Toxicological profile for

gamma-Nonalactone

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

5-Pentyloxolan-2-one (PubChem)

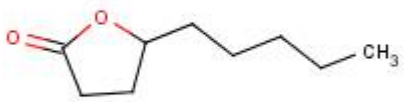
1.2. Synonyms

1,4-Nonalolide; 1,4-Nonyl lactone; 2(3H)-Furanone, dihydro-5-pentyl-; 4-Amyl-4-hydroxybutyric acid lactone; 4-Hydroxynonanoic acid, gamma-lactone; 4-Nonalactone; 4-Nonanolide; 4-Pentyl-butanolide; AI3-02457; Aldehyde C-18; Coconut aldehyde; Cocos aldehyde; delta-n-Amylbutyrolactone; Dihydro-5-pentyl-2(3H)-furanone; EINECS 203-219-1; FEMA No. 2781; gamma-Amyl-gamma-butyrolactone; gamma-N-Amylbutyrolactone; gamma-Nonalactone; gamma-Nonanolactone; gamma-Nonanolide; gamma-Nonylactone; gamma-Nonyllactone; gamma-Pelargolactone; gamma-Pentyl-gamma-butyrolactone; HSDB 5360; Nonan-1,4-olide; Nonanoic acid, 4-hydroxy-, gamma-lactone; NSC 24253; NSC 46099; Prunolide; UNII-I1XGH66S8P; Nonan-4-olide (ChemIDplus)

1.3. Molecular formula

C9-H16-O2

1.4. Structural Formula



1.5. Molecular weight (g/mol)

156.22

1.6. CAS registration number

104-61-0

1.7. Properties

1.7.1. Melting point

(°C): 9.83 (estimated) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 136 at 12.8 mmHg (EPISuite, 2017); 243, 266.6 or 136 (oil) (ChemSpider)

1.7.3. Solubility

1201 mg/L at 25°C (estimated) (EPISuite, 2017; HSDB, 2015); 2180 mg/L at 20°C (GESTIS)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 93.3 or 132 (ChemSpider); 126 (GESTIS)

1.7.6. Flammability limits (vol/vol%)

“Combustible” (PubChem); “Combustible substance, poorly flammable” (GESTIS)

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

Stable at normal temperatures and pressure.

1.7.10. Vapor pressure

0.0±0.5 mmHg at 25°C (estimated) (ChemSpider); 0.0118mmHg at 25°C (estimated) (EPISuite, 2017)

1.7.11. log Kow

2.744 (ChemSpider)

2. General information

2.1. Exposure

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

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	0.22	0.36	Hard candy	6.61	17.59
Baked goods	28.82	50.41	Nonalcoholic beverages	7.22	13.45
Chewing gum	0.54	2.90	Soft candy	27.92	40.52
Frozen dairy	12.09	22.68	Sweet sauce	0.31	0.31

Gelatins, puddings	16.07	26.62			

Estimated intake from flavouring use: 0.007655 mg/kg bw/day.

The FEMA PADI (possible average daily intake) of gamma-nonalactone is 5.518 mg.

As taken from Burdock, 2010.

gamma-Nonalactone is used as a perfuming, fragrance and skin conditioning ingredient in cosmetics in the EU. As taken from CosIng (undated).

Dihydro-5-pentyl-2(3H)-furanone is listed as an ingredient (at given concentrations, where specified) in auto, inside the home (<1%) and pet care (0.024%) products by the CPID.

Occurrence in tobacco products:

In the burned part: Yes

In tobacco naturally: No (Stedman, 1968; Lloyd et al. 1976).

Category: flavor and fragrance agents

Maximised Survey-derived Daily Intakes (MSDI-EU) from use as a flavouring: 1000.00 (µg/capita/day) (EFSA, 2012).

