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore decided that further genotoxicity data are not required and that the substance could be taken through the Procedure.

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern.

It can be anticipated that, at the estimated levels of intake as flavouring substances, the alcohols, aldehydes, acetals, carboxylic acids and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products, many of which are endogenous in humans. The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350]. However, as the estimated levels of intake as flavouring substances are sufficiently low for these two substances, profound GSH depletion is not expected. For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance which has structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Adequate margins of safety could be established for these three substances in step B4 of the Procedure.

Otherwise, it was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was considered that on the basis of the default MSDI approach that the 62 flavouring substances, to which the Procedure have been applied, would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

The mTAMDI for the 54 candidate substances in structural class I, for which use levels information is available, range from 800 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day. For the two substances from structural class III the mTAMDIs are 3800 and 4100, which is above the threshold of 90 microgram/person/day. For two substances [FL-no: 06.135 and 08.113] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000.

Thus, for 60 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. The three candidate substances [FL-no: 05.149, and 07.169 and 09.951] which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

Thus, in conclusion, 62 of the 63 flavouring substances were evaluated through the Procedure (based on MSDI approach), as one flavouring substance, 5-pentyl-3H-furan-2-one [FL-no: 10.170] could not be evaluated through the Procedure until adequate genotoxicity data become available.

In order to determine whether the conclusion for the 62 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 58 flavouring substances. For four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] information on composition of mixture and/or stereoisomerism has not been specified sufficiently. For one substance [FL-no: 10.063] an identity test is missing. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 10.038, 10.040, 10.059 and 10.063], pending further information.

For the remaining 58 candidate substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.088, 06.090, 06.095, 06.097, 06.102, 06.135, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874, 09.916, 09.951, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.068 and 10.168] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN FGE.10REV3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

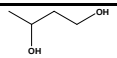
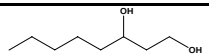
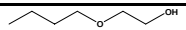
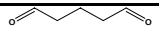
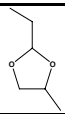
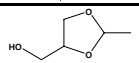
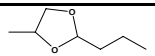
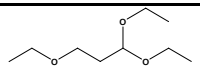
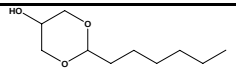
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.132	Butane-1,3-diol		 107-88-0	Liquid $C_4H_{10}O_2$ 90.12	Soluble Freely soluble	102 (13 hPa) MS 95 %	1.436-1.442 0.992-0.998	Racemate.
02.198	Octane-1,3-diol		 23433-05-8	Liquid $C_8H_{18}O_2$ 146.23	Sparingly soluble Freely soluble	82 (7 hPa) MS 95 %	1.452-1.458 0.980-0.986	Racemate.
02.242	2-Butoxyethan-1-ol		 10182 111-76-2	Liquid $C_6H_{14}O_2$ 118.18	Slightly soluble Freely soluble	170 MS 95 %	1.416-1.422 0.899-0.905	
05.149	Glutaraldehyde		 111-30-8	Liquid $C_5H_8O_2$ 100.12	Soluble Freely soluble	188 MS 95 %	1.430-1.436 1.005-1.011	
06.088	2-Ethyl-4-methyl-1,3-dioxolane		 4359-46-0	Liquid $C_6H_{12}O_2$ 116.16	Soluble Freely soluble	116 MS 95 %	1.402-1.408 0.916-0.922	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		 3674-21-3	Liquid $C_5H_{10}O_3$ 118.13	Practically insoluble or insoluble Freely soluble	187 MS 95 %	1.440-1.446 1.120-1.126	Racemate. CASrn in Register to be changed to 3773-93-1 (EFFA, 2006ac). CASrn in Register refers to the (2R, 4S) enantiomer.
06.095	4-Methyl-2-propyl-1,3-dioxolane		 4352-99-2	Liquid $C_7H_{14}O_2$ 130.19	Soluble Freely soluble	143 MS 95 %	1.409-1.415 0.907-0.913	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
06.097	1,1,3-Triethoxypropane		 10075 7789-92-6	Liquid $C_9H_{20}O_3$ 176.26	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.403-1.409 0.890-0.896	
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		 2016 1708-36-7	Solid $C_{10}H_{20}O_3$ 188.22	Practically insoluble or insoluble Freely soluble	255 44 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

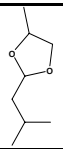
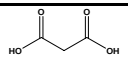
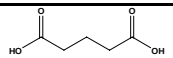
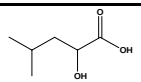
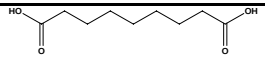
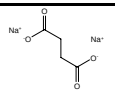
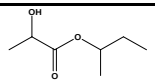
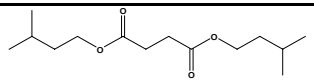
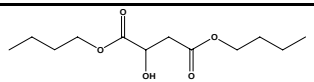
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		4378 18433-93-7	Liquid $C_8H_{16}O_2$ 144.21	Insoluble Soluble	150 MS 96 %	n.a. 0.895	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
07.169	1-Hydroxypropan-2-one		 11101 116-09-6	Liquid $C_3H_6O_2$ 74.08	Soluble Freely soluble	146 MS 95 %	1.420-1.426 1.084-1.090	
08.053	Malonic acid		 2264 141-82-2	Solid $C_3H_4O_4$ 104.16	Soluble Freely soluble	264 135 MS 95 %	n.a. n.a.	
08.082	Glutaric acid		 110-94-1	Solid $C_5H_8O_4$ 132.12	Soluble Freely soluble	303 98 MS 95 %	n.a. n.a.	
08.090	2-Hydroxy-4-methylvaleric acid		 10118 498-36-2	Solid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	249 76 MS 95 %	n.a. n.a.	Racemate.
08.103	Nonanedioic acid		 10079 123-99-9	Solid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	225 (13 hPa) 107 MS 95 %	n.a. n.a.	
08.113	Succinic acid, disodium salt		3277 150-90-3	Solid $C_4H_4Na_2O_4$ 162.05	Soluble Insoluble	426.03 156.43 IR 60	n.a. n.a.	Anhydrous when heated to 120°C. Min.assay: Anhydrous 60 %, hydrate 40 % (Fenaroli, 1995).
09.333	sec-Butyl lactate		 18449-60-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	172 MS 95 %	1.414-1.420 0.970-0.976	Racemate.
09.345	Di-isopentyl succinate		 10555 818-04-2	Liquid $C_{14}H_{26}O_4$ 258.36	Practically insoluble or insoluble Freely soluble	298 MS 95 %	1.431-1.437 0.955-0.961	
09.346	Dibutyl malate		 1587-18-4	Solid $C_{12}H_{22}O_5$ 246.30	Practically insoluble Freely soluble	170 (16 hPa) 82 MS 95 %	n.a. n.a.	CASrn in Register to be changed to 6280-99-5 (racemate).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

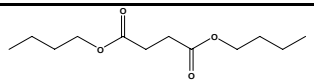
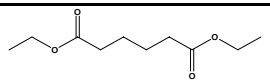
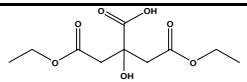
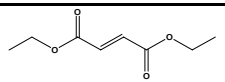
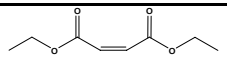
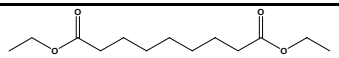
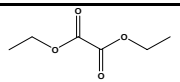
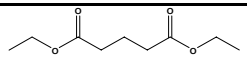
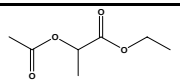
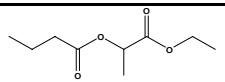
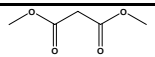
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.347	Dibutyl succinate		 141-03-7	Liquid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	275 MS 95 %	1.426-1.432 0.973-0.979	
09.348	Diethyl adipate		 141-28-6	Liquid $C_{10}H_{18}O_4$ 202.25	Practically insoluble or insoluble Freely soluble	244 MS 95 %	1.425-1.431 1.004-1.010	
09.349	Diethyl citrate		 32074-56-9	Solid $C_{10}H_{16}O_7$ 248.23	Sparingly soluble Freely soluble	354 237 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register refers to incompletely defined substance.
09.350	Diethyl fumarate		 623-91-6	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.444 1.049-1.055	
09.351	Diethyl maleate		 10551 141-05-9	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.445 1.049-1.055	
09.352	Diethyl nonanedioate		 10549 624-17-9	Liquid $C_{13}H_{24}O_4$ 244.33	Practically insoluble or insoluble Freely soluble	290 NMR 95 %	1.432-1.438 0.970-0.976	
09.353	Diethyl oxalate		 95-92-1	Liquid $C_6H_{10}O_4$ 146.14	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.407-1.413 1.076-1.082	
09.354	Diethyl pentanedioate		 818-38-2	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	233 MS 95 %	1.421-1.427 1.019-1.025	
09.360	Ethyl 2-acetoxypropionate		 2985-28-6	Liquid $C_7H_{12}O_4$ 160.17	Practically insoluble or insoluble Freely soluble	76 (13 hPa) MS 95 %	1.405-1.411 1.041-1.047	Racemate.
09.502	Ethyl butyryl lactate		 2242 71662-27-6	Liquid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	208 MS 95 %	1.408-1.414 1.021-1.027	Racemate.
09.558	Dimethyl malonate		 11754 108-59-8	Liquid $C_5H_8O_4$ 132.12	Practically insoluble or insoluble Freely soluble	181 MS	1.411-1.417 1.150-1.156	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

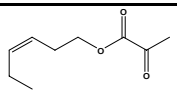
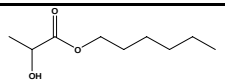
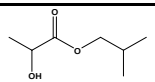
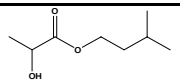
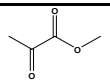
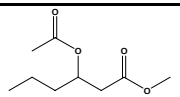
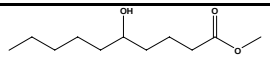
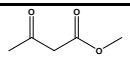
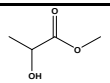
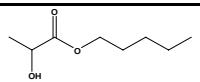
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
09.565 1846	Hex-3-enyl 2-oxopropionate		3934 10684 68133-76-6	Liquid $C_9H_{14}O_3$ 170.21	Practically insoluble or insoluble Freely soluble	76 (0.7 hPa) IR NMR 98 %	1.437-1.445 0.982-0.990	Register name to be changed to Hex-(3Z)-enyl 2-oxopropionate (EFFA, 2010a).
09.580	Hexyl lactate		 20279-51-0	Liquid $C_9H_{18}O_3$ 174.24	Slightly soluble Freely soluble	221 MS 95 %	1.426-1.432 0.951-0.957	Racemate.
09.590	Isobutyl lactate		 10709 585-24-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	182 MS 95 %	1.415-1.421 0.968-0.974	Racemate.
09.601	Isopentyl lactate		 10720 19329-89-6	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	202 MS 97 %	1.421-1.427 0.958-0.974	Racemate.
09.626	Methyl 2-oxopropionate		 10848 600-22-6	Liquid $C_4H_6O_3$ 120.09	Sparingly soluble Freely soluble	137 MS 95 %	1.401-1.407 1.145-1.151	
09.629	Methyl 3-acetoxyhexanoate		 10755 77118-93-5	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	55 (0.7 hPa) MS 95 %	1.420-1.426 1.013-1.019	Racemate. CASrn in Register to be changed to 21188-60-3. CASrn in Register refers to the (R) enantiomer.
09.633	Methyl 5-hydroxydecanoate		 101853-47-8	Solid $C_{11}H_{22}O_3$ 202.29	Practically insoluble or insoluble Freely soluble	278 28 MS 95 %	n.a. n.a.	Racemate.
09.634	Methyl acetoacetate		 105-45-3	Liquid $C_5H_8O_3$ 116.12	Sparingly soluble Freely soluble	169 28 MS 95 %	1.415-1.421 1.073-1.079	
09.644	Methyl lactate		 27871-49-4	Liquid $C_4H_8O_3$ 104.10	Sparingly soluble Freely soluble	244 MS 95 %	1.408-1.414 1.060-1.066	Register name to be changed to (S)-Methyl lactate.
09.683	Pentyl lactate		 6382-06-5	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	206 MS 95 %	1.423-1.429 0.965-0.971	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

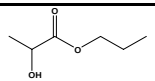
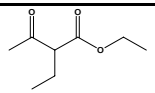
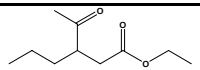
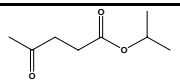
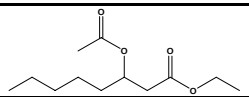
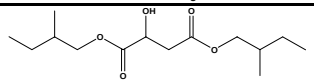
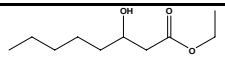
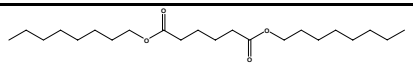
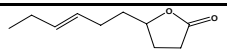
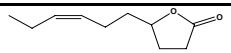
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.815	Propyl lactate		616-09-1	Liquid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	170 MS 95 %	1.414-1.420 1.000-1.006	Racemate.
09.824	Ethyl 2-acetylbutyrate		607-97-6	Liquid $C_8H_{14}O_3$ 158.20	Practically insoluble or insoluble Freely soluble	198 MS 95 %	1.417-1.423 0.982-0.988	Racemate.
09.832	Ethyl 3-acetohexanoate		10566 21188-61-4	Liquid $C_{10}H_{18}O_3$ 186.24	Practically insoluble or insoluble Freely soluble	110 (12 hPa) MS 95 %	1.419-1.425 1.009-1.015	Racemate.
09.833	iso-Propyl 4-oxopentanoate		21884-26-4	Liquid $C_8H_{14}O_3$ 158.20	Sparingly soluble Freely soluble	209 MS 95 %	1.418-1.424 0.981-0.987	
09.862	Ethyl 3-acetoxy octanoate		85554-66-1	Solid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	276 21 MS 95 %	n.a. n.a.	Racemate.
09.874	Di(2-methylbutyl) malate			Solid $C_{14}H_{26}O_5$ 274.35	Sparingly soluble Freely soluble	335 74 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register to be introduced 253596-99-5.
09.916	Ethyl 3-hydroxyoctanoate		10603 7367-90-0	Liquid $C_{10}H_{20}O_3$ 188.27	Practically insoluble or insoluble Freely soluble	118 (12 hPa) MS 95 %	1.421-1.427 0.973-0.979	Racemate (EFFA, 2010a).
09.951 1968	Dioctyl adipate		4476 123-79-5	Liquid $C_{22}H_{42}O_4$ 370.6	Insoluble Soluble	175 (3hPa) -70 MS 99 %	1.443-1.447 0.925	
10.038	Dec-7-eno-1,4-lactone		67114-38-9	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a). Composition of mixture to be specified.
10.039	cis-Dec-7-eno-1,4-lactone		63095-33-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

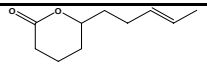
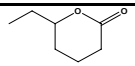
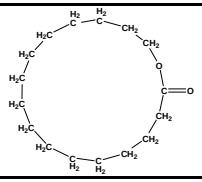
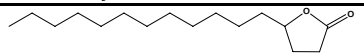
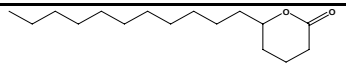
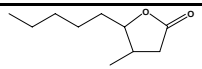
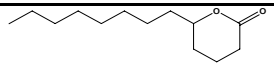
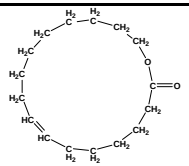
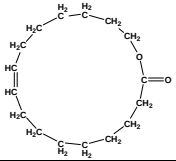
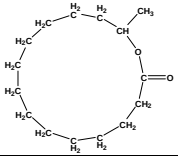
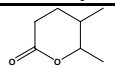
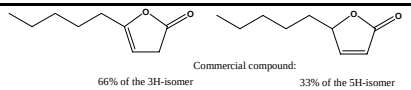
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.040	Dec-8-eno-1,5-lactone		32764-98-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	157 (15 hPa) MS 95 %	1.462-1.468 0.972-0.978	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a). Composition of mixture to be specified.
10.045	Heptano-1,5-lactone		10660 3301-90-4	Liquid $C_7H_{12}O_2$ 128.17	Practically insoluble or insoluble Freely soluble	104 (12 hPa) MS 95 %	1.451-1.457 1.031-1.037	Racemate.
10.047	Hexadecano-1,16-lactone		109-29-5	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	128 (1 hPa) 34 MS 95 %	n.a. n.a.	
10.048	Hexadecano-1,4-lactone		10673 730-46-1	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	185 (5 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.049	Hexadecano-1,5-lactone		10674 7370-44-7	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	130 (1 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.052	3-Methylnonano-1,4-lactone		33673-62-0	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	115 (3 hPa) MS 95 %	1.444-1.450 0.945-0.951	Racemate.
10.055	Pentano-1,5-lactone		10907 542-28-9	Liquid $C_5H_8O_2$ 100.12	Sparingly soluble Freely soluble	219 MS 95 %	1.451-1.457 1.101-1.107	
10.058	Tridecano-1,5-lactone		10902 7370-92-5	Liquid $C_{13}H_{24}O_2$ 212.33	Practically insoluble or insoluble Freely soluble	188 (15 hPa) MS 95 %	1.455-1.463 0.939-0.953	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.059	Hexadec-7-en-1,16-lactone 6)		123-69-3	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	188 (20 hPa) MS 95 %	1.482-1.488 0.955-0.961	CASrn in Register refers to the Z-isomer. Stereoisomeric composition to be specified.
10.063	Hexadec-9-en-1,16 lactone 6)		28645-51-4	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	131 (0.9 hPa) 95 %	1.476-1.482 0.953-0.959	ID 7). CASrn in Register does not specify isomeric composition. Stereoisomeric composition to be specified.
10.068	Pentadecano-1,14-lactone		32539-85-8	Liquid $C_{15}H_{28}O_2$ 240.38	Practically insoluble or insoluble Freely soluble	108 (0.1 hPa) MS 95 %	1.466-1.472 0.942-0.948	Racemate.
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		4141 10413-18-0	Liquid $C_7H_{12}O_2$ 128.17	Slightly soluble Freely soluble	60 NMR MS 98 %	1.452-1.458 1.019-1.025	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
10.170	5-Pentyl-3H-furan-2-one 6)	 Commercial compound: 66% of the 3H-isomer 33% of the 5H-isomer	4323 51352-68-2	Liquid $C_9H_{14}O_2$ 154.2	Sparingly soluble Soluble	73 at 1.2 Torr IR NMR MS 95	1.447-1.459 0.970-0.980	Mixture of 3H and 5H isomer (2:1) (Flavour Industry, 2010g). Stereoisomeric composition to be specified.

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.
7) ID: Missing identification test.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

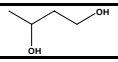
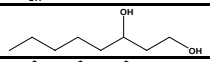
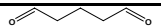
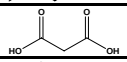
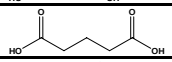
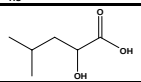
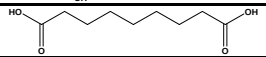
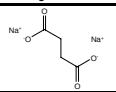
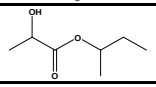
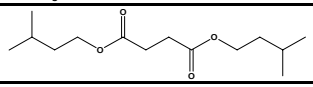
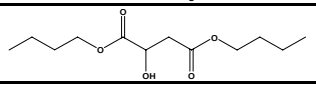
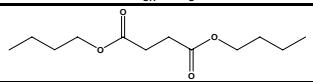
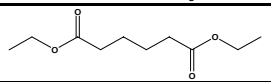
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	
02.198	Octane-1,3-diol		0.0012	Class I A3: Intake below threshold	4)	6)	
05.149	Glutaraldehyde		0.055	Class I A3: Intake below threshold	4)	6)	
07.169	1-Hydroxypropan-2-one		0.22	Class I A3: Intake below threshold	4)	6)	
08.053	Malonic acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.082	Glutaric acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.090	2-Hydroxy-4-methylvaleric acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.103	Nonanedioic acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.113	Succinic acid, disodium salt		1500	Class I A3: Intake below threshold	4)	6)	
09.333	sec-Butyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	
09.345	Di-isopentyl succinate		0.037	Class I A3: Intake below threshold	4)	6)	
09.346	Dibutyl malate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.347	Dibutyl succinate		0.12	Class I A3: Intake below threshold	4)	6)	
09.348	Diethyl adipate		0.027	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

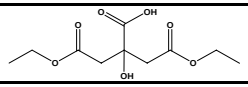
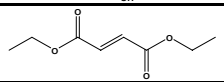
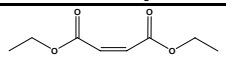
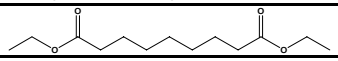
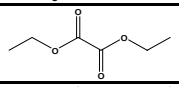
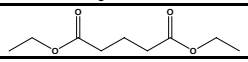
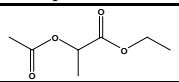
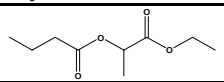
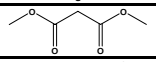
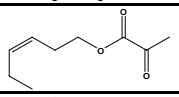
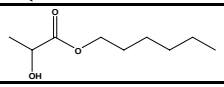
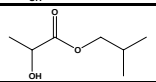
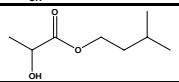
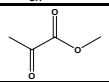
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.349	Diethyl citrate		0.12	Class I A3: Intake below threshold	4)	6)	
09.350	Diethyl fumarate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.351	Diethyl maleate		12	Class I A3: Intake below threshold	4)	6)	
09.352	Diethyl nonanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.353	Diethyl oxalate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.354	Diethyl pentanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.360	Ethyl 2-acetoxypropionate		4.9	Class I A3: Intake below threshold	4)	6)	
09.502	Ethyl butyryl lactate		0.5	Class I A3: Intake below threshold	4)	6)	
09.558	Dimethyl malonate		0.097	Class I A3: Intake below threshold	4)	6)	
09.565 1846	Hex-3-enyl 2-oxopropionate		0.74	Class I A3: Intake below threshold	4)	6)	
09.580	Hexyl lactate		0.49	Class I A3: Intake below threshold	4)	6)	
09.590	Isobutyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	
09.601	Isopentyl lactate		7.2	Class I A3: Intake below threshold	4)	6)	
09.626	Methyl 2-oxopropionate		0.024	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

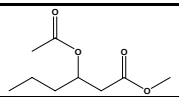
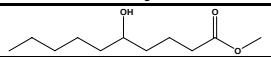
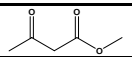
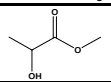
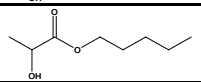
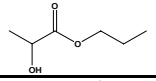
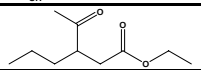
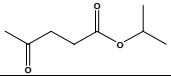
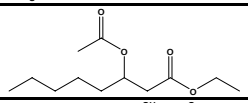
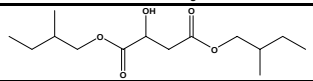
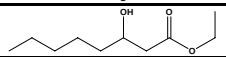
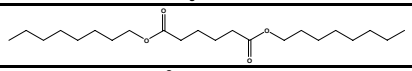
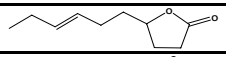
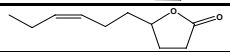
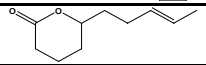
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.633	Methyl 5-hydroxydecanoate		0.24	Class I A3: Intake below threshold	4)	6)	
09.634	Methyl acetoacetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.644	Methyl lactate		0.34	Class I A3: Intake below threshold	4)	6)	
09.683	Pentyl lactate		0.61	Class I A3: Intake below threshold	4)	6)	
09.815	Propyl lactate		0.62	Class I A3: Intake below threshold	4)	6)	
09.832	Ethyl 3-acetohexanoate		0.33	Class I A3: Intake below threshold	4)	6)	
09.833	iso-Propyl 4-oxopentanoate		0.24	Class I A3: Intake below threshold	4)	6)	
09.862	Ethyl 3-acetoxy octanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.874	Di(2-methylbutyl) malate		0.015	Class I A3: Intake below threshold	4)	6)	
09.916	Ethyl 3-hydroxyoctanoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.951 1968	Dioctyl adipate		6.1	Class I A3: Intake below threshold	4)	6)	
10.038	Dec-7-eno-1,4-lactone		0.37	Class I A3: Intake below threshold	4)	7)	
10.039	cis-Dec-7-eno-1,4-lactone		1.2	Class I A3: Intake below threshold	4)	6)	
10.040	Dec-8-eno-1,5-lactone		0.011	Class I A3: Intake below threshold	4)	7)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

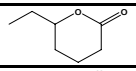
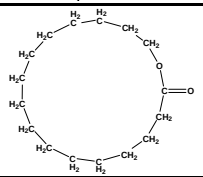
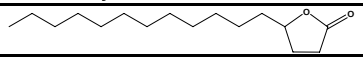
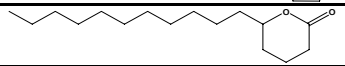
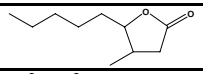
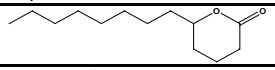
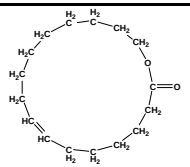
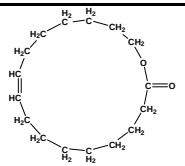
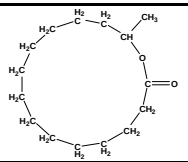
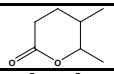
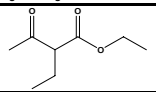
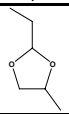
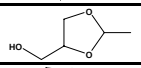
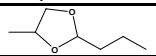
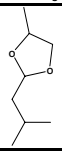
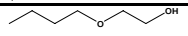
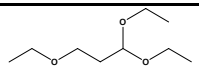
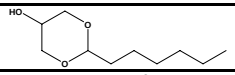
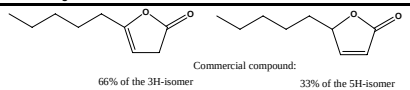
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.045	Heptano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	
10.047	Hexadecano-1,16-lactone		0.024	Class I A3: Intake below threshold	4)	6)	
10.048	Hexadecano-1,4-lactone		0.0061	Class I A3: Intake below threshold	4)	6)	
10.049	Hexadecano-1,5-lactone		0.024	Class I A3: Intake below threshold	4)	6)	
10.052	3-Methylnonano-1,4-lactone		0.61	Class I A3: Intake below threshold	4)	6)	
10.055	Pentano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	
10.058	Tridecano-1,5-lactone		0.61	Class I A3: Intake below threshold	4)	6)	
10.059	Hexadec-7-en-1,16-lactone		1.9	Class I A3: Intake below threshold	4)	7)	
10.063	Hexadec-9-en-1,16 lactone		48	Class I A3: Intake below threshold	4)	7)	
10.068	Pentadecano-1,14-lactone		0.9	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		1.2	Class I A3: Intake below threshold	4)	6)	
09.824	Ethyl 2-acetylbutyrate		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.088	2-Ethyl-4-methyl-1,3-dioxolane		0.0061	Class II A3: Intake below threshold	4)	6)	
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	
06.095	4-Methyl-2-propyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		1.2	Class II A3: Intake below threshold	4)	6)	
02.242	2-Butoxyethan-1-ol		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.097	1,1,3-Triethoxypropane		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		0.011	Class III A3: Intake below threshold	4)	6)	
10.170	5-Pentyl-3H-furan-2-one	 Commercial compound: 66% of the 3H-isomer 33% of the 5H-isomer	1.2	Class III No evaluation			a)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) 1/3 of the named compound correspond to FL-no: 10.054 which is included in FGE.217: additional genotoxicity data required.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

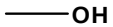
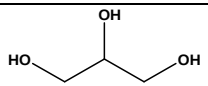
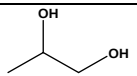
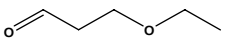
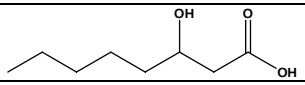
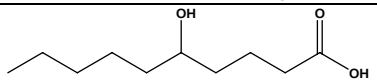
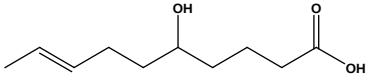
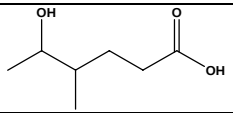
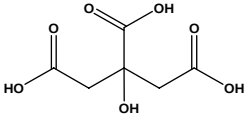
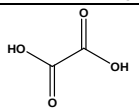
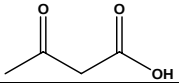
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	3-Ethoxypropan-1-al		Not evaluated as flavouring substance		Not in EU-Register
	3-Hydroxyoctanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxydecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-8-decenoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	Citric acid		Not evaluated as flavouring substance		Not in EU-Register
	Oxalic acid		Not evaluated as flavouring substance		Not in EU-Register
	Acetoacetic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

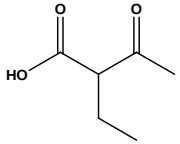
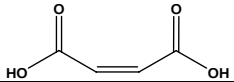
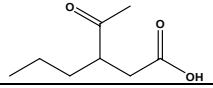
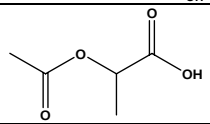
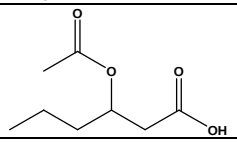
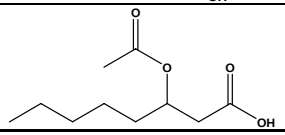
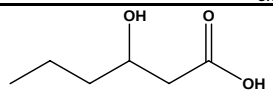
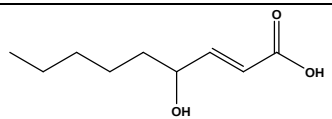
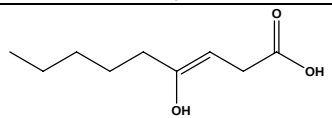
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Acetylbutyric acid		Not evaluated as flavouring substance		Not in EU-Register
	Maleic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetohexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	2-Acetoxypropionic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetoxyhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetoxyoctanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Hydroxyhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxy-2-nonenoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxy-3-nonenoic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

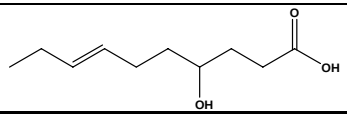
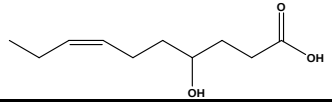
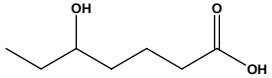
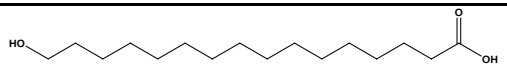
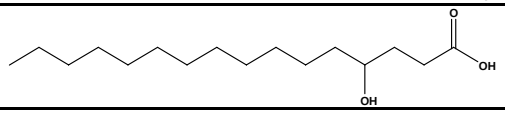
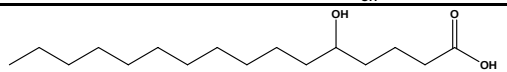
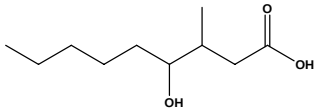
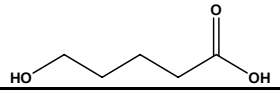
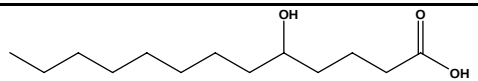
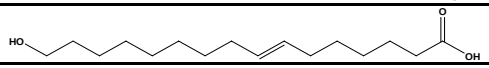
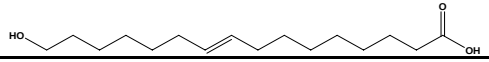
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	(E)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	(Z)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxyheptanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxy-3-methylnonanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxypentanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxytridecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-9-enoic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

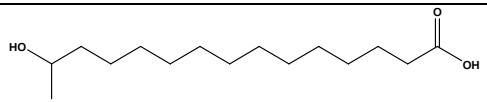
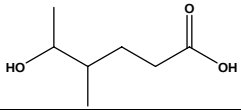
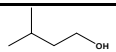
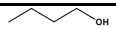
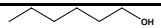
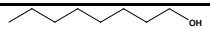
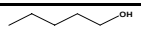
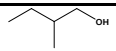
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	14-Hydroxypentadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
02.001	2-Methylpropan-1-ol 251		Category 1 a) Category A b)	Class I A3: Intake above threshold	
02.002	Propan-1-ol 82		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.006	Octan-1-ol 97		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern e) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern d)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

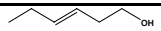
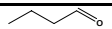
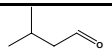
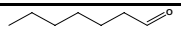
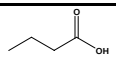
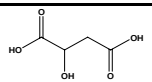
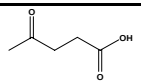
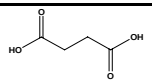
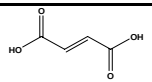
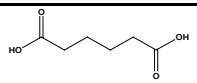
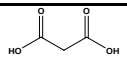
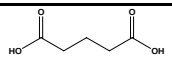
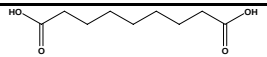
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
					used as flavouring agents.
02.079	Isopropanol 277		Category 1 a) No safety concern f)	Class I A3: Intake above threshold, A4: Endogenous	
02.121	Butan-2-ol		Category 1 a)	No evaluation	
02.159	Hex-3-en-1-ol 315		Category A c)	No evaluation	
05.001	Acetaldehyde 80		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.002	Propanal 83		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.003	Butanal 86		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.006	3-Methylbutanal 258		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.031	Heptanal 95		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern g) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.017	l-Malic acid 619		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.019	Pyruvic acid 936		No safety concern g) Category A c)	Class I A3: Intake below threshold	
08.023	4-Oxovaleric acid 606		No safety concern h) Category A c)	Class I A3: Intake below threshold	
08.024	Succinic acid		Category A c)	No evaluation	
08.025	Fumaric acid 618		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.026	Adipic acid 623		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
08.053	Malonic acid		Category A c) FGE.10	Class I A3: Intake below threshold	
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.103	Nonanedioic acid		FGE.10	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1999b).
c) (CoE, 1992).
d) (JECFA, 1997a).
e) (JECFA, 2004a).
f) (JECFA, 2000a).
g) (JECFA, 2002b).
h) (JECFA, 2000b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

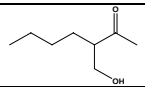
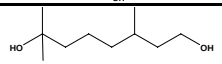
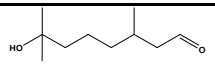
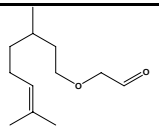
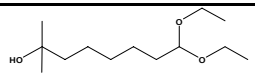
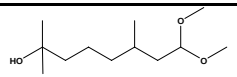
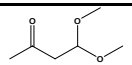
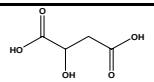
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2003b)	4.6	No safety concern d) Category B	Not in EU-Register.
02.047	3,7-Dimethyloctane-1,7-diol		2586 559 107-74-4	610 JECFA specification (JECFA, 2000d)	9.7	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
05.012	3,7-Dimethyl-7-hydroxyoctanal		2583 100 107-75-5	611 JECFA specification (JECFA, 1999c)	24	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal (CASrn as in Register). CASrn in Register refers to the racemate.
05.079	Citronellyl oxyacetaldehyde		2310 2012 7492-67-3	592 JECFA specification (JECFA, 2003b)	24	No safety concern a) Category B b)	JECFA evaluated citronelloxyacetaldehyde (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.010	1,1-Diethoxy-3,7-dimethyloctan-7-ol		2584 44 7779-94-4	613 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	JECFA evaluated hydroxycitronellal diethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.011	1,1-Dimethoxy-3,7-dimethyloctan-7-ol		2585 45 141-92-4	612 JECFA specification (JECFA, 1999c)	0.037	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal dimethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.038	4,4-Dimethoxybutan-2-one		3381 10029 5436-21-5	593 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	
08.017	l-Malic acid		2655 17 6915-15-7	619 JECFA specification (JECFA, 2000d)	13000	No safety concern a) Category A b)	JECFA evaluated l-malic acid (CASrn 97-67-6). (R)- or (S)- enantiomer not specified by CASrn in Register. GrADI: not specified

Table 3: Supporting Substances Summary

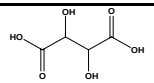
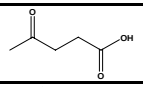
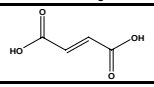
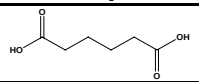
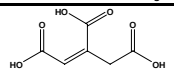
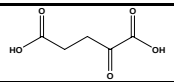
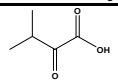
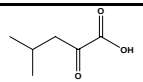
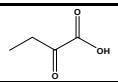
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							(JECFA, 1970a).
08.018	Tartaric acid		3044 18 133-37-9	621 JECFA specification (JECFA, 1999c)	3800	No safety concern a) Category A b)	JECFA evaluated tartaric acid ((+)-, (-)-, (+/-)-, meso-) (CASrn 87-69-4). CASrn in Register refers to (2R,3R)-isomer. No ADI (JECFA, 1978a).
08.023	4-Oxovaleric acid		2627 23 123-76-2	606 JECFA specification (JECFA, 2002d)	190	No safety concern a) Category A b)	
08.025	Fumaric acid		2488 25 110-17-8	618 JECFA specification (JECFA, 2000d)	780	No safety concern a) Category A b)	GrADI not specified (JECFA, 1990a).
08.026	Adipic acid		2011 26 124-04-9	623 JECFA specification (JECFA, 1999c)	11	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1978a).
08.033	Prop-1-ene-1,2,3-tricarboxylic acid		2010 33 499-12-7	627 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category A b)	JECFA evaluated aconitic acid (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
08.037	2-Oxoglutaric acid		3891 653 328-50-7	634 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category A b)	
08.051	3-Methyl-2-oxobutyric acid		3869 2262 759-05-7	631 JECFA specification (JECFA, 1999c)	0.012	No safety concern a) Category B b)	JECFA evaluated 3-methyl-2-oxobutanoic acid (the acid and sodium salt) (CASrn as in Register). CASrn in Register refers to the acid.
08.052	4-Methyl-2-oxovaleric acid		3871 2263 816-66-0	633 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category B b)	JECFA evaluated 4-Methyl-2-oxopentanoic acid and its sodium salt (CASrn 816-66-0 and 4502-00-5).
08.066	2-Oxobutyric acid		3723 600-18-0	589 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	

Table 3: Supporting Substances Summary

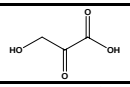
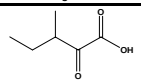
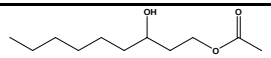
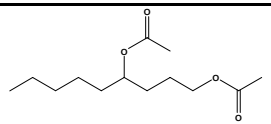
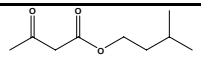
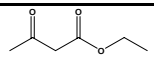
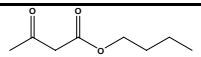
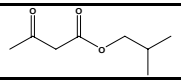
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.086	3-Hydroxy-2-oxopropionic acid		3843 1113-60-6	635 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
08.093	3-Methyl-2-oxovaleric acid		3870 10146 39748-49-7	632 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	JECFA evaluated 3-methyl-2-oxopentanoic acid (the acid and sodium salt) (CASrn 1460-34-0). CASrn 39748-49-7 replaced by CASrn 1460-34-0 in the CASrn system (SciFinder). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.225	1,3-Nonanediol acetate		2783 2075 1322-17-4	605 JECFA specification (JECFA, 2005b)	1.8	No safety concern a) Deleted b)	Reg. CASrn refers to incompletely defined substance (mixed esters). Deleted: Subst. for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available (CoE, 1992).
09.280	Nonane-1,4-diyl diacetate		3579 11927 67715-81-5	609 JECFA specification (JECFA, 2002d)	0.037	No safety concern a)	JECFA evaluated 1,4-nonanediol diacetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.401	Isopentyl acetoacetate		3551 227 2308-18-1	598 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.402	Ethyl acetoacetate		2415 240 141-97-9	595 JECFA specification (JECFA, 1999c)	1200	No safety concern a) Category B b)	
09.403	Butyl acetoacetate		2176 241 591-60-6	596 JECFA specification (JECFA, 2000d)	63	No safety concern a) Category B b)	
09.404	Isobutyl acetoacetate		2177 242 7779-75-1	597 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

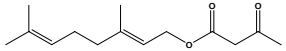
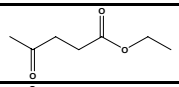
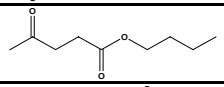
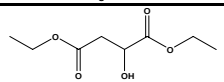
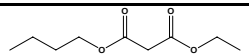
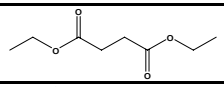
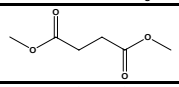
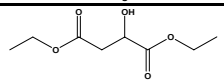
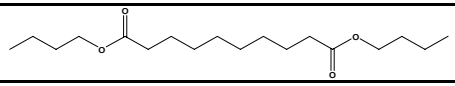
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.405	Geranyl acetoacetate		2510 243 10032-00-5	599 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	
09.435	Ethyl 4-oxovalerate		2442 373 539-88-8	607 JECFA specification (JECFA, 1999c)	470	No safety concern a) Category B b)	
09.436	Butyl 4-oxovalerate		2207 374 2052-15-5	608 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.439	Diethyl malate		2374 382 7554-12-3	620 JECFA specification (JECFA, 2000d)	3.7	No safety concern a) Deleted b)	JECFA evaluated diethyl malate. CASrn in Register refers to the racemate. Deleted: Subst. for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available(CoE, 1992).
09.441	Butyl ethyl malonate		2195 384 17373-84-1	615 Tentative JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	
09.444	Diethyl succinate		2377 438 123-25-1	617 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category B b)	
09.445	Dimethyl succinate		2396 439 106-65-0	616 JECFA specification (JECFA, 2002d)	73	No safety concern a) Category B b)	
09.446	Diethyl tartrate		2378 440 87-91-2	622 JECFA specification (JECFA, 2002d)	15	No safety concern a) Category A b)	JECFA evaluated diethyl tartrate (CASrn as in Register). Register CASrn refers to the (2R,3R)-enantiomer. ADI acceptable (JECFA, 2000b).
09.474	Dibutyl sebacate		2373 622 109-43-3	625 JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

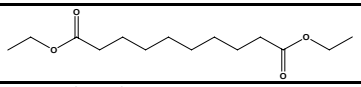
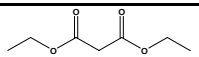
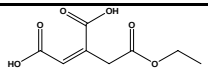
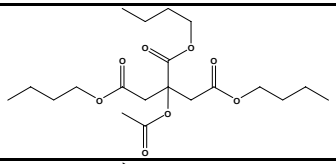
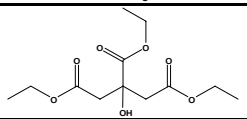
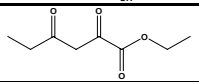
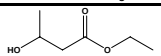
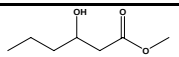
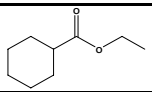
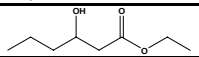
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.475	Diethyl sebacate		2376 623 110-40-7	624 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category A b)	
09.490	Diethyl malonate		2375 2106 105-53-3	614 JECFA specification (JECFA, 2002d)	650	No safety concern a) Category A b)	
09.510	Ethyl aconitate		2417 11845 1321-30-8	628 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	JECFA evaluated ethyl aconitate (mixed esters) (CASrn as in Register). Register CASrn refers to incompletely defined substance.
09.511	Tributyl acetylcitrate		3080 77-90-7	630 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
09.512	Triethyl citrate		3083 11762 77-93-0	629 JECFA specification (JECFA, 2000d)	2900	No safety concern a)	ADI: 0-20 (JECFA, 1984a).
09.514	Ethyl 2,4-dioxohexanoate		3278 11903 13246-52-1	603 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
09.522	Ethyl 3-hydroxybutyrate		3428 10596 5405-41-4	594 JECFA specification (JECFA, 2000d)	7.9	No safety concern a)	JECFA evaluated ethyl 3-hydroxybutyrate (CASrn as in Register). Register CASrn refers to the racemate.
09.532	Methyl 3-hydroxyhexanoate		3508 10812 21188-58-9	600 JECFA specification (JECFA, 2000d)	0.85	No safety concern a)	JECFA evaluated methyl 3-hydroxyhexanoate (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
09.533	Ethyl brassylate		3543 10571 105-95-3	626 JECFA specification (JECFA, 2002d)	3.0	No safety concern a)	
09.535	Ethyl 3-hydroxyhexanoate		3545 11764	601 JECFA specification (JECFA,	60	No safety concern a)	JECFA evaluated ethyl 3-hydroxyhexanoate

Table 3: Supporting Substances Summary

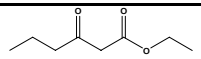
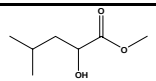
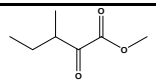
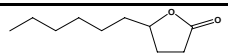
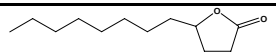
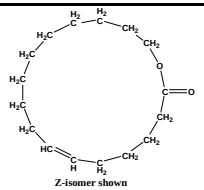
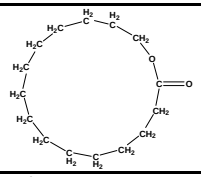
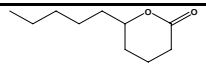
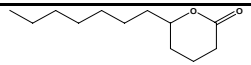
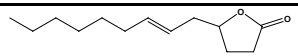
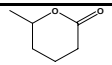
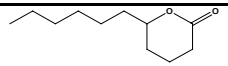
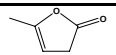
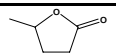
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
			2305-25-1	2002d)			(CASrn as in Register). Register CASrn refers to the racemate.
09.542	Ethyl 3-oxohexanoate		3683 3249-68-1	602 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	
09.548	Methyl 2-hydroxy-4-methylvalerate		3706 40348-72-9	590 JECFA specification (JECFA, 2003b)	0.49	No safety concern a)	JECFA evaluated methyl 2-hydroxy-4-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
09.550	Methyl 2-oxo-3-methylvalerate		3713 3682-42-6	591 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	JECFA evaluated methyl 2-oxo-3-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.001	Nonano-1,4-lactone		2781 178 104-61-0	229 JECFA specification (JECFA, 2000d)	1000	No safety concern c) Category A b)	JECFA evaluated gamma-nonalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn ADI: 0-1.25 (JECFA, 1968).
10.002	Undecano-1,4-lactone		3091 179 104-67-6	233 JECFA specification (JECFA, 1998b)	1200	No safety concern c) Category A b)	JECFA evaluated gamma-undecalactone (CASrn as in Register). Register CASrn refers to the racemate. ADI: 0-1.25 (JECFA, 1968).
10.003	Hexadec-6-eno-1,16-lactone	 Z-isomer shown	2555 180 7779-50-2	240 JECFA specification (JECFA, 2001c)	5.1	No safety concern c) Category B b)	JECFA evaluated omega-6-hexadecenlactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.004	Pentadecano-1,15-lactone		2840 181 106-02-5	239 JECFA specification (JECFA, 2000d)	73	No safety concern c) Category B b)	
10.006	Butyro-1,4-lactone		3291 615 96-48-0	219 JECFA specification (JECFA, 1998b)	110	No safety concern c) Category A b)	
10.007	Decano-1,5-lactone		2361 621 705-86-2	232 JECFA specification (JECFA, 2000d)	7200	No safety concern c) Category B b)	JECFA evaluated delta-decalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.008	Dodecano-1,5-lactone		2401 624 713-95-1	236 JECFA specification (JECFA, 2000d)	5800	No safety concern c) Category B b)	JECFA evaluated delta-dodecalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.009	Dodec-6-eno-1,4-lactone		3780 625 18679-18-0	249 JECFA specification (JECFA, 2001c)	0.012	No safety concern c) Category A b)	JECFA evaluated 1,4-dodec-6-enolactone (CASrn as in Register). Register CASrn refers to the (Z)-isomer.
10.010	Hexano-1,5-lactone		3167 641 823-22-3	224 JECFA specification (JECFA, 1998b)	320	No safety concern c) Category B b)	JECFA evaluated delta-hexalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.011	Undecano-1,5-lactone		3294 688 710-04-3	234 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	JECFA evaluated 5-hydroxyundecanoic acid delta-lactone (CASrn as in Register). Register CASrn refers to the racemate.
10.012	5-Methylfuran-2(3H)-one		3293 731 591-12-8	221 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	
10.013	Pentano-1,4-lactone		3103 757 108-29-2	220 JECFA specification (JECFA, 1998b)	120	No safety concern c) Category A b)	JECFA evaluted gamma-valerolactone (CASrn as in Register). Register CASrn refers to the racemate.

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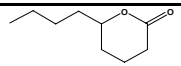
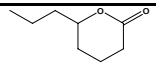
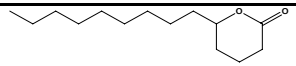
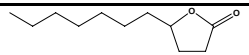
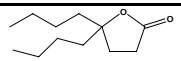
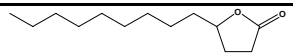
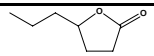
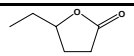
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.014	Nonano-1,5-lactone		3356 2194 3301-94-8	230 JECFA specification (JECFA, 1998b)	130	No safety concern c) Category B b)	JECFA evaluated hydroxynonanoic acid delta-lactone (CASrn as in Register). Register CASrn refers to the racemate.
10.015	Octano-1,5-lactone		3214 2195 698-76-0	228 JECFA specification (JECFA, 2000d)	230	No safety concern c) Category B b)	JECFA evaluated delta-octalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.016	Tetradecano-1,5-lactone		3590 2196 2721-22-4	238 JECFA specification (JECFA, 1998b)	110	No safety concern c) Category B b)	JECFA evaluated delta-tetradecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.017	Decano-1,4-lactone		2360 2230 706-14-9	231 JECFA specification (JECFA, 1998b)	1600	No safety concern c) Category A b)	JECFA evaluated gamma-decalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.018	4-Butyloctano-1,4-lactone		2372 2231 7774-47-2	227 JECFA specification (JECFA, 2000d)	0.12	No safety concern c) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technological and/or toxicological information was available (CoE, 1992).
10.019	Dodecano-1,4-lactone		2400 2240 2305-05-7	235 JECFA specification (JECFA, 1998b)	190	No safety concern c) Category A b)	JECFA evaluted gamma-dodecalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.020	Heptano-1,4-lactone		2539 2253 105-21-5	225 JECFA specification (JECFA, 2000d)	170	No safety concern c) Category A b)	JECFA evaluated gamma-heptalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.021	Hexano-1,4-lactone		2556 2254 695-06-7	223 JECFA specification (JECFA, 1998b)	160	No safety concern c) Category A b)	JECFA evaluted gamma-hexalactone (CASrn as in Register). Register CASrn refers to

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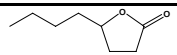
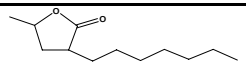
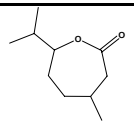
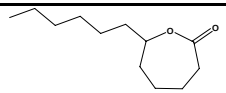
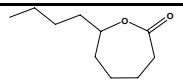
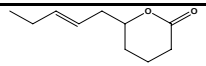
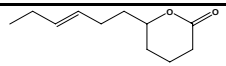
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							the racemate.
10.022	Octano-1,4-lactone		2796 2274 104-50-7	226 JECFA specification (JECFA, 2000d)	430	No safety concern c) Category A b)	JECFA evaluated gamma-octalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.026	3-Heptyldihydro-5-methyl-2(3H)-furanone		3350 10953 40923-64-6	244 JECFA specification (JECFA, 2003b)	0.037	No safety concern c)	JECFA evaluated 3-heptyldihydro-5-methyl-2(3H)-furanone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.027	3,7-Dimethyloctano-1,6-lactone		3355 11833 499-54-7	237 JECFA specification (JECFA, 2003b)	0.012	No safety concern c)	JECFA evaluated 6-hydroxy-3,7-dimethyloctanoic acid lactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.028	Dodecano-1,6-lactone		3610 16429-21-3	242 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated epsilon-dodecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.029	Decano-1,6-lactone		3613 5579-78-2	241 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated epsilon-decalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.033	Dec-7-eno-1,5-lactone		3745 34686-71-0	247 JECFA specification (JECFA, 2000d)	0.22	No safety concern c)	JECFA evaluated 5-Hydroxy-7-decenoic acid delta-lactone (CASrn 25524-95-2 which refers to the (Z)-isomer). Neither (Z)- or (E)-isomer nor (R)- or (S)-enantiomer specified by Register CASrn.
10.035	Undec-8-eno-1,5-lactone		3758 68959-28-4	248 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated 5-hydroxy-8-undecenoic acid delta-lactone (CASrn as in Register). (R)- or (S)-enantiomer

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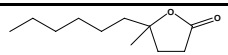
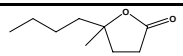
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							not specified by Register CASrn.
10.051	5-Hexyl-5-methyldihydrofuran-2(3H)-one		3786 7011-83-8	250 JECFA specification (JECFA, 1998b)	ND	No safety concern c)	JECFA evaluated gamma-methyldecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.053	3-Methyloctano-1,4-lactone		3803 10535 39212-23-2	437 JECFA specification (JECFA, 1998b)	ND	No safety concern c)	JECFA evaluated 4-hydroxy-3-methyloctanoic acid gamma-lactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2000b).

b) (CoE, 1992).

c) (JECFA, 1999b).

d) (JECFA, 2000c).

ND No intake data reported.

1 REFERENCES

Aeschbacher HU, Wolleb U, Loliger J, Spadone JC and Liardon R, 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem. Toxicol. 27(4), 227-232.

Al-Ani FY and Al-Lami SK, 1988. Absence of mutagenic activity of acidity regulators in the Ames Salmonella/microsome test. Mutat. Res. 206, 467-470.

Albro PW, 1975. The metabolism of 2-ethylhexanol in rats. Xenobiotica 5(10), 625-636.

Aldridge WN, 1953. Serum esterases. 1. Two types of esterase (a and b) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. Biochem. J. 53, 110-117.

Anders MW, 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J and Paulson GD (Eds.). Intermediary xenobiotic metabolism in animals. Taylor and Francis, New York, pp 81-97.

Andersen PH and Jensen NJ, 1984. Mutagenic investigation of flavourings: dimethyl succinate, ethyl pyruvate and aconitic acid are negative in the Salmonella/mammalian-microsome test. Food Addit. Contam. 1(3), 283-288.

Arena C and Fung HL, 1980. Absorption of sodium gamma-hydroxybutyric acid and its prodrug gamma - butyrolactone: relationship between *in vitro* transport and *in vivo* absorption. J. Pharm. Sci. 69, 356-358.

Ashley DL, Bonin MA, Cardinali FL, McCraw JM, and Wooten JV, 1994. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin. Chem. 40, 1401-1404.

Baker RSU and Bonin AM, 1981. Study of 42 coded compounds with the Salmonella/mammalian microsome assay. Prog. Mutat. Res. 1, 249-260.

Ballantyne B and Myers RC, 2001. Related articles: The acute toxicity and primary irritancy of glutaraldehyde solutions. Vet. Hum. Toxicol. 43(4), 193-202.

Bär F and Griepentrog F, 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. [Where we stand concerning the evaluation of flavoring substances from the viewpoint of health]. Med. Ernähr. 8, 244-251.

Barnhart JL and Combes B, 1978. Choleresis associated with metabolism and biliary excretion of diethyl maleate in the rat and dog. J. Pharmacol. Exp. Ther. 206(3), 614-623.

BASF, 1956. Abt. Toxikologie, unveroeffentliche Untersuchung (V/420), 17.04.1956. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 111-76-2, EINECS Name 2-butoxyethanol. Section 5 Toxicity.

BASF, 1978. Abteilung Toxikologie, unveroeffentlichte Untersuchung (XXVI/531), 02/22/78. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 105-45-3, EINECS Name methyl acetoacetate. Section 5.1.1 Acute oral toxicity.