PROBABLE ROUTES OF HUMAN EXPOSURE:

According to the 2012 TSCA Inventory Update Reporting data, 9 reporting facilities estimate the number of persons reasonably likely to be exposed during the manufacturing, processing, or use of dihydro-5-pentyl-2(3H)-furanone in the United States may be as low as < 10 workers and as high as 99 workers per plant; the data may be greatly underestimated due to confidential business information (CBI) or unknown values(1).[(1) US EPA; Chemical Data Reporting (CDR). Non-confidential 2012 Chemical Data Reporting information on chemical production and use in the United States. Available from, as of June 29, 2015: http://www.epa.gov/cdr/pubs/guidance/cdr_factsheets.html] **PEER REVIEWED**

Occupational exposure to dihydro-5-pentyl-2(3H)-furanone may occur through dermal contact with this compound at workplaces where dihydro-5-pentyl-2(3H)-furanone is produced or used. Monitoring data indicate that the general population may be exposed to dihydro-5-pentyl-2(3H)-furanone via ingestion of food containing dihydro-5-pentyl-2(3H)-furanone. Use data indicate that the general population may be exposed to dihydro-5-pentyl-2(3H)-furanone via dermal contact with consumer products containing dihydro-5-pentyl-2(3H)-furanone(SRC).

Uses:

Dihydro-5-pentyl-2(3H)-furanone is used in perfumes and as a food flavoring. It was used as an ingredient in some cat and dog repellents. Dihydro-5-pentyl-2(3H)-furanone is no longer registered for use as a pesticide. It can be used as an inert ingredient at very low concentrations in pesticides containing 2-limonene.

Concentration in final product as reported as percentage ranges from 0.005 usual to 0.1 maximum in soaps; 0.0005 usual to 0.01 maximum in detergents; 0.002 usual to 0.04 maximum in creams, lotions; and 0.04 to 1.0 maximum in perfumes. /From table/ [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593] **PEER REVIEWED**

For dihydro-5-pentyl-2(3H)-furanone (USEPA/OPP Pesticide Code: 122301) there are 0 labels match. /SRP: Not registered for current use in the U.S., but approved pesticide uses may change periodically and so federal, state and local authorities must be consulted for currently approved uses./[National Pesticide Information Retrieval System's Database on Dihydro-5-pentyl-2(3H)-

furanone (104-61-0). Available from, as of April 27, 2015: http://npirspublic.ceris.purdue.edu/ppis/] **PEER REVIEWED**

Perfumery, flavors [Lewis, R.J. Sr.; Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc. New York, NY 2007., p. 906] **PEER REVIEWED**

Exposure:

Workers that use or produce dihydro-5-pentyl-2(3H)-furanone may breathe in vapors or have direct skin contact. The general population may be exposed when eating foods containing dihydro-5-pentyl-2(3H)-furanone or by breathing in vapors or having direct skin contact when using perfume containing dihydro-5-pentyl-2(3H)-furanone.

As taken from HSDB, 2015

Dihydro-5-pentyl-2(3H)-furanone (also known as gamma-nonalactone) is also referred to by the common name furanone. Products containing furanones are registered for use as insecticides, insect and mammal repellents, and mosquito larvicides. Pesticide products containing furanones may be used used as dog and cat repellents; fly, cockroach and ant killers; insect repellents, insect repellent strips, and insect repellent tablecloths; and mosquito larvicides. Formulations include liquid ready-to-use solutions, emulsifiable concentrates, granulars and impregnated material. Furanone is applied by the use of impregnated tablecloths, premoistened applicator puffs, aersol sprayers, non-aerosol hand pump sprayer, knapsack sprayers, and granules which may be applied by hand. Use practice limitations include a label statement prohibiting application directly to treated, finished drinking water reservoirs or drinking water receptacles. Users also must avoid application to man-made surfaces sensitive to mineral oils.

As taken from EPA. 1996. Re-registration Eligibility Decision (RED) available at http://archive.epa.gov/pesticides/reregistration/web/pdf/3138fact.pdf

gamma-Nonalactone (CAS RN 104-61-0) is listed as a fragrance ingredient by IFRA (undated) and the US EPA (US EPA InertFinder Database, 2021).