Bernstein ME, 1984. Agents affecting the male reproductive system: Effects of structure on activity. Drug Metab. Rev. 15, 941-996.

Besrat A, Polan CE and Henderson LM, 1969. Mammalian metabolism of glutaric acid. J. Biol. Chem. 244(6), 1461-1467.

Billecke S, Draganov D, Counsell R, Stetson P, Watson C, Hsu C and La Du B, 2000. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. Drug Metab. Disposition 28(11), 1335-1342.

Bio-Fax, 1971. Bio-Fax Industrial Bio-test Lab., Inc., Data sheets. (1810 Frontage Rd., Northbrook, IL 60062). Cited in The Registry of Toxic Effects of Chemical Substances. Malonic acid. RTECS OO0175000. CAS 141,82-2. Update: January 1997.

Bornmann C, 1954. Grundwirkungen der glykole und ihre Bedeutung für die toxizität. Arzneim.-Forsch./Drug Res. 4(643), 710-715.

Bosron WF and Li TK, 1980. Alcohol dehydrogenase. In: Jakoby WB (Ed.). Enzymatic Basis of Detoxification vol. 1. Academic Press, New York, 231-248.

Boyland E and Chasseaud LF, 1970. The effect of some carbonyl compounds on rat liver glutathione levels. Biochem. Pharmacol. 19(4), 1526-1528.

Boyland E, 1940. 142. Experiments on the chemotherapy of cancer. 4. Further experiments with aldehydes and their derivatives. Biochem. J. 34(8/9), 1196-1201.

Bradford JC, Brown GL, Caldwell JA and Drobeck HP, 1984. Teratology and mutagenicity studies with glutaric acid. Teratology 29(2), 19A.

Brauninger RM, 1995. Clonal transformation assay on RO434.01 DRD:HESE 415 using Syrian golden hamster embryo (SHE) cells with cover letter dated 08/11/95. Ethylene glycol monobutyl ether. EPA Doc 8695000406, microfiche no. OTS0557846. Unpublished data submitted by EFFA to SCF.

Brooks TM and Dean BJ, 1981. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay with preincubation. Prog. Mutat. Res. 1, 261-270.

Bushy Run Research Center, 1989. Glutaraldehyde: ninety day drinking water toxicity study in mice. Unpublished data submitted by Union Carbide, Bound Brook, NJ. Cited in Anonymous, 1996. Final report on the safety assessment of glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Bushy Run Research Center, 1990. Glutaraldehyde: 13 week study in dogs with administration via the drinking water. Unpublished data submitted by Union Carbide, Bound Brook, NJ. Cited in Anonymous, 1996. Final report on the safety assessment of glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Carpenter CP, Pozzani UC, Weil CS, Nair JH, Keck GA and Smyth HF, 1956. The toxicity of butyl cellosolve solvent. Arch. Ind. Health 14, 114-131.

Chiewchanwit T and Au WW, 1995. Mutagenicity and cytotoxicity of 2-butoxyethanol and its metabolite, 2-butoxyacetaldehyde, in Chinese hamster ovary (CHO-AS52) cells. Mutat. Res. 344(3), 341-346.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Cook WM, Purchase R, Ford GP, Creasy DM, Brantom PG and Gangolli SD, 1992. A 28-day feeding study with ethyl acetoacetate in rats. Food Chem. Toxicol. 30(7), 567-573.

Corley RA, Bormett GA and Ghanyem BI, 1994. Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. Toxicol. Appl. Pharmacol. 129(1), 61-79.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

CTFA (Cosmetic, Toiletry and Fragrance Association), 1978. Acute oral toxicity test of products containing butylene glycol. (CTFA code 2-17-79). Unpublished data submitted by EFFA to SCF.

Dambly C, Thoman Z and Radman M, 1981. Zorotest. Prog. Mutat. Res. 1, 219-223.

Dargel R, 1966. Ausscheidung von Dimethylamin unter Zufuhr methylierter Stickstoffverbindungen. Acta Biol. Med. Germ. 16, 474-479. (In German)

Dean BJ, 1981. Activtiy of 27 coded compounds in the RL1 chromosome assay. Prog. Mutat. Res. 1, 570-579.

Deichmann W, Hirose BR and Witherup S, 1945. Observation on the effect of gamma-valerolactone upon experimental animals. J. Ind. Hyg. Toxicol. 27(9), 263-268.

Deisinger PJ, Boatman RJ and Guest D, 1994. Metabolism of 2-ethylhexanol administered orally and dermally to the female Fischer 344 rat. Xenobiotica 24(5), 429-440.

Deuel Jr HJ, 1957. The lipids, their chemistry and biochemistry. Vol. III Biochemistry, Biosynthesis, Oxidation, Metabolism and Nutritional Value. Chapter III: The oxidation and metabolism of triglycerides, fatty acids, and glycerol in the animal body. Interscience Publishers Inc., New York.

Dick RB, Brown WD, Setzer JV, Taylor BJ and Shukla R, 1988. Effects of short duration exposures to acetone and methyl ethyl ketone. Toxicol Lett. 43, 31-49.

Dillon D, Combes R and Zeiger E, 1998. The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. Mutagenesis 13(1), 19-26.

Doherty JD and Roth RH, 1978. Metabolism of gamma-hydroxy-[1-14 C] butyrate by rat brain: relationship to the Krebs cycle and metabolic compartmentation of amino acids. J. Neurochem. 30, 1305-1309.

Dow Chemical Company, 1982a. Unveroeffentlichte Untersung. Zit. In: Clayton, G.D., Clayton, F.E. (Eds.). Patty's Industrial Hygiene and Toxicology 2C. 3rd Ed. John Wiley & Sons, New York, p. 3933.

Eastman Kodak Company, 1984. Toxicity studies with diethylene glycol monobutyl ether with cover letter dated 05/30/84. EPA Doc 40-8478008, microfiche no. OTS0512376. April, 1984. Unpublished data submitted by EFFA to SCF.

Eastman Kodak Company, 1989. Material safety data sheet. And acute oral LD50 for 2-butoxyethanol with cover letter dated 04/19/89. EPA Doc 86-89000019, microfiche no. OTS0516735. December 27, 1988. Unpublished data submitted by EFFA to SCF.

EC, 1996a. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EFFA, 2000c. Submission 2000-1 rev. Assessment of 19 flavouring substances (candidate chemicals) of the chemical groups 1 and 2 (Annex I of 1565/2000/EC), structurally related to esters of aliphatic acyclic primary alcohols and branched-chain aliphatic acyclic carboxylic acids from TRS 884; FAO/JECFA 49/52. December 10, 2000. SCOOP/FLAV/8.1 rev.1. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.

EFFA, 2001a. Submission 2000-2. Assessment of 96 flavouring substances (candidate chemicals) of the chemical groups 1 and 2 (Annex I of 1565/2000/EC), structurally related to esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids from TRS 884; FAO/JECFA 49/52. February 2, 2001. SCOOP/FLAV/8.2.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2003c. Submission 2002-3. Flavouring group evaluation of 49 flavouring substances (candidate chemicals) of the chemical group 9 (Annex I of 1565/2000/EC), structurally related to aliphatic lactones [FAO/WHO JECFA 40/49] and aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups [FAO/WHO JECFA 44/53] used as flavouring substances. November 20, 2002. SCOOP/FLAV/8.16.

EFFA, 2003d. Submission 2002-3. Flavouring group evaluation of 49 flavouring substances (candidate chemicals) of the chemical group 9 (Annex I of 1565/2000/EC), structurally related to aliphatic lactones [FAO/WHO JECFA 40/49] and aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups [FAO/WHO JECFA 44/53] used as flavouring substances. November 20, 2002. SCOOP/FLAV/8.16. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.

EFFA, 2003s. Submission of 2002-Addendum 1+2. Supplement of 22 flavouring substances (candidate chemicals) of the chemical group 1 and 2 (Annex I of 1565/2000/EC) structurally related to to esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids and branched-chain aliphatic acyclic carboxylic acids used as flavouring substances. 20 December 2002. FLAVIS/8.72. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EFFA, 2004ag. Submission 2002-3 Addendum. Supplement of four flavouring substances (candidate chemicals) to the flavouring group evaluation of the chemical group 9 (Annex I of 1565/2000/EC) structurally related to aliphatic lactones [FAO/WHO JECFA 40/49] and aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups [FAO/WHO JECFA 44/53] used as flavouring substances. March 31, 2004. FLAVIS/8.82. Unpublished report submitted by EFFA to FLAVIS secretariat.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFFA, 2006ac. EFFA Letter to EFSA for clarification of specifications and isomerism for which data were requested in Rev10.

EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

EFFA, 2008b. Poundage data on selected substances. Private communication from EFFA to the FLAVIS secretariat. 19 December 2008. FLAVIS/8.113.

EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true

EFSA, 2005b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 10: Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 28 October 2005. EFSA-Q-2003-153a.

EFSA, 2008b. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27 - 29 November 2007. Parma, 7 January 2008. [Online]. Available: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf

Elias Z, Danière MC, Marande AM, Poirot O, Terzetti F and Schneider O, 1996. Genotoxic and/or epigenetic effects of some glycol ethers: Results of different short-term tests. Occup. Hyg. 2(1-6), 187-212.

Ema M, Itami T and Kawasaki H, 1992. Teratological assessment of glutaraldehyde in rats by gastric intubation. Toxicol. Lett. 63(2), 147-153.

Engel K-H, 2003. Personal communication to the FLAVIS working group. 14 November, 2003.

EPA, 1971. Initial submission: Acute oral toxicity of AAD in rats (final report) with cover letter dated 112191 (sanitized). Submitting organization: confidential. 4,4-dimethoxy-2-butanone. EPA Doc 88-920000222S, microfiche no. OTS0534674. June 9, 1971. Unpublished data submitted by EFFA to SCF.

US-EPA, 1999. Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE) (CAS nr 111-76-2) in support of summary information on the Integrated Risk Information System (IRIS), October, 1999. Downloaded from IRIS Home page http://www.epa.gov/iris, October, 2007.

EU-RAR (European Union Risk Assessment Report), 2004a. EU-RAR on 2-butoxyethanol (CAS no: 111-76-2; EINECS no: 203-905-0). Draft human health section. August, 2004. European Chemicals Bureau, Institute for Health and Consumer Protection, Ispra, Italy.

EU-RAR (2007) European Risk Assessment Report 2-butoxyethanol (CAS no: 111-76-2; EINECS No: 203-905-0). Draft human health section, version July 2007. Available through: European Chemicals Bureau, Institute for Health and Consumer Protection, Ispra, Italy.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

Exon JH, Mather GG, Bussiere JL, Olson DP and Talcott PA, 1991. Effects of subchronic exposure of rats to 2-methoxyethanol or 2-butoxyethanol: Thymic atrophy and immunotoxicity. Fundam. Appl. Toxicol. 16(4), 830-840.

Fassett D, 1961. Biological investigation of lactones as flavoring agents for margarine. March 16, 1961. Unpublished data submitted by EFFA to SCF.

Feldman RI and Weiner H, 1972. Horse liver aldehyde dehydrogenase. I. Purification and characterization. J. Biol. Chem. 247(1), 260-266.

Fenaroli's Handbook of Flavor Ingredients, Edited by Burdock GA, Virginia, 3rd Ed., 1995 vol I + II. CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

Fey EG, White HA and Rabin BR, 1981. Development of the degranulation test system. Prog. Mutat. Res. 1, 236-244.

Finkelstein M and Gold H, 1959. Toxicology of the citric acid esters: Tributyl citrate, acetyl tributyl citrate, triethyl citrate, and acetyl triethyl citrate. Toxicol. Appl. Pharmacol. 1, 283-298.

Fishbein WN and Bessman SP, 1966. Purification and properties of an enzyme in human blood and rat liver microsomes catalyzing the formation and hydrolysis of gamma-lactones. I. Tissue location, stoichiometry, specificity, distinction from esterase. J. Biol. Chem. 241, 4835-4841.

Fitzhugh OG and Nelson AA, 1947. The comparative chronic toxicities of fumaric, tartaric, oxalic, and maleic acids. J. Am. Pharm. Assoc. 36, 217-219.

Flavour Industry, 2006a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-05.

Flavour Industry, 2010g. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. FGE.10rev3 and A-13rev2 [Fl-no: 10.170 and 13.135].

Flavour Industry, 2010n. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. A-10rev3 [Fl-no:09.951].

Flavour Industry, 2011a. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. Specifications Succinic acid. A-10rev2 [FL-no: 08.113].

Flavour Industry, 2011g. Unpublished information submitted by Flavour Industry to the FLAVIS Secretariat. Specification. A-10Rev3 [FL-no: 09.951].

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology. 18, 219-232.

Foulger JH, 1947. Preliminary toxicity tests on 15 compounds. Adiponitrile. E.I. Dupont de Nemours & Co. 1947, with cover letter dated 12/18/47. EPA Doc 86-870001072, microfiche no. OTS0514975. December 18, 1947. Unpublished data submitted by EFFA to SCF.

Foureman P, Mason JM, Valencia R and Zimmering S, 1994. Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ. Mol. Mutag. 23, 208-227.

Frankenfeld JW, Mohan RR and Squibb RL, 1975. Preservation of grain with aliphatic 1,3-diols and their esters. J. Agric. Food Chem. 23, 418-425.

Fujita H and Sasaki M, 1987. [Mutagenicity test of food additives with *Salmonella typhimurium* TA97 and TA102]. Ann. Rep. Tokyo Metrop. Res. Lab. Public Health 38, 423-430. (In Japanese)

Fujita H, Aoki N and Sasaki M, 1994. [Mutagenicity test of food additives with *Salmonella typhimurium* TA97 and TA102 (IX*)]. Ann. Rep. Tokyo Metrop. Res. Lab. Public Health 45, 191-199. (In Japanese)

Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P and Zeiger E, 1985. Development of a standard protocol for *in vitro* cytogenetic testing with chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ. Mutag. 7, 1-51.

Garner R, Welch A and Pickering C, 1981. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay. Prog. Mutat. Res. 1, 280-284.

Garst J, Stapleton P and Johnston J, 1983. Mutagenicity of alpha-hydroxy ketones may involve superoxide anion radical. Oxy Radicals and Their Scavenger Systems 2, 125-130.

Gatehouse D, 1981. Mutagenic activity of 42 coded compounds in the "microtiter" fluctuation test. Prog. Mutat. Res. 1, 376-386.

Ghanayem BI, Blair PC, Thompson MB, Maronpot RR and Matthews HB, 1987a. Effect of age on the toxicity and metabolism of ethylene glycol monobutyl ether (2-butoxyethanol) in rats. Toxicol. Appl. Pharmacol. 91, 222-234.

Ghanayem BI, Burka LT and Matthews HB, 1987b. Metabolic basis of ethylene glycol monobutyl ether (2-butoxyethanol) toxicity: role of alcohol and aldehyde dehydrogenases. J. Pharmacol. Exp. Ther. 242(1), 222-231.

Ghanayem BI, Burka LT, Sanders JM and Matthews HB, 1987c. Metabolism and disposition of ethylene glycol monobutyl ether (2-butoxyethanol) in rats. Drug Metab. Disposition 15(4), 478-484.

Gollapudi BB, Barber ED, Lawlor TE and Lewis SA, 1996. Re-examination of the mutagenicity of ethylene glycol monobutyl ether to Salmonella tester strain TA97a. Mutat. Res. 370(1), 61-64.

Green MHL, 1981. A differential killing test using an improved repair-deficient strain of *Eschericia coli*. Prog. Mutat. Res. 1, 184-194.

Guidotti A and Ballotti PL, 1970. Relationship between pharmacological effects and blood and brain levels of gamma-butyrolactone and gamma-hydroxybutyrate. Biochem. Pharmacol. 19, 884-894.

Gulati DK, Hommel L, Poonacha KB, Russell V, Russell S and Lamb JC, 1985b. Ethylene glycol monobutyl ether: Reproduction and fertility assessment in CD-1 mice when administered in drinking water. Environmental Health Research and Testing; NTP PB-85-226827; Report 85-155. Research Triangle Park, NC.

Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB, 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5(2), 141-157.

Hanson H, 1943. Untersuchungen uber Nchweis und Isolierung von im Harn ausgeschiedenen Dicarbonsauren. Cited in Rusoff, I. I., Balldwin, R.R., Dominues, F.J., Monder, C., Ohan, W.J., Thiessen Jr., R., 1960. Intermediary metabolism of adipic acid. Toxicol. Appl. Pharmacol. 2, 316-330.

Hardin BD, Schuler RL, Burg JB, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ and Smith KN, 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog. Carcinog. Mutag. 7, 29-48.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutag.5 (Suppl. 1) 3-142.

Hayashi M, Kishi M, Sofuni T, Ishidate Jr M, 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem. Toxicol. 26(6), 487-500.

Heck JD, Vollmuth TA, Cifone MA, Jagannath DR, Myhr B and Curren RD, 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Heindel JJ, Gulati DK, Russell VS, Reel JR, Lawton AD and Lamb JC, 1990. Assessment of ethylene glycol monobutyl and monophenyl ether reproductive toxicity using a continuous breeding protocol in Swiss CD-1 mice. Fundam. Appl. Toxicol. 15, 683-696.

Hellwig J, 1991a. Study of the prenatal toxicity of glutaraldehyde in rats after oral administration (drinking water) with cover letter dated 12/16/91. EPA Doc 86-920000654, microfiche no. OTS0535537. February 11, 1991. Unpublished data submitted by EFFA to SCF.

Hellwig J, 1991b. Study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (gavage) with cover letter dated 12/16/91. EPA Doc 86-920000655, microfiche no. OTS0535536. February 11, 1991. Unpublished data submitted by EFFA to SCF.

Hemminki K, Falck K and Vainio H, 1980. Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals. Arch. Toxicol. 46, 277- 285.

Henrich RT and McMahon JM, 1988. Genetic evaluation of Dow Corning X2-5327 in bacterial reverse mutation assays with attachments and cover letter dated 06/08/89. 2-butoxyethanol. EPA Doc 86-890000428, microfiche no. OTS0520475. June 8, 1989. Unpublished data submitted by EFFA to SCF.

Hess FG, Cox GE, Bailey DE, Parent RA and Becci PJ, 1981. Reproduction and teratology study of 1,3-butanediol in rats. J. Appl. Toxicol. 1(4), 202-209.

Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

Hiser MF, Markley BJ, Reitz RH and Nieusma JL, 1992. Metabolism and disposition of acetyl tributyl citrate in male Sprague-Dawley rats. Toxicologist 12, 161.

Hjelle J and Peterson D, 1983. Metabolism of monodialdehyde by rat liver aldehyde dehydrogenase. Cited in Anonymous, 1996. Final report on the safety assessment of glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Hoechst, 1995. Material safety data sheet. 3-hydroxy-2-oxopropionic acid. Data submitted by EFFA to SCF.

Hoflack JC, Lambolez L, Elias Z and Vasseur P, 1995. Mutagenicity of ethylene glycol ethers and of their metabolites in *Salmonella typhimurium* his-. Mutat. Res. 341(4), 281-287.

Hogan GK and Rinehart WE, 1979. A twenty-four month oral toxicity/carcinogenicity study of propanedioic acid, (carboxymethoxy)-, trisodium salt in rats with attachments and cover letter dated 08/26/92. Bio/dynamics Inc. EPA Doc 88-920006877, microfiche no. OTS0543874. July 27, 1979. Unpublished data submitted by EFFA to SCF.

Hood DB, 1951. Toxicity tests on diethyl and dimethyl fumurate with cover letter dated 10/15/92. Project no. MR-125. EPA Doc 88-920009858, microfiche no. OTS0571509. January 29, 1951. Unpublished data submitted by EFFA to SCF.

Horn HJ, Holland EG and Hazleton LW, 1957. Safety of adipic acid as compared with citric and tartaric acids. J. Agric. Food Chem. 5, 759-762.

Hubbard SA, Green MHL, Bridges BA, Wain AJ and Bridges JW, 1981. Fluctuation test with S9 and hepatocyte activation. Prog. Mutat. Res. 1, 361-370.

Humbert R, Adler DA, Disteche CM, Hassett C, Omleoinski CJ and Purlong CE, 1993. The molecular basis of the human serum paraoxonase activity polymorphism. Nature Genetics 3, 73-76.

Ichinotsubo D, Mower H and Mandel M, 1981b. Mutagen testing of a series of paired compounds with the Ames Salmonella testing system. In: De Serres, F.J., Ashby, J. (Eds.). Evaluation of short-term tests for carcinogens: report of the international collaborative program. Vol. 1. Elsevier/North Holland, New York, pp. 298-301.

Ikeda M, 1980. List of LD50 values. Oyo Yakuri. Pharmacometrics 19, 503-508. (In Japanese)

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22(8), 623-636.

Ito N, Tsuda H, Tatematsu M, Inoue T, Tagawa Y, Aoki T, Uwagawa S, Kagawa M, Ogiso T, Masui T, Imaida K, Fukushima S and Asamoto M, 1988. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats - an approach for a new medium-term bioassay system. Carcinogenesis 9, 387-394.

Jakoby WB and Scott EM, 1959. Aldehyde oxidation. III. Succinic semialdehyde dehydrogenase. J. Biol. Chem. 234, 937-940.

JECFA, 1968. 11. Report: 11th Report of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 383.

JECFA, 1970s. 13. Report: Thirteenth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report, Toxicological monographs and Specifications: Technical Report Series, no. 445.

JECFA, 1978a. 21. Report: Twenty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 617.

JECFA, 1984a. 28. Report: Twenty-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 710.

JECFA, 1990a. 35. Report: Thirty-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 789.

JECFA, 1993b. 41. Report: Toxicological evaluation of certain food additives. Fourty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Toxicological monographs WHO Food Additives, No 32.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1998a. Safety evaluation of certain food additives and contaminants. The forty-ninth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 40. IPCS, WHO, Geneva.

JECFA, 1998b. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 1999c. Compendium of food additive specifications. Addendum 7. Joint FAO/WHO Expert Committee of Food Additives. 53rd meeting. Rome, 1-10 June 1999. FAO Food and Nutrition paper 52 Add. 7.

JECFA, 2000a. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA, 2000b. Evaluation of certain food additives and contaminants. Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 896. Geneva, 1-10 June 1999.

JECFA, 2000c. Safety evaluation of certain food additives and contaminants. Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 44. IPCS, WHO, Geneva.

JECFA, 2000d. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. 55th meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.

JECFA, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.

JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.

JECFA, 2003b. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.

JECFA, 2004a. Evaluation of certain food additives. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.

JECFA, 2005b. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Johanson G, Wallén M and Nordqvist MB, 1986. Elimination kinetics of 2-butoxyethanol in the perfused rat liver-dose dependence and effect of ethanol. Toxicol. Appl. Pharmacol. 83, 315-320.

Kada T, 1981. The DNA-damaging activity of 42 coded compounds in the rec-assay. Prog. Mutat. Res. 1, 176-182.

Kaneko S, Battino D, Andermann E, Wada K, Kan R, Takeda A, Nakane Y, Ogawa Y, Avanzini G, Fumarola C, Granata T, Molteni F, Pardi G, Minotti L, Canger R, Dansky L, Oguni M, Lopes-Cendas I, Sherwin A, Andermann F, Seni M-H, Okada M and Teranishi T, 1999. Congeital malformations due to antiepileptic drugs. Epilepsy Res. 33, 145-158.

Kaphalia BS, Ghanayem BI and Ansari GAS, 1996. Nonoxidative metabolism of 2-butoxyethanol via fatty acid conjugation in Fischer 344 rats. J. Toxicol. Environ. Health 49(5), 463-479.

Kassinova GV, Kavaltsova SV, Marfin SV and Zakhrov IA, 1981. Activity of 40 coded compounds in differential inhibition and mitotic crossing-over assays in yeast. Prog. Mutat. Res. 1, 434-455.

Katz M, Heddle JA and Salamone MF, 1981. Mutagenic activity of polycyclic aromatic hydrocarbons and other environmental pollutants. Polynuclear Arom. Hydrocarbons 519-528.

Kawachi T, Komatsu T, Kada T, Ishidate M, Sasaki T, Sugiyama T and Tazima Y, 1980b. Results of recent studies on the relevance of various short-term screening tests in Japan. Appl. Methods Oncol. 3, 253-267.

Keith G, Coulais C, Edorh A, Bottin C and Rihn B, 1996a. Ethylene glycol monobutyl ether has neither epigenetic nor genotoxic effects in acute treated rats and in sub-chronic v-HA-ras transgenic mice. Cited in Elliott, B.M., Ashby, J., 1997. Review of the genotoxicity of 2-butoxyethanol. Mutat. Res. 387, 89-96.

Klimisch H-J, Andreae M and Tillmann U, 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology 25, 1-5.

Kopf R, Loeser A and Meyer G, 1950. Untersuchungen über die Pharmakologie und Toxikologie mehrwertiger Alkohole (1,3-butylenglykol). Arch. Exp. Pathol. Pharmakol. 210, 346-360. (In German)

Krasavage WJ, 1983. The subchronic oral toxicity of ethylene glycol monobutyl ether in male rats with cover letter dated 06/03/83. EPA Doc 8EHQ-0683-0475, microfiche no. OTS0503697. Unpublished data submitted by EFFA to SCF.

Krebs HA, Salvin E and Johnson WA, 1938. The formation of citric and alpha-ketoglutaric acids in the mammalian body. Biochem. J. 32, 113-117.

Kronevi T, Holmberg B and Arvidsson S, 1988. Teratogenicity test of gamma-butyrolactone in the Sprague-Dawley rat. Pharmacol. Toxicol. 62, 57-58.

Krop S, Gold H and Paterno CA, 1945. On the toxicity of hydroxyacetic acid after prolonged administration: Comparison with its sodium salt and citric and tartaric acids. J. Am. Pharm. Assoc. 24, 86-89.

Kuroda K, Tanaka S, Yu YS and Ishibashi T, 1984a. [Rec-assay of food additives]. Nippon. Koshu. Eisei. Zasshi 31(6), 277-281. (In Japanese)

Kuroda M, Yoshida D and Mizusaki S, 1986. Bio-antimutagenic effect of lactones on chemical mutagenesis in *Escherichia coli*. Agric. Biol. Chem. 50(1), 243-245.

Kvelland I, 1988. The mutagenic effect of five oil dispersants and of ethyleneglycolmonobutylether in bacteriophage T4D. Hereditas 109, 149-150.

Lawrence WH, Malik M and Autian J, 1974. Development of a toxicity evaluation program for dental materials and products. II. Screening for systemic toxicity. J. Biomed. Mater. Res. 8, 11-34.

Lee CR, 1977. Evidence for the beta-oxidation of orally administered 4-hydroxybutyrate in humans. Biochem. Med. 17, 284-291.

Leegwater DC and VanStraten S, 1979. *In vitro* digestion test on methyl-2-keto-3-methyl valerate. Flavoring Extracts Manufacturers Association. July 10, 1979. Unpublished report submitted by EFFA to SCF.

Lettieri JT and Fung HL, 1978. Improved pharmacological activity via pro-drug modification:comparative pharmacokinetics of sodium gamma-hydroxybutyrate and gamma-butyrolactone. Res. Commun. Chem. Pathol. Pharmacol. 22, 107-118.

Levenstein I, 1973b. Acute oral toxicity reports on rats. Hydroxycitronellol. Leberco Laboratories, Inc. Assay no. 30963. January 9, 1973. Unpublished data submitted by EFFA to SCF.

Levenstein I, 1974c. Acute oral toxicity (rat - 5 gms./kg. Body weight dose). Dermal toxicity (rabbit - 5 gms./kg. Body weight dose). Cyclopentadecanolide. Leberco Laboratories, Inc. Assay no. 41772 March 15, 1974. Upublished data submitted by EFFA to SCF.

Levenstein I, 1975c. Acute oral toxicity (rat - 5 gms./kg. Body weight dose). Dermal toxicity (rabbits-5 gms./kg. Body weight dose). Delta decalactone. Leberco Laboratories, Inc. Assay no. 53804. May 20, 1975. Unpublished data submitted by EFFA to SCF.

Levenstein I, 1976b. Acute oral LD50 in rats. Dimethyl malonate. Cited in Opdyke DLJ, 1979. Food Cosmet. Toxicol. 17, 363.

Levey S, Lasichak AG, Brimi R, Orten JM, Smyth CJ and Smith AH, 1946. A study to determine the toxicity of fumaric acid. J. Am. Pharm. Assoc. 35, 298-304.

Levi PE and Hodgson E, 1989. Metabolites resulting from oxidative and reductive processes. In: Hutson DH, Caldwell J and Paulson GD (Eds.). Intermediary Xenobiotic Metabolism in Animals. Taylor and Francis, London, pp. 119-138.

Levin DE, Hollstein M, Christman MF, Schwiers EA and Ames BN, 1982. A new Salmonella tester strain (TA102) with A-T base pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. USA. 79, 7445-7449.

Lewis CA and Palanker AL, 1979a. Acute toxicity studies in rats and rabbits. Menthone lactone. Consumer Product Testing. Experiment ref. No. 79104-11. May 31, 1979. Unpublished data submitted by EFFA to SCF.

Loeser A, 1949. Über 1,3-butylenglykol. Pharmazie 4, 263-264. (In German)

Loprieno N, 1981. Screening of coded carcinogenic/noncarcinogenic chemicals by a forward-mutation system with the yeast Schizosaccharomyces pombe. Prog. Mutat. Res. 1, 424-433.

Loquet C, Toussaint G and LeTalaer JY, 1981. Studies on the mutagenic constituents of apple brandy and various alcoholic beverages collected in western France, a high incidence area for oesophageal cancer. Mutat. Res. 88, 155-164.

MacDonald DJ, 1981. Salmonella/microsome tests on 42 coded chemicals. Prog. Mutat. Res. 1, 285-297.

Maekawa A, Todate A, Onodera H, Matsushima Y, Nagaoka T, Shibutani M, Ogasawara H, Kodama Y and Hauashi Y, 1990. Lack of toxicity / carcinogenicity of monosodium succinate in F344 rats. Fd. Chem. Toxic. 28 (4), 235-241.

Mankes RF, Renak V, Fieseher J and Lefevre R, 1986, Birthweight depression in male rats contigious to male siblings in utero exposed to high doses of 1,3-butanediol during organogenesis. J. Am. Coll. Toxicol. 5(4), 189-196.

Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H and Ames BN, 1985a. Naturally-occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat. Res. 148, 25-34.

Marshall LM, Orten JM and Smith AH, 1949. The determination of fumaric acid in animal tissues by partition chromatography. J. Biol. Chem. 179, 1127-1139.

Martin CN and McDermid AC, 1981. Testing of 42 coded compounds for their ability to induce unscheduled DNA repair synthesis in HeLa cells. Prog. Mutat. Res. 1, 533-537.

Matsushima T, Takamoto Y, Shirai A, Sawamura M and Sugimura T, 1981. Reverse mutation test on 42 coded compounds with *E. coli* WP2 system. Prog. Mutat. Res. 1, 387-395.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D and Caspary WJ, 1988b. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay II: 18 coded chemicals. Environ. Mol. Mutag. 11, 91-118.

McKelvey JA, Garman RH, Anuszkiewicz CM, Tallant MJ and Ballantyne B, 1992. Percutaneous pharmacokinetics and material balance studies with glutaraldehyde. Cited in Anonymous, 1996. Final report on the safety assessment of glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Medinsky MA, Singh G, Bechtold WE, Bond JA, Sabourin PJ, Birnbaum LS and Henderson RF, 1990. Disposition of three glycol ethers administered in drinking water to male F344/N rats. Toxicol. Appl. Pharmacol. 102(3), 443-455.

Mehlman MA, Tobin RB, Hahn HKJ, Kleager L and Tate RL, 1971. Metabolic fate of 1,3-butanediol in the rat: liver tissue slices metabolism. J. Nutr. 101, 1711-1718.

Merck Index of Chemical and Drugs, 1992. Sicherheitsdatenbank-Programm MS-Safe. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 108-59-8, EINECS Name dimethyl malonate. Section 5.1.1 Acute Oral Toxicity.

Miller SA and Dymsza HA, 1967. Utilization by the rat of 1,3-butanediol as a synthetic source of dietary energy. J. Nutr. 91, 79-88.

Mingrone G, Greco AV, Nazzaro-Porro M and Passi S, 1983. Toxicity of azelaic acid. Drugs Exp. Clin. Res. 9(6), 447-455.

Mirsalis JC, Tyson CK, Steinmetz KL, Loh EK, Hamilton CM, Bakke JP and Spalding JW, 1989. Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following *in vivo* treatment: Testing of 24 compounds. Environ. Mol. Mutag. 14, 155-164.

Möhler H, Patel AJ and Balázs R, 1976. Gamma-hydroxybutyrate degradation in the brain *in vivo*: Negligible direct conversion to GABA. J. Neurochem. 27, 253-258.

Moran EJ, Easterday DD and Oser BL, 1980. Acute oral toxicity of selected flavor chemicals. Drug Chem. Toxicol. 3(3), 249-258.

Moreno OM, 1972b. Acute oral toxicity study in rats. Gamma-Nonalactone. Toxicological Resources. Project no. 847-72. May 5, 1972. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1973d. Acute oral toxicity (rat - 5 g/kg body weight dose). Dermal toxicity (rabbit - 5 g/kg body weight dose). Citronellyl oxyacetaldehyde. MB Research Laboratories, Inc. Project no. MB 72-11. Date 2/1/73. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1974c. Acute oral toxicity in rats. Dermal toxicity in rabbits. Gamma-Octalactone. MB Research Laboratories, Inc. Project no. MB 74-675. December 11, 1974. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1974d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Gamma-Dodecalactone. MB Research Laboratories, Inc. Project no. MB 74-672. December 11, 1974. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1975h. Acute oral toxicity in rats. Dermal toxicity in rabbits. Gamma-Decalactone. MB Research Laboratories, Inc. Project no. MB 75-752. April 9, 1975. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1975i. Acute oral toxicity in rats. Dermal toxicity in rabbits. Delta-Undecalactone. MB Research Laboratories, Inc. Project no. MB 75-814. June 25, 1975. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1976j. Acute oral toxicity in rats. Dermal toxicity in rabbits. G-Methyl decalactone. MB Research Laboratories, Inc. Project no. MB 76-1040. March 13, 1976. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1976k. Acute toxicity studies in rats. Dermal toxicity in rabbits. Geranyl acetoacetate. MB Research Laboratories, Inc. Project no. MB 76-1221. July 31,1976. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1976l. Report on acute dermal toxicity in rabbits. 2-Butoxyethanol. MB Research Laboratories, Inc. EPA Doc 86-890000171, microfiche no. OTS0516708. January 6, 1976. Unpublished data submitted by EFFA to SCF. Attached: 1) Report on oral LD50 in rats. MB Research Laboratories, Inc. Project no. MB 75-988. Date 3/12/76. 2) Report on oral LD50 in rats. MB Research Laboratories, Inc. Project no. MB 77-1820. Date 7/20/77.

Moreno OM, 1977e. Acute oral toxicity rats. Dermal toxicity in rabbits. Delta-Dodecalactone. MB Research Laboratories, Inc. Project no. MB 76-1457. January 24, 1977. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1977f. Acute oral toxicity in rats. Dermal toxicity in rabbits. Gamma-Hexalactone. MB Research Laboratories, Inc. Project no. MB 77-1687. July 20, 1977. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1977g. Acute oral toxicity in rats. Dermal toxicity in rabbits. Gamma-Heptalactone. MB Research Laboratories, Inc. Project no. MB 77-1684. July 5, 1977. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1977h. Acute oral toxicity in rats. Dermal toxicity in rabbits. Delta-Octalactone. MB Research Laboratories, Inc. Project no. MB 77-1888. September 29, 1977. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1977j. Acute oral toxicity in rats. Dermal toxicity in rabbits. Levulinic acid. MB Research Laboratories, Inc. Project no. MB 77-1685. July 6, 1977. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1978e. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Gamma-Valerolactone. MB Research Laboratories, Inc. Project no. MB 78-2646. Date 5/10/78. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1978f. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ethyl levulinate. MB Research Laboratories, Inc. Project no. MB 77-2196. Date 2/01/78. Unpublished data submitted by EFFA to SCF.

Moreno OM, 1979b. Test for oral toxicity in rats. Methyl 2-oxo-3-methylpentanoate. MB Research Laboratories, Inc. Study director: Moreno, M.T. Project no. MB 79-3578. February 5, 1979. Unpublished data submitted by EFFA to SCF.

Morgareidge K, 1962a. *In vitro* digestion of four acetals. Food and Drug Research Laboratories, Inc. Lab. No. 83179. August 7, 1962. Unpublished report submitted by EFFA to SCF.

Morgareidge K, 1962b. *In vitro* digestion of four lactones. Food and Drug Research Laboratories, Inc. Lab. No. 83180. August 7, 1962. Unpublished report submitted by EFFA to SCF.

Morgareidge K, 1963a. *In vitro* digestion of three lactones. Food and Drug Research Laboratories, Inc. Lab. No. 84919. July 23, 1963. Unpublished report submitted by EFFA to SCF.

Morgareidge K, 1973a. Approximate acute LD50 in rats. Pomalus; malic acid. Food and Drug Research Laboratories, Inc. Lab. No. 1763 r. October 16, 1973. Unpublished data submitted by EFFA to SCF.

Morgareidge K, 1973b. Approximate acute LD50 in mice. Pomalus; malic acid. Food and Drug Research Laboratories, Inc. Lab. No. 1762 r. October 16, 1973. Unpublished data submitted by EFFA to SCF.

Morgareidge K, 1973c. Approximate acute LD50 in rabbits. Pomalus; malic acid. Food and Drug Research Laboratories, Inc. Lab. No. 1764 r. November 29, 1973. Unpublished data submitted by EFFA to SCF.

Morgareidge K, 1973d. Teratologic evaluation of FDA 71-50. Adipic acid in rats. Food and Drug Research Laboratories, Inc. Lab. No. 1361 g. February 26, 1973. Unpublished data submitted by EFFA to SCF.

Morgareidge K, 1974a. Teratologic evaluation of compound FDA 71-50. Adipic acid, in rabbits. Food and Drug Research Laboratories, Inc. Lab. No. 1363 g. June 28, 1974. Food and Drug Administration. NTIS PB-267 202. Report no. FDA/BF-77/116. Unpublished data submitted by EFFA to SCF.

Morgott DA, 1993. Acetone. In: Clayton, G.D., Clayton, F.E. (Eds.). Patty's Industrial Hygiene and Toxicology, 4th Ed. Vol. II, Part A, John Wiley & Sons, New York, pp. 149-281.

Müller W, Engelhart G, Herbold B, Jäckh R and Jung R, 1993. Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. Environ. Health Perspec. Suppl. 101(3), 33-36.

Myers RC and Homan ER, 1980. Butyl cellosolve: Range finding toxicity studies with attachments and cover letter dated 06/06/89. Bushy Run Research CTR. EPA Doc 86-890000938, microfiche no. OTS0520376. October 22, 1980. Unpublished data submitted by EFFA to SCF.

Myers RC, Carpenter CP and Cox EF, 1977b. Glutaraldehyde, 50% aqueous solution: Range finding toxicity studies. (Report no. 40-50). Obtained through UCC (Union Carbide Corporation) (1992) with cover letter dated 3/18/92. EPA Doc 88-920001503, microfiche no. OTS0536179. Unpublished data submitted by EFFA to SCF.

Myers RC, Carpenter CP and Cox EF, 1977c. Glutaraldehyde, 25% aqueous solution: Range finding toxicity studies. (Report no. 40-120). Obtained through UCC (Union Carbide Corporation) (1992) with cover letter dated 3/18/92. EPA Doc 88-920001503, microfiche no. OTS0536179. Unpublished data submitted by EFFA to SCF.

Nagano K, Nakayama E, Adachi H and Yamada T, 1977. Testicular dysfunction due to cellosolves. Rodo Eisei, 18, 24-27. Cited in Tyler, T.R., 1984. Acute and subchronic toxicity of ethylene glycol monobutyl ether. Environ. Health Perspect. 57, 185-191.

Nagano K, Nakayama E, Koyano M, Oobayashi H, Adachi H and Yamada T, 1979. Testicular atrophy of mice induced by ethylene glycol mono alkyl ether. Jap. J. Ind. Health 21, 29-35. (In Japanese)

Nagano K, Nakayama E, Oobayashi H, Nishizawa T, Okuda H and Yamazaki K, 1984. Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. Environ. Health Perspec. 57, 75-84.

Nagao M and Takhashi Y, 1981. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay. Prog. Mutat. Res. 1, 302-313.

NAS/COT, 2005. Acetone (CAS Reg. No. 67-64-1). National Academy of Sciences, Committee on Toxicology, Subcommittee for AEGLs. Interim 1: 07/2005.

Nau H and Löscher W, 1986. Pharmacologic evaluation of metabolites and analogs of valproic acid: Teratogenic potencies in mice. Fundam. Appl. Toxicol. 6, 669-676.

Neeper-Bradley TL and Ballantyne B, 2000. Two-generation reproduction study by dosing with glutaraldehyde in the drinking water of CD rats. J. Toxicol. Environ. Health 60(2), 107-29.

Noblitt T, Mansfield G, Dunipace A, Li Y, Origel A and Stookey G, 1992. Mutagenicity of glutaraldehyde in the Ames test. J. Dent. Res. 71, 227.

Noblitt T, Li Y, Dunipace A, Origel A and Stookey G, 1993. Cytogenic effect of glutaraldehyde-micronucleus assay. J Dent. Res. 72, 163.

NTP, 1992e. NTP technical report on the toxicology and carcinogenesis studies of gamma-butyrolactone (CAS no. 96-48-0) in F344/N rats and B6C3F1 mice (gavage studies). March 1992. NTP-TR 406. NIH Publication no. 92-3137.

NTP, 1993a. Toxicity studies of ethylene glycol ethers 2-methoxyethanol, 2-ethoxyethanol and 2-butoxyethanol administered in drinking water to F344/N rats and B6C3F1 mice (Technical report no. 93-3349). Research Triangle park, 122 pp. Cited in Anonymous, 1996. Final report on the safety assessment of butoxyethanol. J. Am. Coll. Toxicol. 15(6), 462-526.

NTP, 2000b. NTP technical report on the toxicology and carcinogenesis studies of 2-butoxyethanol (CAS no. 111-76-2) in F344/N rats and B6C3F1 mice (inhalation studies). March 2000. NTP-TR 484. NIH Publication no. 00-3974.

Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1979. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

OECD SIDS, 2003. Disodium succinate. SIDS Initial Assessment Report. SIAM 16, Paris, France, 27-30 May 2003.

Okamoto K and Riccio ES, 1985. *In vitro* microbiological mutagenicity assays of 3M company's compound T-3722 with cover letter dated 05/17/89. 3M Co. EPA Doc 86-890000242, microfiche no. OTS0516777. Date 4/01/85. Unpublished date submitted by EFFA to SCF.

Önfelt A, 1987. Spindle disturbances in mammalian cells. III. Toxicity, c-mitosis and aneuploidy with 22 different compounds. Specific and unspecific mechanisms. Mutat. Res. 182, 135-154.

Oser BL, Carson S and Oser M, 1965. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 3(4), 563-569.

Osteux R and Laturaze J, 1954. Biological chemistry - Paper chromatography of fixed organic acids found in urine. Comp. Rend. 239, 512-513.

Packman EW, Abbott DD and Harrisson JWE, 1963. Comparative subacute toxicity for rabbits of citric, fumaric, and tartaric acids. Toxicol. Appl. Pharmacol. 5, 163-167.

Passi S, Picardo M, Mingrone G, Breathnach AS and Nazarro-Porro M, 1989. Azeliac acid - biochemistry and metabolism. Acta Derm. Venereol. Suppl., 143, 8-13.

Patty FA, 1963. Patty's Industrial Hygiene and Toxicology, vol. 2. John Wiley & Sons Inc., New York, p. 1546.

Patty FA, 1993. Patty's Industrial Hygiene and Toxicology, 4th Ed. John Wiley & Sons, New York.

Pellmont B, 1973a. Letaldosis an der Maus. Ethyl-3-oxohexanoate. Toxikologisches Labor 256, Bau 69. Date 5/3/1973. Unpublished data submitted by EFFA to FLAVIS Secretariat. (In German)

Pellmont B, 1978. Acute oral toxicity in mice with methyl-2-hydroxy-4-methyl-pentanoate. Toxikologisches Labor 256, Bau 69. Date 25/4/1978. Unpublished data submitted by EFFA to SCF.

Piccirillo VJ and Hartman WC, 1980a. Range-finding oral LD50 determination in rats with 79-051-01. 5-hydroxy-2,4-decadienoic acid delta -lactone. Borriston Research Laboratories, Inc. Project no. 204-P. February 27, 1980. Unpublished report submitted by EFFA to SCF.

Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 7, 405-407.

Posternak J, 1964a. Subacute toxicity (90 days) report on 1-octen-3-ol (amyl vinyl carbinol). Firmenich & Cie. Unpublished report submitted by EFFA to SCF.

Prival MJ, Simmon VF and Mortelmanns KE, 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutat. Res. 260, 321-329.

Putman DL, 1987. Cytogenincity study - bone marrow *in-vivo* (final report) with attachment, cover sheet and letter dated 112691 (sanitized). Glutaraldehyde. Microbiological Associates Inc. EPA Doc 86-920000503s, microfiche no. OTS 0533792. March 9, 1987. Unpublished data submitted by EFFA to SCF.

Ramel C and Magnusson J, 1979. Chemical induction of nondisjunction in Drosophila. Environ. Health Perspect. 31, 59-66.

Rapson WH, Nazar MA and Butzky VV, 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bull. Environ. Contam. Toxicol. 24, 590-596.

Reagan EL and Becci PJ, 1984a. Acute oral LD50 study of filbertone in Sprague-Dawley rats. Food and Drug Research Laboratories, Inc. Study no. 8009 K. August 10, 1984. Unpublished date submitted by EFFA to SCF.

Reuzel PGJ, van Oostrum ECM, Roverts WG and Koeter HBWM, 1978. Initial submission: Subchronic (13-week) feeding study with 1,3-butanediol in dogs (final report) with cover letter. Hoechst Celanese Corp. EPA Doc 88-920001732, microfiche no. OTS0537195. December 13, 1991. Unpublished data submitted by EFFA to SCF.

Richold M and Jones E, 1981. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay. Prog. Mutat. Res. 1, 314-322.

Riebeek WM, 1989. Determination of the acute oral toxicity of "S(-) isopropyl lactate" in rats. TNO Report V89.468. Cited in Clary, J.J., Feron, V.J., van Velthuijsen, J.A., 1998. Safety assessment of lactate esters. Regul. Toxicol. Pharmacol. 27(2), 88-97.

Rosenkranz HS, Hyman J and Leifer Z, 1981. DNA polymerase deficient assay. Prog. Mutat. Res. 1, 210-218.

Roth RH and Giarman J, 1965. Preliminary report on the metabolism of gamma-butyro-lactone and gamma-hydroxybutyric acid. Biochem. Pharmacol. 14(2), 177-178.

Roth RH and Giarman NJ, 1966. Gamma-butyrolactone and gamma-hydroxybutyric acid-I. Distribution and metabolism. Biochem. Pharmacol. 15, 1333-1348.

Rowe VK and Wolf MA, 1982. Derivatives of glycols. In: Clayton, G.D., Clayton, F.E. (Eds.). Patty's Industrial Hygiene and Toxicology. 3rd rev. Ed. Vol. 2C. John Wiley & Sons, New York, p. 3933-3935.

Rowland I and Severn B, 1981. Mutagenicity of carcinogens and noncarcinogens in the Salmonella/microsome test. Prog. Mutat. Res. 1, 323-332.

Ruiz-Rubio M, Alejandre-Duran E and Pueyo C, 1985. Oxidative mutagens specific for A-T base pairs induce forward mutations to L-arabinose resistance in Salmonella typhimurium. Mutat. Res. 147(4), 153-163.

Rusoff II, Balldwin RR, Dominues FJ, Monder C, Ohan WJ and Thiessen Jr R, 1960. Intermediary metabolism of adipic acid. Toxicol. Appl. Pharmacol. 2, 316-330.

Rydén E, Ekström C, Hellmér L and Bolcsfoldi G, 2000. Comparison of the sensitivities of *Salmonella typhimurium* strains TA102 and TA2638A to 16 mutagens. Mutagenesis 15(6), 495-502.

Sakagami Y, Yamasaki H, Yokoyama H, Ose Y and Sato T, 1988. DNA repair test of disinfectants by liquid rec-assay. Mutat. Res. 193, 21-30.

Sakagami Y, Yamasaki H, Ogasawara N, Yokoyama H, Ose Y and Sato T, 1989. Evaluation of genotoxic activities of disinfectants and their metabolites by the umu test. Mutat. Res. 216(6), 373.

Salamone MF, Heddle JA and Katz M, 1981. Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. Prog. Mutat. Res. 1, 686-697.

Samren EB, van-Duijn CM, Koch S, Hiilesmaa VK, Klepel H, Bardy AH, Mannagetta GB, Deichl AW, Gaily E, Granstrom ML, Meinardi H, Grobbee DE, Hofman A, Janz D and Lindhout D, 1997. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European propective study of human teratogenesis associated with maternal epilepsy. Epilepsia 38(9), 981-990.

San Sebastian JR, 1989a. Initial submission: *In vivo* bone marrow cytogenetics rat metaphase analysis with cover letter dated 8/14/92. Glutaric acid. Monsanto Co. EPA Doc 88-920007732, microfiche no. OTS0538652. January 26, 1989. Unpublished date submitted by EFFA to SCF.

Scala RA and Paynter OE, 1967. Chronic oral toxicity of 1,3-butanediol. Toxicol. Appl. Pharmacol. 10, 160-164.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Schafer EW and Bowles WA, 1985. The acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol. 14, 111-129.

Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V and Smith K, 1984. Results of testing of fifteen glycol ethers in a short-term *in vivo* reproductive toxicity assay. Environ. Health Perspect. 57, 141-146.

Schweikl H, Schmalz G and Bey B, 1994. Mutagenicity of dentin bonding agents. J. Biomed. Mater. Res. 28, 1061-1067.

Sharp DC and Parry JM, 1981. Induction of mitotic gene conversion by 41 compounds using the yeast culture JD1. Prog. Mutat. Res. 1, 491-501.

Shelanski MV and Moldovan M, 1973b. Acute oral toxicity (rats - 5 gms/kg body weight dose). Dermal toxicity (rabbits - 5 gms/kg body weight dose). Hydroxycitronellal dimethyl acetal. Food and Drug Research Laboratories. IBL no. 12208-F. 30 January 1973. Unpublished report submitted by EFFA to SCF.

Shellenberger TE, 1971c. Subacute toxicity evaluation of alpha-angelica lactone with rats. Gulf South Research Institute. Final Report: GSRI Project no. NC-403. January 4, 1971. Unpublished report submitted by EFFA to SCF.

Shillinger YI, 1950. [Action of some synthetic substances on animal organism]. Gig. Sanit. 3, 37-41. (In Russian)

Shimizu H, Suzuki Y, Takemura N, Goto S and Matsushita H, 1985. The results of microbial mutation test for forty-three industrial chemicals. Jap. J. Ind. Health 27, 400-419.

Simmon VF and Shephard GF, 1981. Mutagenic activity 42 coded compounds in the Salmonella/microsome assay. Prog. Mutat. Res. 1, 333-342.

Simola PE and Krusius FE, 1938. The formation of alpha-ketoglutaric acid in animal metabolism. Suomen Kemistilehti 11B, 9.

Singh AR, Lawrence WH and Autian J, 1975. Dominant lethal mutations and antifertility effects of di-2-ethylhexyl adipate and diethyl adipate in male mice. Toxicol. Appl. Pharmacol. 32, 566-576.

Sippel ME, 1977. Mutagenic activity of butyl cellosolve in the Salmonella/Microsome assay with attachments and cover sheet dated 06/12/89. 2-Butoxyethanol. E.I. Dupont De Nemour & Co. EPA Doc 86-890000847S, microfiche no. OTS0520963. December 9, 1977. Unpublished data submitted by EFFA to SCF.

Skopeck TR, Andon BM, Kaden DA and Thilly WG, 1981. Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a genetic marker in *Salmonella typhimurium*. Prog. Mutat. Res. 1, 373-375.

Sleet RB, Price CJ, Marr MC, Morrissey RE and Schwetz BA, 1989. Teratologic evaluation of ethylene glycol monobutyl ether administered to Fischer-344 rats in either gestational days 9 through 11 or days 11 through 13. National Institute Of Environmental Health Sciences. NTP Report 89-058.

Slesinski RS and Weil CS, 1980. Butyl cellosolve. *In vitro* mutagenesis studies: 3-test battery. 2-Butoxyethanol. Olin Corp. EPA Doc 86-890000168, microfiche no. OTS0516704. March 25, 1980. Unpublished data submitted by EFFA to SCF.

Slesinski RS, Hengler WC, Guzzie PJ and Wagner KJ, 1983. Mutagenicity evaluation of glutaraldehyde in a battery of *in vitro* bacterial and mammalian test systems. Food Chem. Toxicol. 21(5), 621-629.

Smith JN, 1953a. Studies in detoxication. The glucuronic acid conjugation of hydroxyquinolines and hydroxypyridines in the rabbit. Biochem. J. 55, 156-160.

Smith CC, 1953b. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate and methoxyethyl oleate. Arch. Ind. Hyg. Occup. Med. 7(4), 310-318.

Smith KN, 1983. Determination of the reproductive effects in mice of nine selected chemicals. Diaminotoluene. Bioassay Systems Corp. EPA Doc AR027-115, microfiche no. OTS0528963. January 7, 1983. Unpublished data submitted by EFFA to SCF.

Smyth Jr HF and Carpenter CP, 1948. Further experience with the range-finding test in the industrial toxiclogy laboratory. J. Ind. Hyg. Toxicol. 30, 63-68.

Smyth Jr HF, Seaton J and Fischer L, 1941. The single dose toxicity of some glycols and derivatives. J. Ind. Hyg. Toxicol. 23, 259-268.

Smyth Jr HF, Carpenter CP and Weil CS, 1949. Range-finding toxicity data. List III. J. Ind. Hyg. Toxicol. 31, 60-62.

Smyth Jr HF, Carpenter CP and Weil CS, 1951a. Range finding toxicity data: List IV. Arch. Ind. Hyg. Occup. Med. J. 4, 119-122.

Smyth Jr HF, Carpenter CP, Weil CS and Pozzani UC, 1954. Range-finding toxicity data: List V. Arch. Ind. Hyg. Occup. Med. 10, 61-68.

Smyth Jr HF, Carpenter CP, Weil CS, Pozzani UC and Striegel JA, 1962. Range-finding toxicity data: List VI. Am. Ind. Hyg. J. 23, 95-107.

Smyth Jr HF, Carpenter CP, Weil CS, Pozzani UC, Striegel JA and Nycum JS, 1969a. Range-finding toxicity data: List VII. Am. Ind. Hyg. Assoc. J. 30(5), 470-476.

Spencer PS, Bischoff MC and Schaumburg HH, 1978. On the specific molecular configuration of neurotoxic aliphatic hexacarbon compounds causing central-peripheral distal axonopathy. Toxicol. Appl. Pharmacol. 44, 17-28.

St. Clair MBG, Bermudez E, Gross EA, Butterworth BE and Recio L, 1991. Evaluation of the genotoxic potential of glutaraldehyde. Environ. Mol. Mutag. 18, 113-119.

Stonehill AA, Krop S and Borick PM, 1963. Buffered glutaraldehyde: a new chemical sterilizing solution. Am. J. Hosp. Pharm. 20, 458-465.