“Soft, squashable toys known as squishies have become increasingly popular amongst children. In this study, one such toy was evaluated sensorially by a trained panel and analytically using gas chromatography-mass spectrometry/olfactometry (GC-MS/O) after thermal extraction of the sample. Sensory analysis revealed the presence of an intense and unpleasant odour exhibited by the sample. The smell was dominated by almond- and inflatable swimming aid-like, as well as malty and glue-like notes, but also pleasant odours that were described as caramel-like and coconut-like. GC-MS/O analysis identified 2-butoxyethanol, cyclohexanone, γ-nonalactone, and ethyl maltol as being the main causative substances for the overall odour of the product. The data additionally indicated that the pleasant smelling substances γ-nonalactone (coconut-like smell) and ethyl maltol (caramel-like smell) were intentionally added by the manufacturer to mask the unpleasant odour of the solvents.” As taken from Wiedmer C and Buettner A, 2019. Anal. Bioanal. Chem. 411(17), 3757-3761. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31079176/

Volatile Components (expressed in mg/kg) of Vanilla Planifolia Fruit Extract and Vanilla Tahitensis Fruit Extract (both dichloromethane extracts):

Component	**Vanilla Tahitensis Fruit Extract (fruit from Polynesian Cultivar: Tahiti)**	**Vanilla Tahitensis Fruit Extract (fruit from Polynesian Cultivar: Haapape)**	**Vanilla Tahitensis Fruit Extract (fruit commercial sample from Papua New Guinea)**	**Vanilla Planifolia Fruit Extract (fruit commercial sample from Madagascar)**
γ-	**64**	**52**	**40**	**65**

Nonalactone				

As taken from CIR, 2019.

gamma-Nonalactone (CAS RN 104-61-0) is used as an oral flavour enhancer, and topical fragrance ingredient and skin-conditioning agent in non-medicinal natural health products. It has an upper limit toxicity restriction of 1.25 mg/kg bw/day when used as a flavouring agent (Health Canada, 2021).

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%
gamma-nonalactone	3882265165	92.07	3618982693	99.14
Total ion chromatogram	4216553529	100	3650302287	100

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

Ingredient CAS Number	Chemical Class	Mol. Wt. (M) Bp or mp (°C)	Max. cig. Appln. Level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)	Max. Level in smoke (ug)
gamma-Nonalactone CAS 104-61-0	gamma-Lactone	M=156 bp121 at 6 mm Hg	20	98	gamma-Nonalactone 99.7 Dihydromethylpentylfuranone?0.2 1 unidentified product 0.1	10 0.02 0.01

In a pyrolysis study, 100% of gamma-nonalactone (“nonan-4-olide”) added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

The determination of gamma-nonalactone as one of the important odor-active compounds in freshly cooked non-scented rice is reported. It was evaluated by gas chromatography-olfactometry (GC-O) analysis and identified by gas chromatography-mass spectrometry (GC-MS) analysis in the headspace above the freshly cooked non-scented rice samples extracted by using a modified headspace solid-phase microextraction (SPME) method. This component had a mass spectrum with a characteristic ion peak at m/z 85 (100%) and a linear retention index (RI) of 2,023 on a DB Wax column, consistent with those of an authentic sample of gamma-nonalactone. The odor characterization of a strong, sweet, coconut-like aroma of this compound was also validated by GC-O comparison with the authentic compound.

As taken from Zeng Z, Zhang H, Zhang T, Tamogami S, Chen JY. Molecules. 2009 Aug 10; 14(8):2927-34.

Syntheised by reacting methylacrylate and hexanol in the presence of ditertiarybutyl peroxide or by condensation of undecylenic acid and malonic acid by lactonization of lactonization of nonenoic acid.

Gamma-nonalactone reportedly found in peaches, apricots, roasted barley, rum, tomato, currants, guava, raisins, papaya, pineapple, blackberry, strawberry jam, asparagus, wheat and crispbread, Camembert cheese, butter, milk, chicken, beef, lamb and pork fat, pork, beer, cognac, whiskies, sherry, grape wines, cocoa, green tea, pecans, oats, soybean, avocado, passion fruit, plum, plumcot, beans, mushroom, starfruit, fenugreek, mango, tamarind, rice, prickly pear, buckwheat, licorice, malt, wort, cherimoya, Bourbon vanilla, shrimp, nectarine, mate and sweet grass oil.

As taken from Burdock, 2010.

Occurs naturally in mango (at 0.3-0.4 mg/kg), beer (at 0.3 mg/kg), other ale (at up to 0.1 mg/kg), cooked beef (at 0.07 mg/kg), cooked pork (at 0.1-0.3 mg/kg), green tea (at 0.1-0.3 mg/kg) and cocoa (trace) (CoE, 2000).

Intake of delta-nonalactone (No. 9), gamma-decalactone (No. 11), gamma-undecalactone (No. 13), and delta-undecalactone (No. 14) predominates from flavour use in dairy and butter flavourings (Lawrence, 1991; White and White, 1991).

As taken from Adams T. B et al. Food and Chemical Toxicology. Volume 36, Issue 4, 6 April 1998, Pages 249-278.

Food Survey Values:

Dihydro-5-pentyl-2(3H)-furanone has been detected in the volatile constituents of nectarines (1,2), concentrations were less than 10 ppb in samples obtained from orchards of the Horticultural Crops Research Laboratory of the US Agricultural Department, Fresno, CA (Sunfire, Flavortop, P 62-27, P 89-56)(1). Dihydro-5-pentyl-2(3H)-furanone was detected at a concentration of 5 ug/kg in fresh apricot fruit tissue; it was not detected in 2 other apricot varieties nor in 2 plum varieties(3). Dihydro-5-pentyl-2(3H)-furanone has been identified but not quantified as a volatile component of roasted filbert tree nuts (Corylus avellana)(4). Dihydro-5-pentyl-2(3H)-furanone was detected in white, rose, and red wines (72 total samples) at mean concentrations of 14.77, 19.98, and 42.09 ug/L(5). [(1) Engel KH et al; J Agric Food Chem 36: 549-53 (1988) (2) Takeoka GR; et al J Agric Food Chem 36: 553-560 (1988) (3) Gomez E et al; J Agric Food Chem 41: 1669-76 (1993) (4) Kinlin T et al; J Agric Food Chem 20: 1021-28 (1972) (5) Perez-Olivero SJ et al; J Anal Methods Chem 2014: 863019. Published online: March 20, 2014. Available from, as of Jun 30, 2015: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3980784/

Plant Concentrations:

Dihydro-5-pentyl-2(3H)-furanone has been detected in raw earth almond (Cyperus esculentus), a weed-like plant native to Africa and Southern Europe, and roasted earth almond after 10, 20, 30, 40, 50, and 60 minute roasting times, decreasing in concentration from 0.4% peak area at 10 minutes to <0.01% at 60 minutes(1). [(1) Cantalejo MJ; J Agric Food Chem 45: 1853-60 (1997)] **PEER REVIEWED**

Fish/Seafood Concentrations:

Dihydro-5-pentyl-2(3H)-furanone has been detected in shrimp(1). [(1) Burdock GA, ed; Fenaroli's Handbook of Flavor Ingredients. 6th ed., Boca Raton, FL: CRC Press p. 1480 (2010)] **PEER REVIEWED**

Milk Concentrations:

ENVIRONMENTAL: Dihydro-5-pentyl-2(3H)-furanone is reported present in camembert cheese, butter and milk(1). [(1) Burdock GA, ed; Fenaroli's Handbook of Flavor Ingredients. 6th ed., Boca Raton, FL: CRC Press p. 1480 (2010)] **PEER REVIEWED**