Striegel JA and Carpenter CP, 1964. Initial submission: Letter submitting twelve enclosed toxicology studies on glutaraldehyde. Union Carbide Corp. EPA Doc 88-920001503, microfiche no. OTS0536179. March 18, 1992. Unpublished data submitted by EFFA to SCF.

Styles JA, 1981. Activity of 42 coded compounds in the BHK-21 cell transformation tests. Prog. Mutat. Res. 1, 638-646.

Summer KH, Rozman K and Coulston F, 1979a. Urinary excretion of mercapturic acids in chimpanzees and rats dosed with napthalene and diethylmaleate. Naunyn-Schmiedeberg's Arch. Pharmacol. 307, R8.

Tate RL, Mehlman MA and Tobin RB, 1971. Metabolic fate of 1,3-butanediol in the rat: conversion to beta-hydroxybutyrate J. Nutr. 101, 1719-1726.

Tischer RG, Fellers CR and Doyle BJ, 1942. The non-toxicity of levulinic acid. J. Am. Pharm. Assoc. 31, 217-220.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Topham JC, 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than cacinogens? Mutat. Res. 74, 379-387.

Trueman RW, 1981. Activity of 42 coded compounds in the Salmonella reverse mutation test. Prog. Mutat. Res. 1, 343-350.

Tsuchimoto T and Matter BE, 1981. Activity of coded compounds in the micronucleus test. Prog. Mutat. Res. 1, 705-711.

Turek B, Barta I, Smerak P, Kovacova E, Sedmikova M and Sestakova H, 1997. Mutagenic activity of substances of plant origin. Potravin. Vedy 15(4), 271-288. (In Rumanian)

Tweats DJ, 1981. Actvity of 42 coded compounds in a differential killing test using *Escherichia coli* strains WP2, WP67 (uvrA polA), and CM871 (uvrA lexA recA). Prog. Mutat. Res. 1, 199-209.

Uhde P, 2004a. Unpublished report on the genotoxicity of 5,6-dimethyl-tetrahydro-pyran-2-one.

Union Carbide Corp., 1952. Butyl cellosolve I. Acute and subacute toxicity. II. Evaluation of red blood cell fragility as a measure of initial response. Mellon Institute of Industrial Research, University of Pittsburgh. Report no. 15-37. Cited in Tyler, T.R., 1984. Acute and subchronic toxicity of ethylene glycol monobutyl ether. Environ. Health Perspect. 57, 185-191.

Union Carbide Corp., 1963. Results of three months of inclusions of butyl cellosolve in the diets of rats. Mellon Institute of Industrial Research special report 26-5. Cited in Tyler, T.R., 1984. Acute and subchronic toxicity of ethylene glycol monobutyl ether. Environ. Health Perspect. 57, 185-191.

Union Carbide Corp., 1986. Review of the toxicological studies and human health effects: glutaraldehyde. Cited in Anonymous, 1996. Final report on the safety assessment of glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Union Carbide Corp., 1992. Initial submission: Letter submitting twelve enclosed toxicology studies on glutaraldehyde. EPA Doc 88-920001503, microfiche no. OTS0536179. March 18, 1991. Unpublished data submitted by EFFA to SCF.

Union Carbide Corp., 1993. 2-Year drinking water study on glutaraldehyde. BRRC Project Report 91U0012. Unpublished data submitted by Union Carbide Corporation, Tarrytown, NY. Cited in Anonymous, 1996. Final report on the safety assessment of Glutaraldehyde. J. Am. Coll. Toxicol. 15(2), 98-139.

Van Miller JP, Hermansky SJ, Losco PE and Ballantyne B, 2002. Chronic toxicity and oncogenicity study with glutaraldehyde dosed in the drinking water of Fischer 344 rats. Toxicology 175, 177-189.

Venitt S and Crofton-Sleigh C, 1981. Mutagenicity of 42 coded compounds in a bacterial assay using Escherichia coli and *Salmonella typhimurium*. Prog. Mutat. Res. 1, 351-360.

Vergnes S and Ballantyne B, 2002. Genetic toxicology studies with glutarladehyde. J. Appl. Toxicol. 22, 45-60.

Vergnes JS and Morabit ER, 1993a. UCARCIDE Antimicrobial 250 (glutaraldehyde 50 % aqueous solution): Bone marrow chromosomal aberrations assay in rats with cover letter dated 06/04/93. Union Carbide Corp. EPA Doc 86-930000246, microfiche no. OTS0537689. May 27, 1993. Unpublished data submitted by EFFA to SCF.

Vergnes JS and Morabit ER, 1993b. *In vivo* mouse blood micronucleus test with Swiss-Webster mice with cover letter dated 03/04/93. EPA Doc 86-930000155, microfiche no. OTS0538149. February 26, 1993. Unpublished data submitted by EFFA to SCF.

Vernot EH, Mc Ewen JD, Haun CC and Kinkead ER, 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol. 42(2), 417-423.

Villalobos-Pietrini R, Gomez-Arroyo S, Altamirano-Lozano M, Orozco R and Rios P, 1989. Cytogenic effects of some cellosolves. Res. Int. Contam. Ambient. 5, 41-48. Cited in Elliot, B.M., Ashby, J., 1997. Review of the genotoxicity of 2-butoxyethanol. Mutat. Res. 387, 89-96.

Voet D and Voet JG, 1990. Biochemistry. Chapter 19: Citric Acid Cycle. Chapter 23: Lipid Metabolism, beta-oxidation, cholesterol biosynthesis. Chapter 24: Amino Acid Metabolism, tetrahydrofolate pathway. John Wiley & Sons, New York, pp. 506-527, 623-633, 645-651, 686-700, 761-763.

Wagner VO, 1997. Genetic evaluation of Dow Corning 1-0469 waterborne resin (pentanedial, <0,1 WT.%) in a bacterial reverse mutation assay with cover letter dated 2/6/97. Dow Corning Corp. EPA Doc 56970000441, microfiche no. OTS0573635. January 7, 1997. Unpublished data submitted by EFFA to SCF.

Walkenstein SS, Wiser R, Gudmundsen C and Kimmel H, 1964. Metabolism of gamma-hydroxybutyric acid. Biochim. Biophys. Acta 86, 640-642.

Wang G, Maranelli G, Perbellini L, Raineri E and Brugnone G, 1994c. Blood acetone concentration in "normal people" and in exposed workers 16 h after the end of the workshift. Int. Arch Occup. Environ Health 65, 285-289.

Wangenheim J and Bolcsfoldi G, 1988. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3(3), 193-205.

Watanabe S and Morimoto Y, 1990. Mutagenicity test. Cis-6-dodecen-4-olide. Takasago International Corporation. September 21, 1990. Unpublished data submitted by EFFA to SCF.

Watanabe K, Sakamoto K and Sasaki T, 1998a. Comparisons on chemically-induced mutation among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 uvrA/pKM101: collaborative study II. Mutat. Res. 412(1), 17-31.

Weil CS and Wright GJ, 1967. Intra- and interlaboratory comparative evaluation of single oral test. Toxicol. Appl. Pharmacol. 11, 378-388.

Weiner H, 1980. Aldehyde oxidating enzymes. In: Jakoby WB, (Ed.). Enzymatic Basis of Detoxification. vol 1, 261. Academic Press, New York, pp. 261-280.

Wenzel DG and Koff GY, 1956. H, Am. Pharm. Ass. 45, 669. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 107-88-0, EINECS Name butane-1,3-diol. Section 5.1.1 Acute Oral Toxicity.

WHO, 1998a. Acetone. Environmental Helath Criteria (EHC) 207. International Programme on Chemical Safety (IPCS); World Health Organization, Geneva, Switzerland.

Wier PJ, Lewis SC and Traul KA, 1987. A comparison of developmental toxicity evident at term to postnatal growth and survival using ethylene glycol monoethyl ether, ethylene glycol monobutyl ether, and ethanol. Teratog., Carcinog. Mutag. 7(1), 55-64.

Wilcox P, Naidoo A, Wedd DJ and Gatehouse DG, 1990. Comparison of *Salmonella typhimurium* TA102 with *Escherichia coli* WP2 tester strains. Mutagenesis 5(3), 285-291.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Williams RT, 1959a. Detoxication mechanisms. The metabolism and Detoxification of Drugs, Toxic Substances, and Other Organic Compounds. 2nd Ed. Chapman & Hall Ltd, London.

Wolf MA, 1959. Results of range finding toxicological test on Dowanol EB (sanitized). Dow Chem. Co. EPA Doc 86-890001175S, microfiche no. OTS0520315. March 30, 1959. Unpublished data submitted by EFFA to SCF.

Yamaguchi T and Nakagawa K, 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agric. Biol, Chem. 47(11), 2461-2465.

Yamaguchi T, 1982. Mutagenicity of trioses and methyl glyoxal on *Salmonella typhimurium*. Agric. Biol. Chem. 46(3), 849-851.

Yingnian Y, Yifab D, Ming F and Xingruo C, 1990. ADPRT-mediated decrease of cellular NAD content and the detection of chemically induced DNA damage-development of a new short-term screening test for mutagens. Proc. CAMS PUMC 5, 19-24.

Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Med. J. 34(3-4), 267-288.

Yoon JS, Mason JM, Valencia R, Woodruff RC and Zimmering S, 1985. Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the national toxicology program. Environ. Mutag. 7, 349-367.

Zeiger E and Margolin BH, 2000. The proportions of mutagens among chemicals in commerce. Reg. Toxicol. Pharmacol. 32, 219-225.

Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ. Mol. Mutag. 11(Suppl. 12), 1-158.

Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutag. 19(21), 2-141.

Zimmering S, Mason JM and Valencia R, 1989. Chemical mutagenesis testing in Drosophila. VII. Results of 22 coded compounds tested in larval feeding experiments. Environ. Mol. Mutag. 14, 245-251.

Zlatkis A and Liebich HM, 1971. Profile of volatile metabolites in human urine. Clin. Chem. 17(7), 592-594.

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ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[11] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[12] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[11] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[12] "Endogenous substances": 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 (JECFA, 1997a).

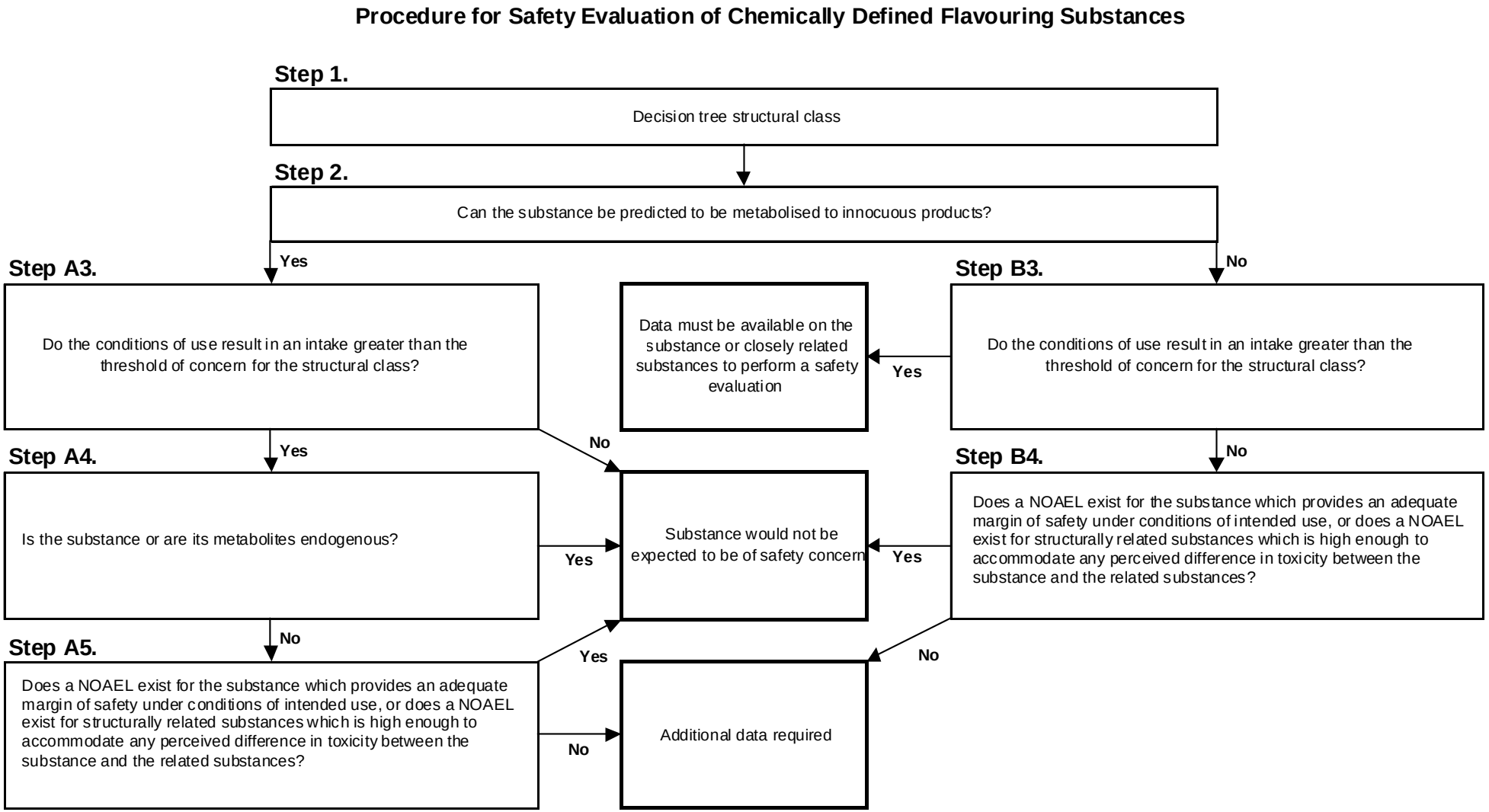

Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for 61 of the candidate substances in the present Flavouring Group Evaluation (Table II.1.2) (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10Rev3

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.132	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.198	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.242	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
05.149	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
06.088	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.090	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.095	7	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10Rev3

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	100	25
06.097	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.102	3	2	3	2	-	10	5	10	2	2	-	-	52	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	5	50	15	50	75	25
07.169	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
08.053	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.082	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.090	3	2	3	2	-	10	5	10	2	-	-	-	5	10	5	10	15	5
	15	10	15	10	-	50	25	50	10	-	-	-	25	50	25	50	75	25
08.103	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
09.333	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.345	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.346	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.347	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.348	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.349	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.350	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.351	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.352	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.353	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.354	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.360	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.502	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.558	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.565	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.580	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	200	10	10	-	-	25	50	25	50	100	25
09.590	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.601	10	5	10	7	-	20	15	15	2	2	-	-	5	10	5	20	20	5
	50	75	50	35	-	100	75	75	10	10	-	-	25	50	50	100	100	25
09.626	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.629	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.633	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.634	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.644	7	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	10	5
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	50	25
09.683	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.815	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.824	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10Rev3

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
09.832	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.833	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.862	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.874	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.916	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.951	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	6
	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	10
10.038	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.039	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.040	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.045	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.047	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.048	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.049	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.055	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.058	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.059	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	30	25	50	100	25
10.063	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.068	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.170	5	2	1	1	1	4	2,2	3	-	-	-	-	101	-	3	2	2	2
	20	10	5	5	5	20	10	15	-	-	-	-	1005	-	10	10	10	10

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories	
15.0	Ready-to-eat savouries		Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food	

The mTAMDI values (see Table II.2.3) are presented for each of the 61 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	3900	Class I	1800
02.198	Octane-1,3-diol	3900	Class I	1800
05.149	Glutaraldehyde	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	1600	Class I	1800
08.053	Malonic acid	3200	Class I	1800
08.082	Glutaric acid	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	3800	Class I	1800
08.103	Nonanedioic acid	3200	Class I	1800
08.113	Succinic acid, disodium salt		Class I	1800
09.333	sec-Butyl lactate	3900	Class I	1800
09.345	Di-isopentyl succinate	3900	Class I	1800
09.346	Dibutyl malate	3900	Class I	1800
09.347	Dibutyl succinate	3900	Class I	1800
09.348	Diethyl adipate	3900	Class I	1800
09.349	Diethyl citrate	3900	Class I	1800
09.350	Diethyl fumarate	3900	Class I	1800
09.351	Diethyl maleate	3900	Class I	1800
09.352	Diethyl nonanedioate	3900	Class I	1800
09.353	Diethyl oxalate	3900	Class I	1800
09.354	Diethyl pentanedioate	3900	Class I	1800
09.360	Ethyl 2-acetoxypropionate	3900	Class I	1800
09.502	Ethyl butyryl lactate	3900	Class I	1800
09.558	Dimethyl malonate	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	3900	Class I	1800
09.580	Hexyl lactate	3900	Class I	1800
09.590	Isobutyl lactate	3900	Class I	1800
09.601	Isopentyl lactate	5100	Class I	1800
09.626	Methyl 2-oxopropionate	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	3900	Class I	1800
09.634	Methyl acetoacetate	3900	Class I	1800
09.644	Methyl lactate	3600	Class I	1800
09.683	Pentyl lactate	3900	Class I	1800
09.815	Propyl lactate	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	3900	Class I	1800
09.951	Dioctyl adipate	800	Class I	1800
10.038	Dec-7-eno-1,4-lactone	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	3900	Class I	1800
10.045	Heptano-1,5-lactone	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	3900	Class I	1800

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
10.049	Hexadecano-1,5-lactone	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	3900	Class I	1800
10.055	Pentano-1,5-lactone	3900	Class I	1800
10.058	Tridecano-1,5-lactone	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane		Class II	540
02.242	2-Butoxyethan-1-ol	3900	Class II	540
06.097	1,1,3-Triethoxypropane	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	4100	Class III	90
10.170	5-Pentyl-3H-furan-2-one	3800	Class III	90

1

ANNEX III: METABOLISM

III.1. Introduction

III.1.1. Equilibrium Between Aliphatic Lactones and Ring-opened Hydroxycarboxylic Acids: Effect of pH

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured (see Figure III.1). Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclisation are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones, with low molecular weight, being uncharged, may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

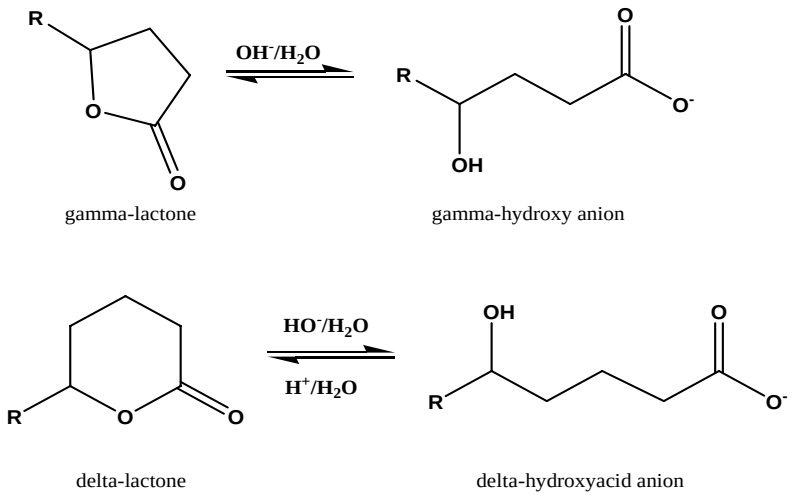

Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion*

III.1.2. Hydrolysis of Aliphatic Lactones

Fifteen candidate substances [FL-no: 10.038, 10.039, 10.040, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068, 10.168 and 10.170] are simple aliphatic lactones that are expected to readily undergo hydrolysis *in vivo*.

Information on the disposition of these substances is mainly derived from studies on a single supporting substance, butyro-1,4-lactone [FL-no: 10.006], which has been extensively studied due to the production of CNS depression, attributed to its hydrolysis product, gamma-hydroxybutyrate. No data on the candidate substances are available.

When 4-hydroxybutanoic acid gamma-lactone (butyro-1,4-lactone) is administered intravenously (Roth and Giarman, 1966), intraperitoneally (i.p.) or orally (Guidotti and Ballotti, 1970) to rats, the open-chain 4-hydroxybutanoate anion is detected in the blood and tissues and the sedative effect produced by 4-hydroxybutanoate was evidenced (Roth and Giarman, 1966; Guidotti and Ballotti, 1970). The half-life for the conversion of the lactone ring to the open-chain anion in the blood is less than one minute. The reaction is catalysed by gamma-lactonase, which shows greater activity in the plasma than in the liver or brain (Fishbein and Bessman, 1966).

Hydrolysis of various aliphatic lactones (1 mM), including those formed from tertiary alcohols, has been described after *in vitro* incubation in basic simulated intestinal fluid and rat liver homogenate, (Morgareidge, 1962a; Morgareidge, 1963a).

Table III.1. Hydrolysis of various aliphatic lactones

Substance	Test System	% Hydrolysis	Time (hr)	Reference
Gamma-Valerolactone	Simulated intestinal fluid	32	4	(Morgareidge, 1962a)
	Rat liver homogenate	93	1	(Morgareidge, 1963a)
Gamma-Nonalactone	Rat liver homogenate (pH= 7.5)	62-94	1	(Morgareidge, 1963a)
	Rat liver homogenate (pH =8)	81-88	1	(Morgareidge, 1963a)
Gamma-Undecalactone	Simulated intestinal fluid	58	1	(Morgareidge, 1962a)
	Rat liver homogenate (pH= 7.5)	26-40	4	(Morgareidge, 1963a)
	Rat liver homogenate (pH= 8)	45-70	1	(Morgareidge, 1963a)
Omega-6-Hexadecenlactone	Simulated intestinal fluid	92	0.25	(Morgareidge, 1962a)
	Simulated intestinal fluid	96	1	(Morgareidge, 1963a)
4,4-Dibutyl-gamma-butyrolactone	Simulated intestinal fluid	92	1	(Morgareidge, 1962a)

As shown in Table III.1, the rate and the extent of hydrolysis differ, depending on the lactone tested. The observation that gamma-lactones, sterically hindered gamma-lactones and omega-lactones are hydrolysed to the ring-opened form under these conditions supports the conclusion that the ring-opened hydroxycarboxylic acid anion exists in body fluids at basic pH. In acidic media, such as the gastric juice and the urine, the lactone form predominates.

Gamma-valerolactone and gamma-hexalactone have been detected in the urine of normal human adults (Zlatkis and Liebich, 1971).

III.1.3.Absorption of Aliphatic Lactones

Aliphatic lactones or the ring-opened hydroxycarboxylic acids are expected to be absorbed from the gastrointestinal tract. In rats, single oral doses >100 mg/kg bw/day of the supporting substance gamma-butyrolactone [FL-no: 10.006] were absorbed rapidly and completely from the intestinal tract (Arena and Fung, 1980; Guidotti and Ballotti, 1970; Lettieri and Fung, 1978). However, the lactone being an uncharged low molecular weight molecule may cross the cell membrane more easily than the ring-opened form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma-, delta- and omega-lactones, lactones fused to alicyclic rings such as 2-(2-hydroxycyclopent-4-enyl)ethanoic

acid gamma-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993).

Incubation of 1 mM of human R-type PON1 with aliphatic lactones gamma-butyrolactone, gamma-valerolactone, gamma-decanolactone and undecano-gamma-lactone resulted in hydrolysis rates of 9.1, 7.0, 19.0 and 13.0 µmol/min/ml substrate, respectively (Billecke et al., 2000). Hydrolysis is slower for the alicyclic fused-ring lactone, 2-(2-hydroxycyclopent-4-enyl)ethanoic acid gamma-lactone, with a hydrolysis rate of less than 3 µmol/min/ml substrate in the Q and R isoenzymes of PON1 (Billecke et al., 2000).

Based on these data, it is concluded that a wide variety of lactones readily hydrolyse in human blood serum support either prior to absorption or upon entering systemic circulation.

III.1.4.Metabolism of Lactones Formed From Linear and Branched-chain Aliphatic Hydroxy-carboxylic Acids

No literature data on the candidate substances are available; however, due to the simple structure of the substances, information on their metabolic fate may be derived from text books.

Linear aliphatic hydroxycarboxylic acids are hydrolysed and rapidly oxidised *via* the fatty acid pathway. Linear saturated 5-hydroxycarboxylic acids formed from delta-lactones are converted, *via* acetyl coenzyme A (CoA), to hydroxythioesters, which then undergo beta-oxidation and cleavage to yield an acetyl CoA fragment and a new beta-hydroxythioester reduced by two carbons. Even numbered-carbon acids continue to be oxidised and cleaved to yield acetyl CoA while odd numbered-carbon acids yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly while propionyl CoA is transformed into succinyl CoA, which then enters the citric acid cycle (Voet and Voet, 1990).

Linear saturated 4- or 6-hydroxycarboxylic acids formed from gamma- or epsilon-lactones participate in the same pathway as linear saturated 5-hydroxycarboxylic acids; however, loss of an acetyl CoA fragment produces an alpha-hydroxythioester, which undergoes oxidation and alpha-decarboxylation to yield a linear carboxylic acid and eventually carbon dioxide (Voet and Voet, 1990). In rats and dogs, the supporting substances, $^{14}CO_1$-gamma-decalactone and $^{14}CO_1$-gamma-dodecalactone, are metabolised in a manner similar to $^{14}CO_1$-lauric acid, with approximately 75 % of the labeled ^{14}CO being eliminated as carbon dioxide within 48 hours (Fassett, 1961).

The metabolic fate of the supporting substance butyro-1,4-lactone [FL-no: 10.006] has been extensively studied in animals and humans. The majority of ^{14}C-labeled 4-hydroxybutanoate administered by intravenous injection to rats was recovered as $^{14}CO_2$ within 2.5 hours (Roth and Giarman, 1965). Oxidation of gamma-butyrolactone to succinate by alcohol dehydrogenase and succinic semialdehyde dehydrogenase occurs primarily in the liver (Jakoby and Scott, 1959); succinate then participates in the citric acid cycle (Doherty and Roth, 1978; Lee, 1977; Möhler et al., 1976; Walkenstein et al., 1964). However, this pathway accounts for only a limited proportion of the metabolised compound. The main biotransformation route through which gamma-butyrolactone is metabolised is beta-oxidation as indicated by the presence of (S)-3,4-dihydroxybutyric acid, glycolic acid and 3-oxobutyric acid in the urine of human volunteers given orally 1.0 g gamma-butyrolactone [FL-no: 10.006] (Lee, 1977); other intermediates derived from beta-oxidation have previously been detected in samples of human urine (Walkenstein et al., 1964).

If the lactone is formed from a linear hydroxycarboxylic acid containing unsaturation, cleavage of acetyl CoA units will continue along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl CoA fragmentation will eventually yield a 3-enoyl CoA, which is converted to the *trans*-Δ_2-enoyl CoA before entering the fatty acid pathway. If unsaturation

begins at an even-numbered carbon, acetyl CoA fragmentation yields a Δ_2-enoyl CoA product, which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, hydration yields (R)-3-hydroxyacyl CoA, which is isomerised to (S)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering into normal fatty acid metabolism (Voet and Voet, 1990).

The principal metabolic pathways utilized for detoxication of branched-chain hydroxycarboxylic acids are influenced by the chain length and the position and size of alkyl substituents. Short-chain (< C6) branched aliphatic hydroxycarboxylic acids may be excreted conjugated mainly with glucuronic acid, or undergo alpha- or beta-oxidation followed by cleavage and complete metabolism to CO_2 (Voet and Voet, 1990; Williams, 1959a) via the fatty acid pathway and the tricarboxylic acid cycle. Alternatively, as chain length, substitution and lipophilicity increase, the hydroxycarboxylic acid may undergo a combination of omega-, omega-1 and beta-oxidation to yield polar hydroxyacid, ketoacid and hydroxydiacid metabolites that may be excreted as the glucuronic acid or sulphate conjugates in the urine and, to a lesser extent, in the faeces. Methyl substituted carboxylic acids are, to some extent, omega-oxidised in animals to form diacids, which can be detected in the urine (Williams, 1959a).