As taken from HSDB, 2015

“Selaginella doederleinii (SD) is a perennial medicinal herb widely distributed in China. In this study, the volatile components of SD from two regions (24 batches), namely Zhejiang and Guizhou, were determined by combining headspace solid phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS). After investigating different influence factors, the optimal conditions for extraction were as follows: The sample amount of 1 g, the polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber of 65 μm, the extraction time of 20 min, and the extraction temperature of 100 °C. Based on the above optimum conditions, 58 volatiles compounds, including 20 terpenes, 11 alkanes, 3 alcohols, 6 ketones, 3 esters, 11 aldehydes, 1 ether, 1 aromatic, 1 phenol, and 1 furan, were found and identified in SD. Furthermore, hierarchical cluster analysis and principal component analysis were successfully applied to distinguish the chemical constituents of SD from two regions. Additionally, anethol, zingerone, 2,4-di-tert-butylphenol, ledene, hexyl hexanoate, α-cadinol, phytone, hinesol, decanal, octadecene, cedren, 7-tetradecene, copaene, β-humulene, 2-butyl-2-octenal, tetradecane, cedrol, calacorene, 6-dodecanone, β-caryophyllene, 4-oxoisophorone, γ-nonanolactone, 2-pentylfuran, 1,2-epoxyhexadecane, carvacrol, n-pentadecane, diisobutyl phthalate, farnesene, n-heptadecane, linalool, 1-octen-3-ol, phytane, and β-asarone were selected as the potential markers for discriminating SD from 24 habitats in Zhejiang and Guizhou by partial least squares discrimination analysis (PLS-DA). This study revealed the differences in the components of SD from different regions, which could provide a reference for the future quality evaluation.” As taken from Ma X-K et al. 2019. Molecules 25(1), 115. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31892247/

3. Status in legislation and other official guidance

JECFA number	229
CAS number	104-61-0
FEMA number	2781
COE number	178

As taken from FAO, undated, accessed June 2021, available at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/details/en/c/44/

The acceptable daily intake of gamma-nonalactone is between 0-1.25 mg/kg bw. As taken from FAO Nutrition Meetings Report Series 44a, available at http://www.inchem.org/documents/jecfa/jecmono/v44aje29.htm

The compounds dihydro-5-heptyl-2(3H)-furanone (also known as gamma-undecalactone) and dihydro-5-pentyl-2(3H)-furanone (also known as gamma-nonalactone) are compounds that appear on the 'GRAS' ("generally recognized as safe") list of the Expert Panel of the Flavoring Extract Manufacturers' Association of the UnitedStates (FEMA). These same compounds are not on the FDA 'GRAS' list, but are approved as food additives by the FDA. As taken from EPA, 1996. Re-registration Eligibility Decision (RED): Furanone. Available at http://archive.epa.gov/pesticides/reregistration/web/pdf/3138fact.pdf

gamma-Nonalactone 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, 2022a,b).

gamma-Nonalactone is included on the US EPA’s list of Safer Chemical Ingredients (US EPA, 2020).

There is a REACH dossier on nonan-4-olide (ECHA, 2022a)

Nonanoic acid, 4-hydroxy-,.gamma.-lactone (CAS RN 104-61-0) is listed in the US EPA InertFinder Database (2021) as approved for non-food and fragrance use pesticide products.

Dihydro-5-pentyl-2(3H)-furanone (CAS RN 104-61-0) is listed on the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2012 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.

The TSCA inventory and 2012 CDR list are available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Nonano-1,4-lactone (CAS RN 104-61-0) is authorised for use as a flavouring substance in all categories of flavoured foods in the EU under Regulation No 872/2012 (European Commission, 2012).

Nonan-4-olide (CAS RN 104-61-0) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022b).

gamma-Nonalactone (FEMA no. 2781) has been given GRAS (generally recognized as safe) status by FEMA (Hall RL and Oser BL, 1965).