Carboxylic acids with a methyl substituent located at an even-numbered carbon (e.g. 2-methylpentanoic acid or 4-methyldecanoic acid) are metabolised extensively in the fatty acid pathway to CO_2 via beta-oxidation and cleavage of the longer branched-chain. If the methyl group is located at an odd-numbered carbon such as the 3-position, beta-oxidation is inhibited and omega-oxidation predominates, primarily leading to polar, acidic metabolites capable of being excreted in the urine as such or as conjugates (Williams, 1959a). Larger alkyl substituents (> C2) located at the alpha- or beta-position inhibit metabolism to CO_2 (Albro, 1975; Deisinger et al., 1994; Deuel, 1957) in which case there is either direct conjugation of the acid with glucuronic acid or omega-oxidation leading to diacid metabolites, which may be conjugated and excreted.

III.2. Absorption, Metabolism and Elimination of: Esters, Acetals, Aliphatic Primary Alcohols, Aldehydes, and Carboxylic Acids Containing Additional Oxygenated Functional Groups

III.2.1.Mono- and Di-esters

Thirty-two candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874 09.916 and 09.951]. They are expected to undergo hydrolysis in humans to yield their corresponding alcohol (linear or branched-chain aliphatic alcohols) and acid components (i.e. alpha-, beta- or gamma-keto or hydroxy acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised. The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters; therefore the discussion and conclusions presented in previous evaluations (FGE.01 and FGE.02) apply equally well to the candidate esters in the present evaluation.

Hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases or esterases (Heymann, 1980), the most important of which are the B-esterases (Anders, 1989; Heymann, 1980). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals, these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980).

The majority of degradation products yielded from the candicate ester hydrolysis are endogenous in mammals and are known to be completely metabolised, through different reactions, depending on their chain length and degree of branching and functional groups. It is likely that multiple metabolic reactions will occur for some hydrolysis products. The most probable metabolic reactions are the following:

- Oxidation of alcohols to aldehydes and acids.
- Conjugation of alcohols and acids to glucuronides and sulphates.
- Beta-oxidation of carboxylic acids.
- Omega-oxidations of carboxylic acids.

However, the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, has some structural similarities to valproic acid, which together with a number of its derivatives has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Although it can be predicted that 2-acetyl butyric acid is further metabolised through the above mentioned pathways of detoxication for carboxylic acids, the structural similarity with valproic acid does no allow to anticipate that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocuous products.

While no hydrolysis data have been provided for the esters of the present group of flavourings, information on some structurally related esters could be found.

In vitro incubation of the supporting substance methyl 2-oxo-3-methylvalerate [FL-no: 09.550], with a 2 % pancreatin solution (pH = 7.5), resulted in virtually complete hydrolysis (> 98 %) within 80 minutes (Leegwater and VanStraten, 1979). The supporting substance dibutyl sebacate [FL-no: 09.474] in 10 % acacia solution, was hydrolysed *in vitro* in a 10 % crude pancreatic lipase solution (Smith, 1953b).

The supporting substance ^{14}C-tributyl acetylcitrate [FL-no: 09.511], administered to male Sprague-Dawley rats by gavage at a dose level of 70 mg/kg bw, was rapidly absorbed ($t_{½}$ = 1 hour) and partially hydrolysed. More than 87 % of the administered radioactivity was eliminated within 24 hours after dosing. At least nine urinary metabolites (59 - 70 %) were detected. Five metabolites were positively identified as the partially hydrolysed mono-, di- and tri-alkylesters of citric acid. Three metabolites (25 - 26 %) were identified in the faeces; approximately 2 % of the administered dose was eliminated as $^{14}CO_2$ (Hiser et al., 1992).

III.2.2.Acetals

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption.

In vitro experiments using simulated gastric fluid revealed the rates of hydrolysis of acetals to be dependent on the structures of the aldehyde and alcohol moieties. Acetals derived from short (< C8) chain saturated aldehydes were hydrolysed almost instantly (Engel, 2003).

Hydroxycitronellal dimethyl acetal similar to the supporting substance hydroxycitronellal diethyl acetal was > 99 % hydrolysed *in vitro* to the terpenoid hydroxycitronellal and methanol in simulated gastric juice (pH about 2.1) after 1 hour and > 6 % hydrolysed in intestinal fluid (pH = 7.5) after 2 hours (Morgareidge, 1962b).

Once hydrolysed, the component alcohol, aldehydes and acids are expected to be completely metabolised, through the above mentioned common routes of biotransformations and excreted.

III.2.3.Alpha-hydroxy- and Alpha-keto-acids and Their Esters

One candidate substance [FL-no: 08.090] is an alpha-hydroxyacid. In addition alpha-keto- and alpha-hydroxyacids are formed by hydrolysis of candidate esters [FL- No: 09.333, 09.346, 09.353, 09.565, 09.580,

09.590, 09.601, 09.626, 09.644, 09.683, 09.815 and 09.874]. They would be expected to be metabolised like endogenous alpha-ketoacids formed from oxidative deamination of amino acids such as isoleucine, methionine and valine *in vivo*.

The supporting substance, 2-oxobutyric acid [FL-no: 08.066] (i.e. alpha-ketobutyric acid), is endogenous in humans as a product of methionine degradation and undergoes alpha-decarboxylation to yield propionyl CoA. Propionyl CoA ultimately enters the tricarboxylic acid cycle as succinyl CoA (Voet and Voet, 1990).

III.2.4.Beta-keto- and Beta-hydroxyacids and Their Esters

One candidate substance [FL-no: 08.053] is a beta-ketoacid. In addition eight candidate substances [FL-no: 09.346, 09.558, 09.629, 09.634, 09.824, 09.862, 09.874 and 09.916] are precursor of acetoacetic acid or its beta-hydroxy or aldehyde precursor. [FL-no: 09.346, 09.629, 09.862, 09.874 and 09.916] can be oxidised *in vivo* to acetoacetic acid. Acetoacetic acid is endogenous in humans and is formed from the condensation of two acetyl CoA units in the fatty acid pathway. It is released from the liver into the bloodstream and transported to peripheral tissues where it is converted to acetyl CoA and is completely metabolised. At elevated endogenous levels, beta-ketoacids may undergo non-enzymatic decarboxylation, which, for acetoacetic acid, yields acetone and CO_2 (Voet and Voet, 1990).

III.2.5.Gamma-keto- and Gamma-hydroxyacids and Their Esters

Gamma-hydroxy and gamma-keto acids are produced by hydrolysis of two candidate substances [FL-no: 09.832 and 09.833]. They are expected to be completely metabolised to CO_2 at low levels of exposure from use as flavouring substances. At elevated levels of exposure, the ketone function may be reduced to the corresponding secondary alcohol (Bosron and Li, 1980) and excreted as the glucuronic acid conjugate (Williams, 1959a).

Products of partial beta-oxidation or glucuronic acid conjugation have also been identified in the urine. When 1.0 g of the structurally related substance gamma-hydroxybutyrate [FL-no: 10.006] was administered to humans, it was excreted in the urine as *S*-3,4-dihydroxybutyrate, 3-oxobutyric acid and glycolate (Lee, 1977).

III.2.6.Aliphatic Di- and Tricarboxylic Acids and Their Esters

Among candidate substances the aliphatic di- and tri-carboxylic acids and their precursors [FL-no: 05.149, 08.053, 08.082, 08.103, 08.113, 09.345, 09.346, 09.347, 09.348, 09.349, 09.350, 09.351, 09.352, 09.353, 09.354, 09.558, 09.874 and 09.951] either occur endogenously in humans or are structurally related to endogenous substances. Succinic acid (from [FL-no: 09.345 and 09.347]), fumaric acid (from [FL-no: 09.350]), *l*-malic acid (from [FL-no: 09.346 and 09.874]), maleic acid (from [FL-no 09.351]) and citric acid (from [FL-no: 09.349]), are components of the tricarboxylic acid cycle (Voet and Voet, 1990). Fumaric acid is present in the blood, brain, liver, muscle and kidney of normal rats (Marshall et al., 1949). Moreover, the following acids are present in the urine of normal adults, citric, tartaric, malic, aconitic, fumaric and adipic (Hanson, 1943; Osteux and Laturaze, 1954). Alpha-ketoglutaric acid is an intermediate metabolite of citric acid, fumaric acid and succinic acid, and is formed via alpha-oxidation (Krebs et al., 1938; Simola and Krusius, 1938).

Simple aliphatic di- and tricarboxylic acid candidate substances and component acids of the candidate esters are metabolised in the fatty acid beta-oxidation pathway or tricarboxylic acid cycle. When the supporting substance ^{14}C-*l*-malic acid [FL-no: 08.017] was administered to male albino Wistar rats by gavage at a dose level of 2.5 mg/kg bw, 93 % of the radioactivity was recovered in expired air, urine and faeces (Dargel, 1966).

After the administration of the radioactive supporting substance adipic acid [FL-no: 08.026] to rats by stomach tube at a dose level of 200 - 300 mg/kg bw, the compound was extensively metabolised. Labelled products identified in the urine included glutamic acid, lactic acid, beta-ketoadipic acid and citric acid. The presence of the beta-oxidation metabolite, beta-ketoadipic acid, indicates that adipic acid participates in beta-oxidation in the fatty acid pathway (Rusoff et al., 1960).

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids (Bosron and Li, 1980; Feldman and Weiner, 1972; Levi and Hodgson, 1989). The resulting carboxylic acids would be metabolised in the fatty acid pathway and tricarboxylic acid cycle (Voet and Voet, 1990) or conjugated to glucuronides and sulphates and excreted. Branched-chain diols or keto alcohols may undergo oxidation to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted, through the common routes of biotransformation of carboxylic acids.

III.2.7.Aliphatic Alkoxy- alcohol and Diols

Among candidate substances, one is an alkoxy-alcohol [FL-no: 02.242] and two are diols [FL-no: 02.132 and 02.198].

The metabolism and disposition of 2-butoxyethanol [FL-no: 02.242] were extensively studied, and details are reported below. However, it can be anticipated that the major metabolite is butoxyacetic acid, which is primarily responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

1-Hydroxypropan-2-one [FL-no: 07.169] (acetol) is an endogenous metabolite of acetone which is also an endogenous substance formed from the degradation of body fat/fatty acids.

The metabolism in mammals of acetone, which at low concentrations, primarily occurs in the liver, is shown in Figure III.2. At low acetone concentrations in blood, i.e. in healthy humans not exposed to external sources, in amounts of approximately 4 - 12 mg per person corresponding to 0.7 to 2 mg/l blood (Ashley et al., 1994; Dick el al., 1988; Wang et al, 1994c), the major pathway is via the methylglyoxal route. At higher acetone concentrations in the blood, e.g. after acetone exposure, after fasting or in relation to certain deceases the propan-1,2-diol route is the dominating pathway. In the fist step acetone is oxidized to 1-hydroxypropan-2-one via acetone monooxygenase (p-450 IIE1). 1-Hydroxypropan-2-one is oxidised to 2-oxopropanal via acetol monooxygenase (p-450 IIE1), or at higher acetone concentrations to propan-1,2-diol. 2-Oxopropanal is then oxidised to pyruvate leading to glucose formation (Morgott, 1993; WHO, 1998a; NAS/COT, 2005).

The diols are anticipated to be metabolised by the common route of alcohol biotransformation, i.e. direct conjugation or oxidation by alcohol-dehydrogenase to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted.

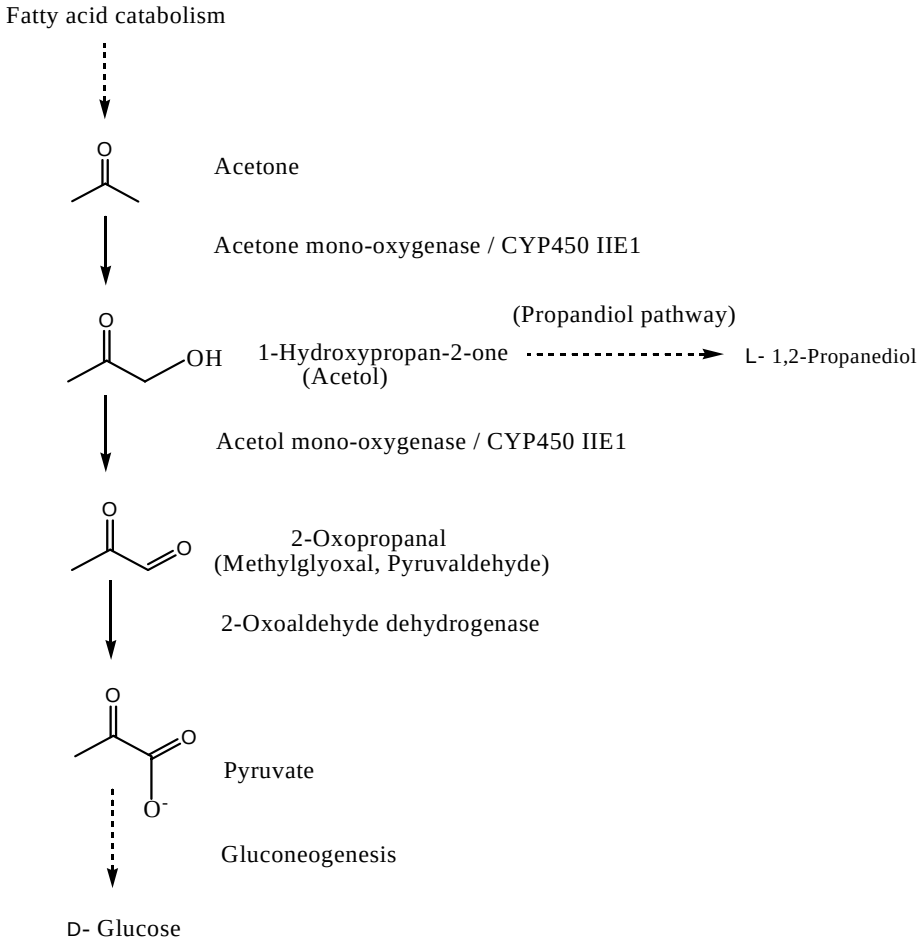

Figure III.2. *Acetone metabolism (methylglyoxal pathway)*

III.3. Studies on Candidate Substances

2-Butoxyethan-1-ol [FL-no: 02.242]

Several experiments by the oral route of administration have been conducted, indicating that 2-butoxyethan-1-ol is rapidly absorbed, metabolised and eliminated. Butoxyacetic acid is its major metabolite, metabolism being mainly catalysed by hepatic alcohol dehydrogenase; most excretion is in the urine (Corley et al., 1994; Ghanayem et al., 1987a; Ghanayem et al., 1987b; Ghanayem et al., 1987c; Medinsky et al., 1990).

The distribution and excretion of ^{14}C-butoxyethanol and its metabolites was evaluated using male F344 rats (9 - 13 weeks old). A single 125 or 500 mg/kg dose of ^{14}C-butoxyethanol was administered to each animal via gavage. Animals were killed 48 hours post-administration and tissues excised. At 48 hours, approximately 18 % and 10 % of the administered dose was exhaled as $^{14}CO_2$ for the 125 and 500 mg/kg doses, respectively; whereas only between 2 and 3 % was excreted in the faeces. The percentage of the 125 mg/kg dose excreted in the urine (70 %) was significantly greater than the percentage excreted after the 500 mg/kg dose (40 %). Butoxyacetic acid was the only urinary metabolite detected for the 125 mg/kg dose; the glucuronide conjugates of butoxyethanol and butoxyacetic acid (23 %) were also detected in the urine of animals dosed with the higher dose. A small portion (8 %) of the 500 mg/kg dose was excreted in the bile 8 hours after dosing. Compared to the 125 mg/kg dose group, tissue concentrations of ^{14}C-butoxyethanol 48

hours after administration were significantly greater in specific organs of rats that received the 500 mg/kg dose. In both dose groups the highest concentration of radioactivity was detected in the forestomach, followed by the liver, kidneys, spleen and the glandular stomach (Ghanayem et al., 1987c).

The metabolism and excretion of 2-butoxyethan-1-ol [FL-no: 02.242] were evaluated using both young (4 to 5 weeks old) and adult (9 to 13 weeks old) male F344 rats with the same experimental design described in Ghanayem *et al*. (1987c), except that ^{14}C-butoxyethanol was administered at a single oral dose (500 mg/kg). There was a significantly higher proportion of the administered dose eliminated as CO_2 in young rats as compared to older rats. Similarly, a significantly higher proportion of the administered dose was excreted in the urine of the young rats. The butoxyacetic acid/butoxyethanol-glucuronide + butoxyethanol-sulphate ratio was significantly greater in older rats (Ghanayem et al., 1987a), which are consistently more susceptible to the toxic action of 2-butoxyethan-1-ol . There was a strong correlation between the amount of butoxyacetic acid in the urine and 2-butoxyethanol-induced haematotoxicity. Moreover, metabolic activation via alcohol and aldehyde dehydrogenases is a prerequisite for the induction of toxic effects, since pre-treatment of rats with pyrazole (alcohol dehydrogenase inhibitor) or cyanamide (aldehyde dehydrogenase inhibitor) protected rats against 2-butoxyethanol-induced haematotoxicity and increased the urinary amount of butoxyethanol-conjugates (glucuronide and sulphate) (Ghanayem et al., 1987b).

2-Butoxyethan-1-ol [FL-no: 02.242] was administered to male F344/N rats (11 to 12 weeks old) at concentrations in drinking water of 290, 860 and 2590 ppm over a 24 hours period. Butoxyethanol was administered as 2-butoxy[U-^{14}C]ethanol, and exhaled air, urine and faeces were collected over a 72 hours period. Most ^{14}C was excreted either in the urine or exhaled as CO_2: 50 - 60 % of the administered dose was eliminated in the urine as butoxyacetic acid and 8 to 10 % as CO_2. Analysis of urine samples collected during the 12 - 24 hours after dosing indicated that the majority of the radioactivity was associated with butoxyacetic acid while 10 % of the administered dose was identified as glycol ether. Minor levels of glucuronide conjugate of butoxyethanol and unmetabolised butoxyethanol were also reported (Medinsky et al., 1990).

Non-oxidative metabolism of 2-butoxyethan-1-ol [FL-no: 02.242] via fatty acid conjugation was also investigated in the liver of F344 male rats following a single oral administration of 500 mg/kg [ethyl-1,2-^{14}C] 2-butoxyethanol. Animals were killed two hours after treatment and samples prepared for analysis. It was demonstrated that 2-butoxyethan-1-ol is metabolised non-oxidatively via conjugation with long-chain fatty acids, and the formation of these esters appears to be catalysed by the enzymes involved in fatty acid conjugation of xenobiotic alcohols. However, the biological significance of 2-butoxyethan-1-ol conjugation with fatty acids remains unclear, although several such lipid conjugates were found to be toxic in laboratory animals and cell lines (Kaphalia et al., 1996).

The elimination kinetics of 2-butoxyethan-1-ol were studied in a once-through isolated perfused rat liver system in the presence and absence of ethanol. Dose-dependent Michaelis-Menten kinetics were observed in the elimination of 2-butoxyethan-1-ol. The apparent K_m ranged from 0.32 to 0.70 mM and the maximum elimination rate ranged from 0.63 to 1.4 micromol/min/g liver in six experiments. The results support the hypothesis that 2-butoxyethan-1-ol is metabolised mainly via oxidation by alcohol dehydrogenase in the rat liver at concentration which can be considered representative of human exposure (Johanson et al., 1986).

Butane-1,3-diol [FL-no: 02.132]

Two groups of 14 rats were administered a control diet (70 % carbohydrate and 30 % fat) or a treatment diet (45 % carbohydrate, 30 % fat and 25 % butane-1,3-diol). Blood acetoacetate and beta-hydroxybutyrate concentrations were increased significantly and blood pyruvate concentration was decreased significantly in rats administered the treatment diet. Addition of butane-1,3-diol to *in vitro* liver tissue slices, as they were metabolising glucose to lactate and pyruvate, greatly decreased pyruvate levels and significantly increased lactate/pyruvate ratios. When butane-1,3-diol and glucose were used as substrates, there was a large increase in acetoacetate and beta-hydroxybutyrate formation in liver tissue slices with butane-1,3-diol. Therefore,

butane-1,3-diol is metabolised in the cytosol and converted by the liver *in vivo* and *in vitro* to ketones prior to its oxidation in the tricarboxylic acid cycle (Mehlman et al., 1971).

Tate *et al.* (1971) found that the conversion of butane-1,3-diol to beta-hydroxybutyrate in rat liver was strongly dependent in NAD^+ and it was inhibited by pyrazole. Since pyrazole is a specific inhibitor of alcohol dehydrogenase (ADH), this inhibition indicated ADH as the catalyst in the catabolism in the cytosol of butane-1,3-diol to an intermediate, aldol. Aldol is then further oxidised to beta-hydroxybutyrate (Tate et al., 1971).

Diethyl maleate [FL-no: 09.351]

Traditionally diethyl maleate [FL-no: 09.351] has been utilised to acutely deplete reduced glutathione (GSH) in the tissues, since it forms GHS-conjugates very rapidly, causing a significant decrease in GSH content (Boyland & Chasseaud, 1970). The liver is the most sensitive organ to diethyl maleate-induced GSH depletion, generally occurring 30 - 90 minutes after intraperitoneal injection of the compound. In the rat, the formed GSH-conjugates are excreted in bile or as mercapturates in urine (Barnhart and Combes, 1978).

The excretion of mercapturic acid was determined in chimpanzees and rats after the administration of diethyl maleate [FL-no: 09.351]. The excretion rate of endogenous thioethers in the urine of untreated chimpanzees and rats was 18.0 and 94.4 micromol/kg bw/24 hours, respectively. The value in man was nearly the same as found in chimpanzees. The administration of diethyl maleate at 30, 75 and 200 mg/kg bw led to a dose-dependent increase in the excretion of urinary mercaptic acids in both species, but the increase in rats was about twice that of chimpanzees. Additional experiments indicate that the observed species differences are due to differences in the glutathione conjugation (Summer et al., 1979a).

Glutaric acid [FL-no: 08.082]

Rat liver mitochondria metabolise glutarate [FL-no: 08.082] at a slow rate as compared with glutaryl CoA. The stimulatory effect of citric acid cycle intermediates, NAD and CoA on glutarate metabolism was interpreted as a manifestation of their involvement in the activation of glutarate by a thiol transferase with succinyl CoA as the coenzyme A donor (Besrat et al., 1969).

Glutaraldehyde [FL-no: 05.149]

Material mass balance and pharmacokinetics studies were conducted with glutaraldehyde [FL-no: 05.149] in groups of F344 rats (four/sex) and New Zealand white rabbits (two/sex) using the intravenous route of exposure at dose volumes of 0.2 ml and 2.5 ml, respectively. Rats and rabbits received intravenous doses of 0.075 and 0.75 % glutaraldehyde in the tail vein or ear vein, respectively. Glutaraldehyde was distributed rapidly and eliminated when administered intravenously to rats and rabbits. When a single infusion of 0.075 % glutaraldehyde was administered, 75 to 80 % of the dose in the rat and 66 to 71 % in the rabbit were recovered as $^{14}CO_2$ during the first 24 hours following administration, with 80 % of the $^{14}CO_2$ being recovered during the first four hours. When a single infusion of 0.75 % glutaraldehyde was administered, the proportion of the dose recovered as $^{14}CO_2$ decreased and the amount of radioactivity recovered in urine, tissues and carcass increased as compared to the 0.075 % glutaraldehyde infusion. Also the average plasma concentration of radioactivity increased 10-fold in rats and rabbits with a 10-fold increase in dose, but the tissue concentration increased by an even greater amount. The results suggest that the mechanisms involved in the disposition of glutaraldehyde were saturated when the higher dose was administered and resulted in a shift in the elimination pathway (McKelvey et al., 1992). Although the metabolism of glutaraldehyde has not been studied in detail, it has been suggested that it is oxidised first to a mono- or dicarboxylic acid by aldehyde dehydrogenase (Weiner, 1980; Hjelle and Peterson, 1983) and then further oxidised through an acidic intermediate to CO_2 (McKelvey et al., 1992).

Nonanedioic acid [FL-no: 08.103]

Following intravenous administration in human volunteers, nonanedioic acid [FL-no: 08.103] and its major catabolite, pimelic acid, are found in serum and urine indicating transformation by mitochondrial beta-oxidative enzymes. Serum levels of nonanedioic acid are short-lived following a single 5 or 10 g intravenous (i.v.) infusion over 1-hour. In the first hour after the cessation of i.v. administration, serum levels of nonanedioic acid decreased to about 25 % of their peak values. Administration of multiple intravenous doses at the same concentrations as the one-hour doses produces sustained higher levels of nonanedioic acid in the serum during the period of administration (Passi et al., 1989).

III.4. Conclusions

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured, while in acidic media, such as gastric juice and urine, the lactone ring is favoured.

Lactones formed from linear saturated and branched-chain aliphatic hydroxycarboxylic acids are hydrolysed to the corresponding hydroxycarboxylic acid that then enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by two-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then completely metabolised in the citric acid cycle.

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (linear or branched-chain aliphatic alcohols) and acid components (i.e. alpha-, beta- or gamma-keto- or hydroxy-acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised and excreted through the common pathways of detoxication of aliphatic alcohols and carboxylic acids). The hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, which shows some structural similarities to valproic acid, which together with a number of its derivatives, has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Therefore, it cannot be anticipated that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocuous products.

The presence of a second oxygenated functional group has little, if any, effect on hydrolysis of these esters. The most probable metabolic reactions of the hydrolysis products are: oxidation of alcohols to aldehydes and acids; conjugation of alcohols and acids to glucuronides and sulphates; beta-oxidation of carboxylic acids; omega-oxidations of carboxylic acids.

Beta-keto acids and derivatives like acetoacetic acid undergo decarboxylation. Along with alpha-keto and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways. The gamma-keto-acids and related substances may undergo complete or partial beta-oxidation to yield metabolites that are eliminated in the urine. Omega-substituted derivatives are readily oxidised and/or excreted in the urine. Simple aliphatic di- and tricarboxylic acids participate in the tricarboxylic acid cycle.

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may be expected to undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption. Once hydrolysed, the component alcohols and aldehydes are expected to be metabolised primarily through the above mentioned common routes of biotransformations and excreted.

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their

corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation.

Among candidate substances, an alkoxy-alcohol 2-butoxyethanol [FL-no: 02.242] is mainly metabolised to butoxyacetic acid, which has been identified as the major responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products many of which are endogenous in humans, at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351]; as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to the structurally related ethoxypropanoic acid and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], whose hydrolysis gives rise to 2-acetylbutyric acid, with some structural similarities to valproic acid, a known teratogenic compound.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 16 candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30, for 43 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Methyl 2-hydroxy-4-methylpentanoate [09.548])	Mouse	NR	Oral	4000[1]	(Pellmont, 1978)
(Methyl 2-oxo-3-methylvalerate [09.550])	Rat	M	Gavage	> 5000	(Moreno, 1979b)
(Butyro-1,4-lactone [10.006])	Mouse	NR	Gavage	1245	(Schafer and Bowles, 1985)
(Pentano-1,4-lactone [10.013])	Rat	NR	Oral	> 5000	(Moreno, 1978e)
	Rat	NR	Gavage	8800	(Deichmann et al., 1945)
	Rabbit	NR	Gavage	2480	(Deichmann et al., 1945)
(Hexano-1,4-lactone [10.021])	Rat	NR	Oral	> 5000	(Moreno, 1977f)
(Hexano-1,5-lactone [10.010])	Rat	M	Gavage	13,030	(Smyth et al., 1962)
(Heptano-1,4-lactone [10.020])	Rat	NR	Oral	> 5000	(Moreno, 1977g)
(Octano-1,4-lactone [10.022])	Rat	NR	Oral	> 5000	(Moreno, 1974c)
(Octano-1,5-lactone [10.015])	Rat	NR	Oral	> 5000	(Moreno, 1977h)
(Nonano-1,4-lactone [10.001])	Rat	M, F	Gavage	9780	(Jenner et al., 1964)
	Rat	M	Oral	6600	(Moreno, 1972b)
	Guinea pig	M, F	Gavage	3440	(Jenner et al., 1964)
(Decano-1,4-lactone [10.017])	Rat	NR	Oral	> 5000	(Moreno, 1975h)
(Decano-1,5-lactone [10.007])	Rat	NR	Oral	> 5000	(Levenstein, 1975c)
(Decano-1,6-lactone [10.029])	Mouse	M, F	Gavage	5252	(Moran et al., 1980)
(Undecano-1,4-lactone [10.002])	Rat	M, F	Gavage	18500	(Jenner et al., 1964)
(Undecano-1,5-lactone [10.011])	Rat	NR	Oral	> 5000	(Moreno, 1975i)
(Dodecano-1,4-lactone [10.019])	Rat	NR	Oral	> 5000	(Moreno, 1974d)
(Dodecano-1,5-lactone [10.008])	Rat	NR	Oral	> 5000	(Moreno, 1977e)
(Dodecano-1,6-lactone [10.028])	Mouse	M, F	Gavage	7898	(Moran et al., 1980)
(Pentadecano-1,15-lactone [10.004])	Rat	NR	Oral	> 5000	(Levenstein, 1974c)
(5-Methylfuran-2(3H)-one [10.012])	Mouse	M, F	Gavage	2800	(Moran et al., 1980)
(Dodec-6-eno-1,4-lactone [10.009])	Rat	M, F	Oral	> 5000	(Watanabe and Morimoto, 1990)
(3,7-Dimethyloctano-1,6-lactone [10.027])	Rat	M, F	Gavage	> 5000	(Lewis and Palanker, 1979a)
(5-Hexyl-5-methyldihydrofuran-2(3H)-one [10.051])	Rat	NR	Oral	> 5000	(Moreno, 1976j)
(Citronellyl oxyacetaldehyde [05.079])	Rat	NR	Oral	> 5000	(Moreno, 1973d)
1-Hydroxypropan-2-one [07.169]	Rat	NR	Oral	2200[2]	(Smyth and Carpenter, 1948)
(4,4-Dimethoxybutan-2-one [06.038])	Rat	M	Gavage	6200	(EPA, 1971)
(Ethyl acetoacetate [09.402])	Rat	NR	Oral	3980[3]	(Smyth et al., 1949)
Methyl acetoacetate [09.634]	Rat	NR	Oral	3000	(Smyth and Carpenter, 1948)
	Rat	NR	Oral	2800	(BASF, 1978)
(Butyl acetoacetate [09.403])	Rat	F	Gavage	11260	(Smyth et al., 1954)
(Geranyl acetoacetate [09.405])	Rat	NR	Oral	> 5000	(Moreno, 1976k)
(Ethyl 3-oxohexanoate [09.542])	Mouse	NR	Oral	4000 – 8000	(Pellmont, 1973a)
2-Butoxyethan-1-ol [02.242]	Rat	M	Gavage	1480	(Smyth et al., 1941)
	Rat	NR	Oral	1174	(BASF, 1956)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
	Rat	NR	Oral	620	(Rowe and Wolf, 1982)
	Rat	M, F	Oral	2800	(Carpenter et al., 1956)
	Rat	M	Gavage	2680	(Myers and Homan, 1980)
	Rat	NR	Oral	470	(Wolf, 1959)
	Rat	M	Gavage	1190 – 2800	(Weil and Wright, 1967)
	Rat	M	Gavage	1590	(Moreno, 1976l)
	Rat	M	Gavage	7500	(Moreno, 1976l)
	Rat	NR	Oral	1746	(Eastman Kodak Co., 1989)
	Rat	M	Gavage	7292	(Eastman Kodak Co., 1984)
	Mouse	NR	Oral	1230	(Carpenter et al., 1956)
	Mouse	NR	Oral	1170 – 1700	(Dow Chemical Company, 1982a)
	Mouse	NR	Oral	1519	(Eastman Kodak Co., 1989)
	Mouse	M	Gavage	2406	(Eastman Kodak Co., 1984)
	Rabbit	M	Oral	320 – 370	(Carpenter et al., 1956)
	Guinea pig	M, F	Oral	1200	(Carpenter et al., 1956)
	Guinea pig	M, F	Gavage	1200	(Smyth et al., 1941)
Butane-1,3-diol [02.132]	Rat	F	Gavage	> 5000	(CTFA, 1978)
	Rat	M	Gavage	18610	(Smyth et al., 1941)
	Rat	M	Gavage	22800	(Smyth et al., 1951a)
	Rat	NR5	Oral	29590	(Bornmann, 1954)
	Mouse	NR5	Oral	23440	(Bornmann, 1954)
	Mouse	NR	Oral	23310	(Kopf et al., 1950; Loeser, 1949)
	Mouse	NR	Oral	12980	(Wenzel and Koff, 1956)
	Guinea pig	M, F	Gavage	11460	(Smyth et al., 1941)
(4-Oxovaleric acid [08.023])	Rat	NR	Oral	1850	(Moreno, 1977j)
(Ethyl 4-oxovalerate [09.435])	Rat	NR	Oral	> 5000	(Moreno, 1978f)
Octane-1,3-diol [02.198]	Rat	NR	Oral	> 20000	(Frankenfeld et al., 1975)
(3,7-Dimethyloctane-1,7-diol [02.047])	Rat	M, F	Gavage	> 5000	(Levenstein, 1973b)
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Rat	NR	Oral	> 5000	(Shelanski and Moldovan, 1973b)
1,1,3-Triethoxypropane [06.097]	Rat	M	Gavage	1600	(Smyth et al., 1951a)
Diethyl oxalate [09.353]	Rat	NR	Oral	400 – 1600	(Patty, 1963)
Malonic acid [08.053]	Rat	NR	Oral	1310	(Bio-Fax, 1971)
Dimethyl malonate [09.558]	Rat	NR	Oral	4620	(Levenstein, 1976b)
	Rat	NR	Oral	5331	(Merck Index, 1992)
(Diethyl malonate [09.490])	Rat	NR	Oral	14900	(Smyth et al., 1969a)
	Mouse	NR	Gavage	5400	(Wolven and Levenstein, 1969)
(Diethyl succinate [09.444])	Rat	NR	Oral	8530[3]	(Smyth et al., 1951a)
(Fumaric acid [08.025])	Rat	M, F	Oral	M: 10700; F: 9300	(Vernot et al., 1977)
Diethyl fumarate [09.350]	Rat	NR	Oral	1500	(Hood, 1951)
(l-Malic acid [08.017])	Rat	NR	Oral	3500	(Morgareidge, 1973a)
	Mouse	NR	Oral	2660	(Morgareidge, 1973b)
	Rabbit	NR	Oral	3000	(Morgareidge, 1973c)
Diethyl maleate [09.351]	Rat	M	Gavage	3200	(Smyth et al., 1949)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Rat	NR	Oral	7500[6]	(Foulger, 1947)
Glutaric acid [08.082]	Mouse	NR	Oral	6000	(Boyland, 1940)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
Glutaraldehyde [05.149]	Rat	NR	Gavage	252	(Stonehill et al., 1963)
	Rat	M	Gavage	733[7]	(Ballantyne and Myers, 2001)
	Rat	M	Gavage	2380[8]	(Smyth et al., 1962)
	Rat	M	Gavage	540[9]	(Striegel and Carpenter, 1964)
	Rat	M, F	Oral	M: 134; F: 165	(Ikeda, 1980)
	Rat	M	Gavage	1300[7]	(Myers et al., 1977b)
	Rat	M	Gavage	1870[8]	(Myers et al., 1977c)
	Mouse	NR	Gavage	352	(Stonehill et al., 1963)
	Mouse	M, F	Oral	M: 100; F: 110	(Ikeda, 1980)
	Mouse	M, F	Gavage	M: 152[7] ; F: 113[7]	(Ballantyne and Myers, 2001)
	Mouse	M, F	Gavage	M: 151[8] ; F: 115[8]	(Union Carbide Corp., 1992)
(Adipic acid [08.026])	Mouse	M	Oral	1900[10]	(Horn et al., 1957)
Diethyl adipate [09.348]	Rat	NR	Oral	> 1600	(Patty, 1963)
Nonanedioic acid [08.103]	Rat	M, F	Gavage	> 4000	(Mingrone et al., 1983)
	Rabbit	M, F	Gavage	> 4000	(Mingrone et al., 1983)
(Diethyl sebacate [09.475])	Rat	M, F	Gavage	14470	(Jenner et al., 1964)
	Rat	M	Oral	32000[11]	(Smith, 1953b)
	Mouse	NR	Gavage	> 32000	(Lawrence et al., 1974)
(Triethyl citrate [09.512])	Rat	NR	Gavage	7000[4]	(Finkelstein and Gold, 1959)
(Tributyl acetylcitrate [09.511])	Rat	NR	Gavage	> 30000[12]	(Finkelstein and Gold, 1959)
(3-Hydroxy-2-oxopropionic acid [08.086])	Rat	NR	Oral	2000	(Hoechst, 1995)
Succinic acid, disodium salt [08.113]	Rat	NR	Oral	>1200	MHLW Japan 2002 in: (OECD, 2003)

M = Male; F = Female
NR: Not reported.
[1] Dosed in 5 % gum arabic.
[2] Data derived from a range-finding study.
[3] Actual LD_{50} not reported.Study conducted as a dose range-finder (DRF).
[4] Actual LD_{50} not reported.Value reported as approximate LD_{50}.
[5] Data point not verified.
[6] Actual LD_{50} not reported.Value reported as MFD (assumed to be Median Fatal Dose).
[7] Glutaraldehyde dosed as a 50 % (w/w) solution.The LD_{50} is expressed as mg of actual active ingredients.
[8] Test substance administered as a 25 % solution. The LD_{50} is expressed as mg of actual active ingredients.
[9] Test substance administered as a 45 % aqueous solution.The LD_{50} is expressed as mg of actual active ingredients.
[10] Dosed as a 6 % suspension in 0.5 % methyl cellulose.
[11] Actual LD_{50} not reported.Value represents lowest dose level tested causing mortality. Animals dosed at 16,000 mg/kg had 100 % survival rate, while animals dosed at 32,000 mg/kg had 100 % fatality. Acute lethal dose for dibutyl sebacate is between 16,000 and 32,000 mg/kg.
[12] Value represents the maximum dose level tested. Animals dosed at 30,000 mg/kg had 100 % survival rate.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 and for 20 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for two structurally related substances. The supporting and structurally related substances are listed in brackets.

Table IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Mouse; M, F 5/20	Gavage	90	525	(NTP, 1992e)	a)
	Rat; M, F 5/20	Gavage	90	450	(NTP, 1992e)	a)
	Mouse; M, F 2/100	Gavage	2 years	262	(NTP, 1992e)	a)
	Rat; M, F 2/100	Gavage	2 years	112	(NTP, 1992e)	a)
	Rat; M, F 1/7	Diet	4 – 6 months	100[2]	(Fassett, 1961)	
Pentano-1,4-lactone [10.013])	Rat; M, F 1/30	Diet	90	M: 49[2] ; F: 51.1[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/10	Diet	90	500[2]	(Hagan et al., 1967)	a)
(Octano-1,5-lactone [10.015])	Rat; M, F 1/7	Diet	4 - 6 months	32[2]	(Fassett, 1961)	
(Nonano-1,4-lactone [10.001])	Rat; M, F 1/30	Diet	90	M: 62.8[2] ; F: 72.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
(Decano-1,4-lactone [10.017])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
(Decano-1,5-lactone [10.007])	Rat; M, F 1/NR	Diet	49 weeks	150[2]	(Fassett, 1961)	
	Dog; M, F 1/NR	Diet	38 weeks	250[2]	(Fassett, 1961)	
(Undecano-1,4-lactone [10.002])	Rat; M, F 1/30	Diet	90	M: 14.6[2] ; F: 16.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
	Rat; M, F NR[4]	Diet	90	14.1[2, 3]	(Shillinger, 1950)	
(Dodecano-1,4-lactone [10.019])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
(Dodecano-1,5-lactone [10.008])	Rat; M, F 1/NR	Diet	49 weeks	300[2]	(Fassett, 1961)	
	Dog; M, F	Diet	38 weeks	150[2]	(Fassett, 1961)	

Table IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	1/NR					
(5-Methylfuran-2(3H)-one [10.012])	Rat; M, F 1/NR	Diet	90	M: 17.4[2]; F: 17.7[2]	(Shellenberger, 1971c)	a)
(Ethyl acetoacetate [09.402])	Rat; M, F 3/32	Diet	28 - 29	300	(Cook et al., 1992)	a)
2-Butoxyethan-1-ol [02.242]	Rat; M, F 4/20	Diet	91 – 93	40	(Union Carbide Corp., 1963)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	No NOAEL derived [13]	(Union Carbide Corp., 1952)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	76	(Carpenter et al., 1956)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/20	Drinking water	13 weeks	1500 ppm (150 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M 3/10	Gavage	6 weeks	222	(Krasavage, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/10	Drinking water	14	400	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/20	Drinking water	13 weeks	6000 ppm (1200 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/6[4]	Drinking water	21	M: < 2000 ppm (200 mg/kg/day); F: < 1600 ppm (160 mg/kg/day)	(Exon et al., 1991)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/10	Drinking water	14	< 150[5]	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M NR	Oral	5 week	1000	(Bernstein, 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/5	Gavage	5 weeks[6]	< 500	(Nagano et al., 1977)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/NR	Gavage	5 weeks	1000[7]	(Nagano et al., 1979)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M3/NR	Gavage	5 weeks	< 500[8]	(Nagano et al., 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/50	Inhalation	2 years		(NTP, 2000b)	
	Mouse; M, F 4/50	Inhalation	2 years		(NTP, 2000b)	
Butane-1,3-diol [02.132]	Rat; M 15/10	Diet	30 weeks	200000 ppm (10000 mg/kg/day)	(Miller and Dymsza, 1967)	Study aimed at elucidating the usability of butane-1,3-diol as synthetic energy source. It is of limited value for toxicological evaluation.
	Rat; M, F 3/60	Diet	2 years	100000 ppm (5000 mg/kg/day)	(Scala and Paynter, 1967)	Some details of results not reported (e.g. consumption, histopathological evaluation), limited value.
	Dog; M, F 3/8	Diet	2 years	30000 ppm (750 mg/kg/day)	(Scala and Paynter, 1967)	
	Dog; M, F 4/8	Diet	13 weeks	6000	(Reuzel et al., 1978)	Methods, results, discussion comprehensible. Valid study.
(4-Oxovaleric acid [08.023])	Rat: NR	Diet	16	1000[2]	(Tischer et al., 1942)	a)

Table IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	2/3					
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Rat; M, F 1/20 1/60	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
Malonic acid [08.053]	Rat; M, F 3/140	Diet	2 years	10[9]	(Hogan and Rinehart, 1979)	
(Diethyl malonate [09.490])	Rat; M, F 2/20	Diet	13 weeks	< 500	(Posternak, 1964a)	a)
	Rat; M, F 1/20-32	Diet	90	40[2]	(Posternak et al., 1969)	a)
(Fumaric acid [08.025])	Rat 2/14 1/20	Diet[10]	2 years	1380[2]	(Levey et al., 1946)	a)
	Guinea pig; M, F 1/NR	Diet	1 year	400[2]	(Levey et al., 1946)	a)
	Rat; M, F Rat; M 4/12 3/12	Diet	2 years	1200	(Fitzhugh and Nelson, 1947)	a)
	Rabbit; NR 3/15	Diet[10]	150	2070[2]	(Packman et al., 1963)	a)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Dog; NR 1/4	Oral	90-114	< 990	(Krop et al., 1945)	a)
	Rat; M, F 4/12	Diet	2 years	1200[2]	(Fitzhugh and Nelson, 1947)	a)
	Rabbit: NR 3/15	Diet[2]	150	2310[2]	(Packman et al., 1963)	a)
Glutaraldehyde [05.149]	Rat; M, F 4/10	Diet	7	1.0	(Union Carbide Corp., 1986)	
	Rat; M, F 3/NR	Drinking water	14	100 ppm (10 mg/kg/day)	(Union Carbide Corp., 1993)	
	Rat; NR 3/3	Drinking water	11 weeks	5000 ppm (500 mg/kg/day)	(Spencer et al., 1978)	
	Mouse; M, F 3/40	Drinking water	90	100 ppm (20 mg/kg/day)	(Bushy Run Research Center, 1989)	
	Rat; M, F 3/NR	Drinking water	13 weeks	50 ppm (5 – 7 mg/kg/day)	(Union Carbide Corp., 1986)	
	Dog;, M, F 3/8	Drinking water	13 weeks	50 ppm (3.2 mg/kg/day)	(Bushy Run Research Center, 1990)	
	Rat; M, F 3/200	Drinking water	2 years	50 ppm (4 mg/kg/day)	(Van Miller et al., 2002)	Large Granular Lymphocytic Leukemia in treated as well as control rats; no clear dose-resposne relationship. Otherwise no significant increase in neoplasia.
(Adipic acid [08.026])	Rat; M, F 4/20-39	Diet	2 years	~ 1500[11]	(Horn et al., 1957)	a)
Nonanedioic acid [08.103]	Rat; M, F 2/30	Diet	90 and 180	280	(Mingrone et al., 1983)	Details of methods not reported, study not performed according to appropiate

Table IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
						guidelines. Study of limited value.
	Rabbit; M, F 2/20	Diet	90 and 180	400	(Mingrone et al., 1983)	
	Rat; F 1/10	Diet	3 month[12]	140	(Mingrone et al., 1983)	
	Rabbit; F 1/10	Diet	3 months[12]	200	(Mingrone et al., 1983)	
(Diethyl sebacate [09.475])	Rat; M, F 2/10	Diet	17-18 wks or 27-28 wks	1000[2]	(Hagan et al., 1967)	a)
	Rat; M 4/10	Diet	1 year	1250[2]	(Smith, 1953b)	a)
	Rat; M 5/16	Diet	2 years	6250[2]	(Smith, 1953b)	a)
(Triethyl citrate [09.512])	Rat; M, F 3/7	Diet	2 months	4000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 1/6	Gavage	2 months	< 285	(Finkelstein and Gold, 1959)	a)
(Tributyl acetylcitrate [09.511])	Rat; M, F 2/4	Diet	2 months	5000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 2/4	Gavage	2 months	< 5700	(Finkelstein and Gold, 1959)	a)
(Succinate, monosodium)	Rat; M,F 10/10	Drinking water	13 weeks	1250	(Maekawa et al., 1990) in (OECD, 2003)	
	Rat; M,F 50/50	Drinking water	2 years	2000	(Maekawa et al., 1990) in (OECD, 2003)	Monosodium succinate was given ad libitum in drinking water at levels of 0, 1, or 2 % to F344 rats (50 males, 50 females). No toxic lesion specifically caused by long-term administration of monosodium succinate was detected.
(Succinate, disodium hexahydrate)	Rat; M,F 12 /12	Gavage 0, 100,300, 1000 mg/kg)	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	Males: 100 Females: 300	MHLW, Japan 2002 in (OECD, 2003)	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: males 60 mg/kg; females, 180 mg/kg.

NR: Not reported.

M = Male; F = Female.

a) Study summarised by JECFA at the 49th or 53rd meetings (JECFA, 1998a; JECFA, 2000c).

[1] Number of groups represents the number of treatment groups investigated.Control groups are not reported.

[2] This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.

[3] Article published in Russian. Data point not verified.

[4] Six animals per treatment group. The treatment groups for males were not the same as the females. Males were administered 2000 or 6000 ppm of the test substance, while the corresponding dose levels for the females were 1600 and 4800 ppm, respectively.

[5] Compared to the control group absolute and relative thymus weights were significantly lower in males. These findings were not seen in females receiving up to 650 mg/kg/day.

[6] Animals dosed 5 days a week for five weeks.

[7] Changes in absolute or relative testis weights were not observed.

[8] A decrease in red cell count was noted in the 500 mg/kg dose group and higher dose groups.

[9] No treatment related effects were noted upon mortality, ophthalmology or body weights in the males. Microscopic evaluation noted that the transitional cell carcinomas were found in the urinary bladder. The findings were indicated to be dose related.

[10] Administered as the sodium salt.

[11] Rats fed a maximum dose of ca. 2500 mg/kg/day over a two-year period showed no gross or microscopic changes to their organs. There was no change in the incidence of tumours and mortality was unaffected. There was a slight reduction in body weight in animals dosed at ca. 1500 mg/kg/day and above.

[12] Animals were dosed for 19 gestational days prior to the three month exposure period that is reported.

[13] The value of the study is limited by high mortality in all treatment and control groups.

Developmental and reproductive toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from groups 9, 13 and 30 of the present Flavouring Group Evaluation and for two supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for one structurally related substance. The supporting and structurally related substances are listed in brackets.

Table IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Rat; F	Gavage	5/10	Developmental toxicity: Gestation days 6-15	500	(Kronevi et al., 1988)	
2-Butoxyethan-1-ol [02.242]	Mouse; M, F	Drinking water	5/16	FACB: (Task 1) 2 weeks	0.5 %[2] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	3/40	FACB: (Task 2) 14 weeks[3]	Reproductive: 0.5 %[4] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	1/40	FACB: (Task 3) 14 weeks[3]	M: 1.0 % F: < 1.0 %[5] (2000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Lactation/ Drinking water	1/40	FACB: (Task 4) 32 weeks	0.5 %[6] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; F	Gavage	3/45-47 3/52-59	Developmental toxicity: Gestation days 9 – 11 and 11 - 13	Maternal: 30 Fetal: 100	(Sleet et al., 1989)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	5/6	Developmental toxicity: Gestation days 8 - 14	Maternal: 1000 Fetal: 650	(Wier et al., 1987)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	1/50	Developmental toxicity: Gestation days 6 – 13	Maternal: < 1180[7] Fetal: 1180[7]	(Hardin et al., 1987; Schuler et al., 1984; Smith, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	4/20	During 7 days premating and 98 days cohabitation	Maternal: 720 Fetal: none	(EU_RAR, 2004a)	
Butane-1,3-diol [02.132]	Rat; M, F	Diet	3/50	Five generations ~ 2 years	Reproduction: 5 %[8] (5000 mg/kg/day) Teratogenicity: 5 % (5000 mg/kg/day)	(Hess et al., 1981)	
	Rat; M, F	Gavage	3/10	Developmental toxicity: Gestation days 6 – 15	Maternal: 706; Fetal: 706	(Mankes et al., 1986)	
Glutaric acid [08.082]	Rat; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 1300 Fetal: 1300	(Bradford et al., 1984)	
	Rabbit; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 500 Fetal: 500	(Bradford et al., 1984)	
Glutaraldehyde [05.149]	Rat; M, F	Drinking water	3/56	Reproductive toxicity: 39 weeks[9]	Adult: 50 ppm (5.6 mg/kg/day) Fetal: 250 ppm (24.3 mg/kg/day) Reproductive: > 1000 ppm (84.5mg/kg/day)	(Neeper-Bradley and Ballantyne, 2000)	
	Rat; F	Drinking water	3/25	Developmental toxicity: Gestation days 6 – 16	Maternal: 50 ppm (5 mg/kg/day); Fetal: 750 ppm(68 mg/kg/day)[10]	(Hellwig, 1991a)	
	Rat; F	Gavage	3/21 – 26	Developmental toxicity: Gestation days 6 – 15	Maternal: 50; Fetal: 100	(Ema et al., 1992)	
	Mouse; F	Oral	3/NR	Developmental toxicity: Gestation days 7 – 12	Embryotoxicity: 30; Fetal: 30, Teratogenicity: 30	(Union Carbide Corp., 1986)	

Table IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
	Rabbit; F	Gavage	3/15	Developmental toxicity: Gestation days 7 – 19	Maternal: 15; Fetal: 15	(Hellwig, 1991b)	
(Adipic acid [08.026])	Rat; F	Gavage	4/24-28	Developmental toxicity: Gestation days 6 – 15	288	(Morgareidge, 1973d)	
	Mouse; F	Gavage	4/20 – 21	Developmental toxicity: Gestation days 6 – 15	263	(Morgareidge, 1973d)	
	Rabbit; F	Gavage	4/10 – 14	Developmental toxicity: Gestation days 6 – 18	250	(Morgareidge, 1974a)	
Nonanedioic acid [08.103]	Rat; F	Diet	1/20	Developmental toxicity: Gestation days 0 - 19	140	(Mingrone et al., 1983)	
	Rabbit; F	Diet	1/30	Developmental toxicity: Gestation days 0 - 19	200	(Mingrone et al., 1983)	
(Succinate, disodium hexahydrate)	Rat; M,F	Gavage (0, 100,300, 1000 mg/kg)	4 per sex/ 12	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	M, F: 1000	MHLW, Japan 2002 in (OECD, 2003)	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: m, 600 mg/kg.

M = Male; F = Female.

NR = Not Reported.

FACB = Fertility Assessment by Continuous Breeding.

[1] Number of groups represents the number of treatment groups investigated. Control groups are not reported.

[2] Dose range-finding phase: Based on the results of this dose range-finding study the highest concentration investigated further was 2 % in the drinking water.

[3] Mice were exposed to the test article for a seven day premating period, followed by a 14 week cohabitation/breeding period.

[4] Continuous breeding phase: All breeding pairs in the 0.5 % treatment group were fertile (delivered at least one litter). The fertility of the 1.0 and 2.0 % treatment groups was significantly affected.

[5] Crossover mating trial: Reproductive capacity of female mice is relatively more susceptible than males under the same exposure conditions.

[6] Offspring reproductive performance phase: Reproductive performance was not affected, but the mean liver and kidney weights for females was significantly different from that of the control group when organ weight was adjusted for body weight.

[7] 1180 mg/kg/day was the only dose level tested. Compared to the control group the 1180 mg/kg/day decreased the number of viable litters; therefore increasing the number of failed pregnancies. There were no significant observations noted in the liveborn pups.

[8] Dose related reproductive effects were noted after five successive matings of the F1A generation.

[9] F_0 and F_1 animals dosed for a 10 week pre-breeding period and through mating, and gestation and lactation of offspring.

[10] Glutaraldehyde was evidentially unpalatable, as water consumption was reduced in the mid- and high-dose groups; however, no signs of toxicity were observed at these dose groups.