Dihydro-5-pentyl-2(3H)-furanone is included on the New Zealand Inventory of Chemicals and may be used as a single component chemical under appropriate group standard (NZ EPA, 2006).

2(3H)-Furanone, dihydro-5-pentyl- (CAS RN 104-61-0) is listed by the US EPA Office of Pesticide Programs (2021) and was first registered as a “conventional chemical” pesticide on 8 August 1995. It is noted as being under registration review. One of its stereoisomers is listed separately, under the name “use code no. 122301”.

FL No	In register	Flavis name	CAS No	CE No	JECFA No	FEMA No	Chemical group number	Status	Remarks	Ref short
10.001	Yes	Nonano-1,4-lactone	104-61-0	178	229	2781	09	No further evaluation needed	JECFA evaluated gamma-nonalactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn ADI: 0-1.25 [REF:0562].	JECFA, 1999b

As taken from EFSA, 2012.

Chemical group name: “Primary aliphatic saturated or unsaturated alcohols/aldehydes/ acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones.”

As taken from the FI@vouring substances database (Flavis). Accessed March 2020. Available at: http://ec.europa.eu/food/food/chemicalsafety/flavouring/database/dsp_search.cfm

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

gamma-NONALACTONE

General Information

Synonyms:	ALDEHYDE C-18 (SO-CALLED), NONA-1,4-LACTONE
Chemical Names:	4-NONANOLIDE; 5-PENTYLDIHYDRO-2(3H)-FURANONE
JECFA number:	229
COE number:	178
FEMA number:	2781
Functional Class:	Flavouring Agent LAVOURING_AGENT

Evaluations

Evaluation year:	**1997**
ADI:	0-1.25 mg/kg bw (1967)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1967 ADI of 0-1.25 mg/kg bw was maintained at the forty-ninth meeting (1997).
Report:	TRS 884-JECFA 49/42
Tox Monograph:	FAS 40-JECFA 49/231
Specification:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/138 (2000)
Previous Years:	1998, COMPENDIUM ADDENDUM 6/FNP 52 Add. 6/172. R 1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/40, FAS 68.33/NMRS 44A-JECFA 11/67. 0-1.25. FU. N

As taken from WHO, 2021

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Aliphatic Lactones

In general, lactones are formed by acid-catalysed intramolecular cyclisation of gamma- or deltahydroxycarboxylic 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 favored (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 & Ballotti, 1970).

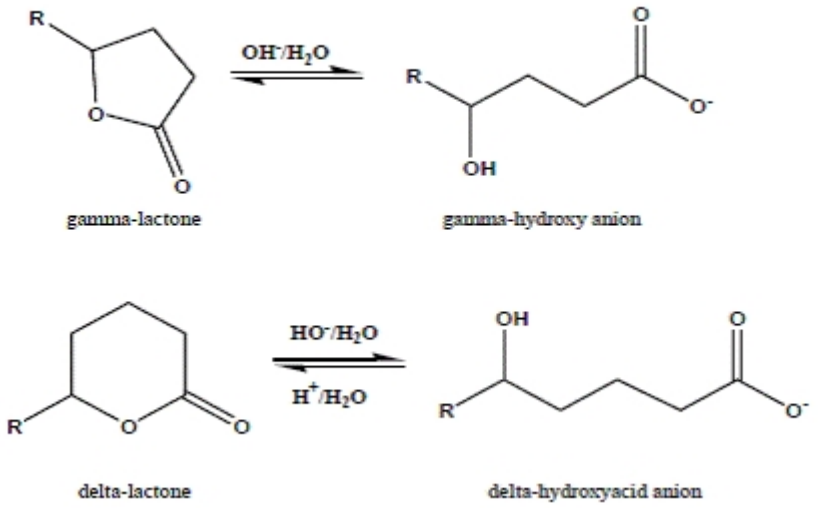


Figure III.1. Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion.