In vitro mutagenicity/genotoxicity data are available for nine candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for 22 supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for one structurally related substance. Supporting and structurally related substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	Ames test	*S. typhimurium* TA98, TA100, TA1535	0.1 - 50 µmoles/plate (8.6 - 4305 µg/plate)	Negative[1]	(Loquet et al., 1981)	No control values are given for inactive compounds. Conclusion not comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA102	0.013 - 1.3 mmol (11.2 - 1120 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(NTP, 1992e)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537,	5,000 or 2000 µg/plate	Negative[1]	(MacDonald, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0 - 10000 µg/plate	Negative[1]	(Haworth et al., 1983)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Negative[1]	(Garner et al., 1981)	
	Ames test	*S. typhimurium* TA98,TA100, TA1535, TA1537, TA1538	4 - 2500 µg/plate	Negative[1]	(Trueman, 1981)	
	Ames test	*S. typhimurium* TA92, TA98, TA100, TA1535, TA1537, TA1538	0.2 - 2000 µg/plate	Negative[1]	(Brooks and Dean, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/ml	Negative[1]	(Baker and Bonin, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Rowland and Severn, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Simmon and Shephard, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537	NR	Negative[1]	(Nagao and Takahashi, 1981)	
	Ames test	*S. typhimurium* TA98, TA100,	1000 mg	Negative[1]	(Ichinotsubo et al., 1981b)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 10000 µg/plate	Negative[3]	(Richold and Jones, 1981)	
	Reverse bacterial mutation assay	*E. coli* WP2 (p)	up to 500 µg/plate (high dose studies) up to 100 µg/plate (low dose studies)	Negative[3]	(Venitt and Crofton-Sleigh, 1981)	
	Reverse bacterial mutation assay	*E. coli* SA500	NR	Lethal[4]	(Dambly et al., 1981)	Authors state "toxic, preventing adequate testing".
	Reverse mutation assay	*E. coli* WP2 *uvrA* pKM102	NR	Negative[1]	(Matsushima et al., 1981)	
	Forward mutation assay	*S. typhimurium* TM677	1000 µg/ml	Negative[3]	(Skopeck et al., 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA1535, TA1537	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA100	NR	Negative[3]	(Hubbard et al., 1981)	
(Butyro-1,4-lactone [10.006]) continued	Microtiter fluctuation test	*E. coli* WP2 *uvrA*	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Rec-assay	*Bacillus subtilis* H17, M45	20 µl (20000 µg)	Positive[1]	(Kada, 1981)	Reliable study, conclusion comprehensible.
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA*, *LexA*	NR	Negative[1]	(Green, 1981)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA*, *LexA*	1000 µg/ml	Negative[2]	(Tweats, 1981)	
	Mitotic crossing-over	*S. cerevisiae*	1000 µg/ml	Negative[1]	(Kassinova et al., 1981)	
	Mitotic gene conversion	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	Cell growth inhibition	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	DNA polymerase I inhibition test	*E. coli* W3110 & P3478	10 µl (10000 µg)	Positive[2] Negative[3]	(Rosenkranz et al., 1981)	Reliable study, conclusion comprehensible.
	Forward mutation assay	*S. Pombe*	20 µg/ml[1]	Negative[3]	(Loprieno, 1981)	
	Unscheduled DNA synthesis	Human HeLa S3 cells	0.1 - 100 µg/ml	Negative[1]	(Martin and McDermid, 1981)	
	ADP-ribosyl transferase activity	Human FL cells	10^{-3} to 10^{-7} mol/L (0.0086 – 86 µg/ml)[3]	Negative	(Yingnian et al., 1990)	
	Clastogenic activity	Rat liver cell line RL1	250 µg/ml	Negative	(Dean, 1981)	
	Mammalian cell transformation	BHK-21 hamster kidney cells	250 µg/ml	Positive[1]	(Styles, 1981)	No specific genotoxicity endpoint.
	Degranulation assay	Rat	25 mg/ml (25000 µg/ml)	Positive	(Fey et al., 1981)	No genetic endpoint (displacement of polysomes from ER).
	Sister chromatid exchange	Chinese hamster ovary cells	494 - 4940 µg/ml 494 - 1480 µg/ml 3010 - 4940 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible.
	Chromosomal aberration	Chinese hamster ovary cells	400 - 2580 µg/ml 400 - 1500 µg/ml > 2580 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible. Cells were selected for scoring on the basis of good morphology and completeness of karyotype.
Pentano-1,5-lactone [10.055]	Microbial assay	*E. coli* B/rWP2(*trp*$^-$), WP2(*trp*$^-$), WP2(*uvrA*$^-$)	1 - 3 mg/plate (1000-3000 µg/plate)	Negative[5]	(Kuroda et al., 1986)	Review, data cannot be validated.
(Hexano-1,5-lactone [10.010])	Ames test	*S. typhimurium* TA98, TA100	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Rec-assay	*B. subtilis*	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Sister chromatid exchange	Hamster lung fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Hamster lung fibroblast cells	NR	Positive[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Human embryo fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Heptano-1,4-lactone [10.020])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	100,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Unscheduled DNA synthesis	Rat hepatocytes	3000 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
(Nonano-1,4-lactone [10.001])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	37500 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mammalian	Mouse lymphoma L5178y TK$^{+/-}$	1000 µg/ml	Negative[2]	(Heck et al., 1989)	Abstract only, study cannot be validated.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
			600 µg/ml	Positive[3]		
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mutation assay	*E.coli* WP2 *uvrA*	0.2 - 1.6 mg/plate (200-1600 µg/plate)	Negative[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
	Rec-assay	*B. subtilis* M45 & H17	20 µl/disk (20000 µg/disk)	Positive[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
(Undecano-1,4-lactone [10.002])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637	5 mg/plate (5000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102	0.1 mg/disk (100 µg/disk)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[6]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[3] Negative[2]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast	0.5 mg/ml (500 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
(Undecano-1,5-lactone [10.011])	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*	10 µl/plate (10000 µg/plate)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
(Pentadecano-1,15-lactone [10.004])	Ames test	*S. typhimurium* TA98, TA100, TA102	50 µmol (12 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
(5-Methylfuran-2(3H)-one [10.012])	Ames test	*S. typhimurium* TA98, TA100	5 - 50 µg/plate	Negative[1]	(Turek et al., 1997)	
(Dodec-6-eno-1,4-lactone [10.009])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
	Rec-assay	*E. coli* WP2 *uvrA*	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
1-Hydroxypropan-2-one [07.169]	Ames test	*S. typhimurium* TA100	20 - 400 µg/plate	Positive[1]	(Yamaguchi, 1982)	Effect dose-dependent, conclusion comprehensible.
	Ames test	*S. typhimurium* TA104	68 µmoles (5 µg/ml)	Positive[2]	(Marnett et al., 1985a)	Authors state that each compound was tested to its toxic limits, data for maximum non-toxic dose given only.
	Ames test	*S. typhimurium* TA100	500 µg/plate	Positive[1]	(Yamaguchi and Nakagawa, 1983)	Numerical value given was obtained from dose-response curves of five concentration levels.
	Ames test	*S. typhimurium* TA100	NR	Positive[2]	(Garst et al., 1983)	Appropriate controls (idomethan for volatile compounds, sterility of compounds and solvent). Test compound judged positive when dose-related doubling of revertants were found.
(Ethyl 3-hydroxybutyrate [09.522])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535	NR	Negative[4]	(Zeiger and Margolin, 2000)	
(Ethyl acetoacetate [09.402])	Ames test; preincubation protocol	*S. typhimurium* TA92, TA100, TA1535, TA1537, TA94 and TA98	25 mg/plate (25000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test; preincubation protocol	*S. typhimurium* TA97, TA102	0.1 - 10 mg/plate (10 - 10000 µg/plate)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis*; H17, M45	20 µg/disk	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*; H17, M45	20 µl/disk (20000 µg/disk)	Positive	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*E. coli*; WP2 *uvrA*	200 - 1600 µg/plate	Positive[8]	(Yoo, 1986)	Methods in Japanese, tables only in

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						English. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20 µl/ml (10 - 20 µg/ml)	Negative[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20 µl/ml (10 - 20 µg/ml)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast cells	1 mg/ml (2000 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
Methyl acetoacetate [09.634]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *E. coli* WP2 *uvrA*	1 - 5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	Modified Ames, reincubation. Reliable study, conclusion comprehensible.
2-Butoxyethan-1-ol [02.242]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 5000 µg/plate	Negative[1]	(Okamoto and Riccio, 1985)	Study performed in compliance with US-FDA GLP standards. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA*	9.8 - 156.3 µg/plate	Negative[1]	(Henrich and McMahon, 1988)	Test material: mixture of 2-butoxyethanol (2 % w/v) with tricholorbenzene and anionic emulsifiers. Test compound produced no revertants vs solvent control.
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(Zeiger et al., 1992)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, TA1538	5000 - 20000 µg/plate	Negative[1]	(Sippel, 1977)	Negative as defined by less than 2-times of the spontaneous reversion rate. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA100 *E. coli* WP2uvrA	500 - 1000 µg/plate	Negative[1]	(Gollapudi et al., 1996)	Re-examination of EGBE to valdazte report by Hoflack et al (1995) on mutagenicity of the compound in a test with TA97a. reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, TA102	14 mg/plate (14000 µg/plate) conc. range: 0,8 - 115 micromol/plate, positive ab 19 micromol = 2,2mg/plate	Negative with TA98, TA100,TA102, positive with TA97a[1]	(Hoflack et al., 1995)	Positive with TA97a, but not reproduced in study specifically addressing this finding (Gollapudi et al., 1996).
	Mutagenicity Assay	Bacteriophage *T4D E. coli CR63* and *K12*	19.6 - 111.1 µl/ml	Negative[9]	(Kvelland, 1988)	Highly toxic at all concentrations tested, bacteriophage yield less than 1 %.
	Forward mutation assay	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)[3]	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to currunt OECD guidelines.
	Forward mutation assay	Chinese hamster ovary cells V79	1 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study (5 concentrations each test, 1 % without S9 (non-toxic), 0,3 % with S9), conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells AS52	0.38 - 7.6 mM (898 µg/ml)	Negative[1]	(Chiewchanwit and Au, 1995)	Non-cytotoxic concentration range. Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells	0.007 - 0.25 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Positive[2, 10]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to current OECD Guidelines.
	Sister chromatid exchange	Human peripheral lymphocytes	3000 ppm	Positive[1]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						program. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing programme. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	Reliable report with details on purity of test compounds, methods and results. 50 % growth inhibition (at 24 hours) approx. at 90 mM, but value cannot be precisely derived from the graphic presentation.
	Chromosomal aberrations	Human peripheral lymphocytes	3000 ppm	Negative[2]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
2-Butoxyethan-1-ol [02.242] continued	Chromosomal aberrations	Human lymphocytes	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	No information on growth inhibition/ survival of treated human lymphocytes given.
	In vitro micronucleus test	V79 cells	16.92 mM (2000 µg/ml)	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to current OECD Guidelines.
	Unscheduled DNA synthesis	Rat hepatocytes	0.1 - 100 x 10^{-3} %	Positive[1, 11]	(Slesinski and Weil, 1980)	The interpretation of these findings is equivocal due to the methodology applied (liquid scintillation) and the absence of relation with dose.
	Embryo Transformation Assay	Syrian hamster embryo cells	NR	Negative[2]	(Elias et al., 1996)	No specific genotoxic endpoint.
	Embryo Transformation Assay	Syrian hamster embryo cells	500 - 1500 µg/ml	Positive[4]	(Brauninger, 1995)	No specific genotoxic endpoint.
(3,7-Dimethyloctane-1,7-diol [02.047])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Diethyl malonate [09.490])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537.	3 µmol/plate (480 µg/plate)	Negative[1]	(Florin et al., 1980)	
(Dimethyl succinate [09.445])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984a)	
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538	10 mg/plate (10000 µg/plate)	Negative[1]	(Zeiger et al., 1992)	
(Fumaric acid [08.025])	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative[4]	(Rapson et al., 1980)	
	Ames test (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	2000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	10 mg/plate (10000 µg/plate)	Negative	(Ishidate et al., 1984)	
	Chromosomal aberrations	Chinese Hamster fibroblast cells	0.5 mg/ml (500 µg/ml)	Negative	(Ishidate et al., 1984)	
(l-Malic acid [08.017])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA104	2000 µg/plate	Negative[1]	(Al-Ani and Al-Lami, 1988)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
Diethyl maleate [09.351]	Forward mutation assay	Mouse lymphocytes L5178Y TK+/-	2.250 - 9.750 x 10^{-4} mol/l (387 - 1679 µg/ml)	Positive[1]	(Wangenheim and Bolcsfoldi, 1988)	No S9 at 2.25 - 9.75 x 10^{-4} mol/L, doubling of the mutation rate at 6 x 10^{-4} mol/L and above, but growth reduction of 70 % or more. Study of insufficient value.
	Aneuploidy test	Chinese hamster lung cells V79	5.2 x 10^{-6} M 8.7 x 10^{-6} M	Negative[4] Positive[4]	(Önfelt, 1987)	Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	REC assay Ames	*B subtilis* M45 & H17 *S. typhimurium* TA98, TA100	NR	Negative[1]	(Sakagami et al., 1989)	Abstract, data cannot be validated.
Glutaraldehyde [05.149]	Ames test	*S. typhimurium* TA104	0.5 µmoles (50.06 µg/ml)	Positive[2]	(Marnett et al., 1985a)	TA104 tested to reassess mutagenic potency of 28 carbonyl compounds. Dose-dependent increase toxic limits of glutaraldehyde. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98	10 mg/plate (10000 µg/plate)	Equivocal[12] Positive[12]	(Haworth et al., 1983)	Part of ring study for re-assessment of 250 chemicals. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA100, TA102, TA104	25 - 300 µg/plate	Positive[1]	(Dillon et al., 1998)	Comparative analysis of TA100, TA102 and TA104 for sensitivity to 13 aldehydes and 4 peroxides. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638, *E. coli* WP2/pKM101, WP2 *uvrA*	20 - 1000 µg/plate	Positive[3, *]	(Watanabe et al., 1998a)	*Cytotoxicity noted in doses as low as 250 µg/plate. Ring study (22 laboratories) for comparative analysis of TA102, TA2638, *E. coli* WP2/pKM101 and WP2 uvrA/pKM101. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, *E. coli* WP2/pKM101, WP2 *uvrA*	5 - 100 µg/plate	Positive[2]	(Wilcox et al., 1990)	Comparative analysis of TA102 and E.coli WP2 strains. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	1000 µg/plate	Positive[13]	(Müller et al., 1993)	Ring study (3 laboratories) to evaluate TA102. Reliable, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638a	76 µg/plate	Positive[3, 14]	(Rydén et al., 2000)	Comparative analysis on the sensitivity of bacterial strains and the possibility of using TA2638a. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	25 µg/plate	Positive[1]	(Levin et al., 1982)	Test of TA102 for detection of oxidative mutagens. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, TA102, TA104	0.1 - 60 µg/plate	Positive[1]	(Noblitt et al., 1992)	Abstract, data cannot be validated.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 *uvrA*	100 - 5000 µg/plate	Negative[1]	(Wagner, 1997)	Study in compliance with inter-national (US-FDA, US-EPA, UK, Japan) GLP Guidelines. Negative result not discussed in view of positive results in other studies. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100,	15.4 µg/plate[2, 15]	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
		TA1537, TA1538, TA98	51.6 µg/plate[3]			due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, A102, TA104	0.050 % in 100 µl/plate (100000 µg/plate)	Positive[14]	(Schweikl et al., 1994)	Study aimed at elucidating the mutagenic potency of 3 different dentin bonding agents, pure glutaraldehyde was tested as one of the ingredients of these materials. Conclusion comprehensible.
Glutaraldehyde [05.149] continued	Ames test	*S. typhimurium* TA100, TA98	20 µg/plate	Negative[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration Ames test less sensitive than REC-assay (see below).
	Ames test	*E. coli* WP2 *uvrA*	20 - 10000 µM (2 - 1001 µg/ml)	Negative[2]	(Hemminki et al., 1980)	Study aimed at comparison of alkylation rate with mutagenicity of directly acting chemicals, glutaraldehyde served as reference compound.
	Rec-assay	*B. subtilis*, M-45 (*Rec*$^-$), H-17 (*Rec*$^+$)	300 µg/ml	Positive[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration REC-assay more sensitive than Ames test (see above).
	L-arabinose resistance forward mutation test	*S. typhimurium:* BA9, BA13	62 - 250 nmoles/ml (6.2 - 25 µg/ml)	Negative[15] Positive[15]	(Ruiz-Rubio et al., 1985)	
	Forward mutation assay	Mouse lymphocytes: L5178Y TK+/-	8 µg/ml	Positive[2]	(McGregor et al., 1988b)	Reliable study, conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells	40.8µM (4.08 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	2.5 µM (.25 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	0.5 - 16 µg/ml	Negative/positive[2] Positive[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol.11-16 µg/ml, with S9 positive (at least with one dose) results in both laboratories. 0,36-16 µg/ml, without S9 results not consistent.
	Chromosomal aberrations	Chinese hamster ovary cells	0.5 - 30 µg/ml	Negative/positive[2] Negative[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol. 1-16 µg/ml, with S9 negative results in both laboratories: 0,3-30 µg/ml, without S9 results not consistent.
	Alkaline elution assay	Human TK6 lymphoblasts	25 µM (0.25 µg/ml)[2]	Positive[2]	(St. Clair et al., 1991)	Linear increase in DNA cross linking between 1-25 µM. At 20 µM 10 % survival only.
	TK6 mutation assay	Human TK6 lymphoblasts	20 µM (2 µg/ml)	Positive	(St. Clair et al., 1991)	Majority of trifluorothymidine resistant colonies displayed normal growth, slow-growing colonies small contribution to overall mutant fraction.
Glutaraldehyde [05.149] continued	Unscheduled DNA synthesis	Primary rat hepatocytes	51 µM (5.1 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Unscheduled DNA synthesis	Rat hepatocytes	100 µM (10 µg/ml)	Positive[2]	(St. Clair et al., 1991)	Significant increase over controls at 100 µM, this concentration tolerated without morphological signs of toxicity.
(Adipic acid [08.026])	Ames test	*E. coli* WP2 *uvrA*	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98, *E. coli* WP2 *uvrA*	10 mg/plate (10000 µg/plate)	Negative[1]	(Prival et al., 1991)	
	Ames test (preincubation method)	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
(Dibutyl sebacate [09.474])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Ethyl brassylate [09.533])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Prop-1-ene-1,2,3-tricarboxylic acid [08.033])	Ames test	*S. typhimurium* TA100, TA1535, TA1537, TA98	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984a)	
5,6-Dimethyl-tetrahydro-pyran-2-one [10.168]	Ames test	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5000 microgram/plate	Negative[1]	(Uhde, 2004a)	Test performed both in the incorporation and preincubation assays.
Succinic acid, disodium salt [08.113]	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537	5000 microgram/plate	Negative3	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Ames test	*S.typhimurium* TA97, TA102	10000 microgram /plate	Negative[1]	(Fujita et al., 1994) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	15000 microgram/ml	Equivoval[2]	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 473.
(Disodium succinate hexahydrate)	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537	5000 microgram/plate	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	5000 microgram/ml	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	

NR: Not reported.

[1] With and without S-9 metabolic activation.

[2] Without S-9 metabolic activation.

[3] With S-9 metabolic activation.

[4] Presence or absence of metabolic activation not specified.

[5] Anti-mutagenic effects study.

[6] Presence or absence of metabolic activation not specified.

[7] 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one did not form DNA adducts, but 2,5-DMHF does. Study addresses mechanism of chemical reaction of 2,5-dimethyl-4-hydroxy-3(2H)-furanone with DNA.

[8] The concentrations used were 10-fold higher than that of spontaneous revertants.

[9] The test substance had a severe toxic effect on phage yield.

[10] Weak positive results were detected.

[11] The test substance induced statistically significant levels of unscheduled DNA synthesis in two of the six dose levels tested. Therefore, the test substance is considered a weak mutagen.

[12] This test compared the results at two different laboratories. Results were equivocal at Case Western Reserve University, while they were positive at Microbiological Associates.

[13] Article presents the results from three different laboratories. Results were positive in both water and ethanol; however, it was concluded that TA102 is not sufficiently matured to be employed routinely.

[14] Maximum non-toxic dose.

[15]Results were negative in BA9, not BA13.

In vivo mutagenicity/genotoxicity data are available for six candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for eight supporting substances evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Supporting substances are listed in brackets.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	*In vivo* Bone- marrow micronucleus assay	B6C3F1 mice	Single dose *via* intraperitoneal injection	80 % of LD_{50}	Negative	(Salamone et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo Bone- marrow micronucleus assay	CD-1 mice		0.11-0.44 ml/kg (110 – 440 mg/kg)	Negative	(Tsuchimoto and Matter, 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo micronucleus assay	Mice (B6C3F1/BR hybrid)		80 % of LD_{50}	Negative	(Katz et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo sperm abnormality	Mice (CBA X Balb/c)F1	Daily exposure for five days *via* intraperitoneal injection	0.1-1.0 mg/kg bw/day	Negative	(Topham, 1980)	Sperm head abnormality test does not make use of a genetic endpoint.
	In vivo sex-linked recessive test	*D. melanogaster*	A: *via* diet B: injection	A: 20000 or 28000 ppm B. 15.000 ppm	Negative	(Foureman et al., 1994)	Study in compliance with OECD 477.
(Hexano-1,5-lactone [10.010])	Chromosomal aberration *in vivo*	Rat bone-marrow cell		NR	Negative[1]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Undecano-1,4-lactone [10.002])	*In vivo* mouse micronucleus test	2-6 ddY male mice	*Via* intraperitoneal injection	250-2000 mg/kg	Negative	(Hayashi et al., 1988)	Single application, only one sampling time. Not in compliance with current OECD 474.
2-Butoxyethan-1-ol [02.242]	*In vivo* mouse micronucleus test	Mouse bone marrow	Single dose *via* intraperitoneal injection	1000 mg/kg	Negative	(Elias et al., 1996)	Reliable report, decreased PCE/NCE ratio demonstrates bioavailability of compound at target compartment. Conclusion comprehensible.
	In vivo mouse micronucleus test	Mouse bone marrow	3 doses *via* intraperitoneal injection	450 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo micronucleus test	Rat bone marrow	3 doses *via* intraperitoneal injection	550 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo DNA adducts	Rat brain, kidney, liver, spleen and testes	Single dose *via* oral route	120 mg/kg	Negative	(Keith et al., 1996a)	The method (based on ^{32}P-postlabelling) is aimed at detecting hydrophobic DNA

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							adducts resulting from CytP450 induction, not from binding of 2-butoxyethan-1-ol to DNA .
	In vivo DNA methylation	Rat brain, kidney, liver, spleen and testes,	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo DNA adducts	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Detection of hydrophobic DNA adducts such as modified nucleotides with aliphatic side chains.
	In vivo DNA methylation	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo tumour formation	Mouse	Daily dose for two weeks *via* oral route	120 mg/kg/day	Inconclusive	(Keith et al., 1996a)	No difference in tumor incident observed. However no conclusion on the oncogenic potential of 2-butoxyethan-1-ol can be drawn because of the limitations of the experimental protocol (treatment, sample size, duration of the study, reporting, etc.).
Butane-1,3-diol [02.132]	*In vivo* cytogenetic assay	Rat femur bone marrow	*Via* diet[2]	5, 10, 24 %	Negative	(Hess et al., 1981)	F1A, F2A, F3A generations in a multigeneration reproductive toxicity study. PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo dominant lethal assay	Rat	Animals exposed for eight weeks *via* diet	5, 10, 24 %	Negative	(Hess et al., 1981)	F1B generation in a multigeneration reproductive toxicity study.
(3,7-Dimethyloctane-1,7-diol [02.047])	*In vivo* micronucleus test	Mouse		516, 860, 1204 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		10 mM (1743 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	*In vivo* Basc test	*D. melanogaster*		37 mM (6374 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		345, 603, 861 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							clear whether the substance had reached the bone marrow.
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	*In vivo* Basc test	*D. melanogaster*		25 mM (5459 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		327, 545, 763 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
Malonic acid [08.053]	*In vivo* mutagenicity assay	Rat hepatocytes	400 mg/kg/day exposure for 6 weeks *via* diet	4000 ppm	Negative	(Ito et al., 1988)	GST-P foci assay following diethyl nitrosamine exposure. Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	*In vivo* bone marrow chromosomal aberrations	Rat bone marrow	Single dose *via* oral gavage	Males: 2750 mg/kg Females: 1375 mg/kg	Negative	(San Sebastian, 1989a)	Reliable study, e.g. cells with gaps excluded. Selected copy of report without data tables.
Glutaraldehyde [05.149]	*In vivo* chromosomal aberration	Rat bone marrow	Single dose *via* oral gavage	Males: 120 mg/kg/bw Females: 80 mg/kg/bw	Negative	(Vergnes and Morabit, 1993a)	Study in compliance with international (FDA, TSCA, OECD) GLP guidelines. Selected copy of report (12 of 100 pages) available.
	In vivo chromosomal aberration	Rat bone marrow	A single dose or daily for five days *via* oral gavage	Single dose: 0.55 ml/kg (males), 0,4 ml/kg (females) of a 6, 12 or 36 % solution. Repeated dose: 0,55 ml/kg (males) of a 5 % solution	Negative	(Putman, 1987)	Time points of investigation: single dose: 8, 12 hours. Repeated dose: 12hours. Well conducted study, conclusion comprehensible. Selected copy of report available.
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* oral gavage	250 mg/kg	Negative	(Vergnes and Morabit, 1993b)	Selected pages of report available (29 of 88 pages).
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* intraperitoneal injection	4, 8, 15 mg/kg/bw	Positive	(Noblitt et al., 1993)	Abstract, study cannot be validated.
	In vivo unscheduled DNA synthesis	Rat	Single dose *via* oral gavage	30, 150, 600 mg/kg	Negative	(Mirsalis et al., 1989)	Reliable part of *In vivo* tumour formation study, conclusion comprehensible.
	In vivo SLRL test	*D. melanogaster*	Three day exposure *via* diet	3500 ppm	Negative	(Zimmering et al., 1989)	Study in compliance with OECD 477.
	In vivo SLRL test	*D. melanogaster*	Single dose *via* intraperitoneal injection three day exposure *via* diet	Injection: 4000 ppm Diet: 10,000 ppm	Negative	(Yoon et al., 1985)	Study in compliance with OECD 477.
(Adipic acid [08.026])	*In vivo* chromosomal nondisjunction	*D. melanogaster*		4000 ppm	Negative	(Ramel and Magnusson, 1979)	
Diethyl adipate [09.348]	*In vivo* dominant lethal assay	Mouse	(Single1460 mg/kg dose *via* intraperitoneal injection)	1.46 ml/kg	Negative	(Singh et al., 1975)	Reliable study, conclusion comprehensible.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Dibutyl sebacate [09.474])	*In vivo* micronucleus test	Mouse		943, 1886, 2829 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		19 mM (4642 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.

NR: Not reported.
[1]Presence or absence of metabolic activation not specified.
[2] Length of exposure not specified in report. Cytogenetic assay conducted on F1A, F2A and F3A generations of a multiple generation study.

ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADI	Acceptable Daily Intake
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CNS	Central Nervous System
CoA	Coenzyme A
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DRF	Dose Range Finder
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	Environmental Protection Agency
ER	**E**ndoplasmic Reticulum
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
I.V.	Intravenous
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
LOAEL	Lowest Observed Adverse Effect Level
MFD	Median Fatal Dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake

mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
RfD	Reference dose
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.6
Revision Date 21.02.2022
Print Date 21.11.2023
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	δ-Decalactone
Product Number	:	W236101
Brand	:	Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	705-86-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Merck Life Science Sp.z.o.o. Szelągowska 30 PL-61-626 POZNAN
Telephone	:	+48 61 8290-100
Fax	:	+48 61 8290-120
E-mail address	:	TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+(48)-223988029 (CHEMTREC) 112 (numer alarmowy)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	(±)-5-Decanolide (±)-δ-Pentyl-δ-valerolactone (±)-6-Pentyltetrahydro-2H-pyran-2-one 5-Hydroxydecanoic acid δ-lactone δ-Decanolactone
Formula	:	$C_{10}H_{18}O_2$
Molecular weight	:	170,25 g/mol
CAS-No.	:	705-86-2
EC-No.	:	211-889-1

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides
Combustible.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
In the event of fire, wear self-contained breathing apparatus.

5.4 Further information
Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Do not breathe vapors, aerosols. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed.

Heat sensitive. Light sensitive.

Storage class
Storage class (TRGS 510): 10: Combustible liquids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
not required

Respiratory protection
Not required; except in case of aerosol formation.

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid, clear Color: colorless
b)	Odor	No data available
c)	Odor Threshold	No data available
d)	pH	5,54 at 9,3 g/l at 25 °C acidic
e)	Melting point/freezing point	Melting point/range: -27 °C
f)	Initial boiling point and boiling range	117 - 120 °C at 0,03 hPa - lit.
g)	Flash point	> 113,00 °C - closed cup
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	No data available
l)	Vapor density	No data available
m)	Density	0,954 g/mL at 25 °C - lit.
	Relative density	No data available
n)	Water solubility	4 g/l at 28 °C
o)	Partition coefficient: n-octanol/water	log Pow: 2,27 at 28 °C
p)	Autoignition temperature	not auto-flammable
q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: 19,753 mPa.s at 28 °C
s)	Explosive properties	No data available
t)	Oxidizing properties	Oxidizing properties

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
no information available

10.5 Incompatible materials
Oxidizing agents

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - > 5.000 mg/kg
Inhalation: No data available
LD50 Dermal - Rat - > 5.000 mg/kg

Skin corrosion/irritation
Skin - Rabbit
Result: Mild skin irritation - 24 h

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Mild eye irritation - 24 h

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: UQ1355000
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties
Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number

ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: - IMDG: - IATA: -

14.4 Packaging group

ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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