As taken from Flavouring Group Evaluation 10 Revision1 (FGE.10 Rev1), the EFSA Journal (2009) 934, 66-114; available at http://www.efsa.europa.eu/fr/scdocs/doc/934.pdf

Hydrolysis of Aliphatic Lactones

Ten candidate substances [FL-no: 10.038, 10.039, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058 and 10.068] 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 central nervous system depression, attributed to its alcaline 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 & Giarman, 1966), intraperitoneally or orally (Guidotti & Ballotti, 1970) to rats, the openchain

4-hydroxybutanoate anion is detected in the blood and tissues and the sedative effect produced by 4-hydroxybutanoate was evidenced (Roth & Giarman, 1966; Guidotti & 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 & Bessman, 1966).

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)

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 omegalactones 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 & Liebich, 1971).

The principal metabolic pathways utilised for detoxication of branched-chain hydroxycarboxylic acids are influenced by the chain length and the position and size of alkyl substituents. Short-chain (C2) located at the alpha- or beta-position inhibit metabolism to CO2 (Deisinger et al., 1994; Deuel, 1957; Albro, 1975), in which case there is either direct conjugation of the acid with glucuronic acid or omegaoxidation leading to diacid metabolites, which may be conjugated and excreted.

The degree of breakdown of gamma-nonalactone under in vitro digestion was determined using rat liver homogenate. It is seen that there are marked difference between the three lactones with respect to the ease of ring cleavage. While both gamma-valerolactone and gamma-nonalactone opened rather readily, at pH 7.5, the gamma-undecalactone is more resistant to the enzymes of rat liver homogenate. [ECHA/European Chemicals Agency, Registered substances (Last updated: July 21, 2015) Nonan-4-olide (CAS: 104-61-0) (EC/List No.: 203-219-1). Available from, as of July 30, 2015: http://echa.europa.eu/] **PEER REVIEWED**

As taken from HSDB, 2015.

4.2. Absorption, distribution and excretion

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 (Guidotti & Ballotti, 1970; Lettieri & Fung, 1978; Arena & Fung, 1980). 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 & 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, gammavalerolactone, 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 gammalactone, 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.

As taken from “Flavouring Group Evaluation 10 Revision1 (FGE.10 Rev1)”, the EFSA Journal (2009) 934, 66-114; available at http://www.efsa.europa.eu/fr/scdocs/doc/934.pdf

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**
guinea pig	LD50	oral	3440mg/kg (3440mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) GASTROINTESTINAL: CHANGES IN STRUCTURE OR FUNCTION OF SALIVARY GLANDS	Food and Cosmetics Toxicology. Vol. 2, Pg. 327, 1964.
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 13, Pg. 889, 1975.
rat	LD50	oral	6600mg/kg (6600mg/kg)		Food and Cosmetics Toxicology. Vol. 13, Pg. 889, 1975.

As taken from ChemIDplus, available at https://chem.nlm.nih.gov/chemidplus/

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - mouse	4390 mg/kg	Details of toxic effects not reported other than lethal dose value	TOVEFN Toksikologicheskii Vestnik. (18-20 Vadkovskii per. Moscow, 101479, Russia) History Unknown Volume(issue)/page/year: (4),50,2003
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	>6000 mg/kg	Details of toxic effects not reported other than lethal dose value	As above
TDLo - Lowest published toxic dose	Ocular	Rodent - rabbit	100 pph	Sense Organs and Special Senses (Eye) - conjunctive irritation	As above
TCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	96 mg/m3/4 H	Behavioral - alteration of classical conditioning Liver - liver function tests impaired Kidney/Ureter/Bladder - urine volume decreased	As above
TCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	96 mg/m3/4 H	Kidney/Ureter/Bladder - renal function tests depressed Kidney/Ureter/Bladder - proteinuria	As above

As taken from RTECS, 2007.

Rat Oral LD_{50} >5000 mg/kg bw. As taken from WHO Food Additives Series 40, available at http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm

Species	Route	Dose data
Rat	Gavage	LD_{50}: 9780 mg/kg bw
Rat	Gavage	LD_{50}: 6600 mg/kg bw
Guinea pig	Gavage	LD_{50}: 3440 mg/kg bw

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

Level causing no toxicological effect in rat: 5000ppm of the diet, equivalent to 250 mg/kg bw.

As taken from FAO Nutrition Meetings Report Series 44a, available at http://www.inchem.org/documents/jecfa/jecmono/v44aje29.htm

In an acute dermal toxicity (limit test) study performed similarly to OECD Guideline 402, a group of 10 New Zealand White rabbits were administered a single dermal dose of gamma-Nonalactone at 5000 mg/kg bw on clipped abraded abdominal skin for 24 hr. Animals were wrapped with binders of rubber dam, gauze and adhesive tape. Following exposure, the binders were removed and observations made for mortality and toxic effects for a period of 7 days. Gross necropsy was performed on all animals at the termination of the study. During the observation period, one animal died on Day 2. All survivors were essentially normal at the termination of the study. No abnormalities were noted at necropsy. Slight to moderate erythema and slight atonia were observed in some rabbits.[ECHA/European Chemicals Agency, Registered substances (Last updated: July 21, 2015) Nonan-4-olide (CAS: 104-61-0) (EC/List No.: 203-219-1). Available from, as of July 30, 2015: http://echa.europa.eu/] **PEER REVIEWED**

In an acute oral toxicity study, groups of guinea-pigs consisting of both males and females were given a single oral dose of gamma-Nonalactone by oral intubation. Animals were then observed for mortality and clinical signs for 2 weeks.... Mortality was observed from 4 hr to 6 days after administration of test material. Major signs of toxicity noted were depression and salivation.[ECHA/European Chemicals Agency, Registered substances (Last updated: July 21, 2015) Nonan-4-olide (CAS: 104-61-0) (EC/List No.: 203-219-1). Available from, as of July 30, 2015: http://echa.europa.eu/] **PEER REVIEWED**

In an acute oral toxicity study conducted similarly to OECD test guideline No. 401, groups (5/sex) of Osborne-Mendel rats were given a single oral dose of gamma-Nonalactone by oral intubation. Animals were then observed for mortality and clinical signs for 2 weeks.... Mortality was observed from 4 -18 hr after administration of test material. Major signs of toxicity noted were depression and coma.[ECHA/European Chemicals Agency, Registered substances (Last updated: July 21, 2015) Nonan-4-olide (CAS: 104-61-0) (EC/List No.: 203-219-1). Available from, as of July 30, 2015: http://echa.europa.eu/] **PEER REVIEWED**

In an acute oral toxicity study performed similarly to OECD test Guideline No. 401, groups of Wistar rats (10 males/dose) were given a single oral dose of gamma-Nonalactone at 4000, 5000, 6250, 7800 or 9700 mg/kg bw. Animals were then observed for mortality at 1 and 6 h after dosing and daily thereafter for 14 days. Gross necropsies were performed on all survivors. Mortalities in rats were 0, 30, 40, 80 and 100 % at 4000, 5000, 6250, 7800 and 9700 mg/kg bw, respectively. Clinical signs noted were piloerection and lethargy. [ECHA/European Chemicals Agency, Registered substances (Last updated: July 21, 2015) Nonan-4-olide (CAS: 104-61-0) (EC/List No.: 203-219-1). Available from, as of July 30, 2015: http://echa.europa.eu/] **PEER REVIEWED**

As taken from HSDB, 2015

5.2. Repeated dose toxicity

Non-Human Toxicity Excerpts:

A 90-day feeding study on 15 male and 15 female rats fed 0 or 67.5 mg/kg Dihydro-5-pentyl-2(3H)-furanone body wt/day showed no adverse effect. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593]

In a 2-wk study, 61.4 mg/kg Dihydro-5-pentyl-2(3H)-furanone fed to rats in the diet produced no effects. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593]

In a 12-week feeding study on 15 male and 15 female /rats/, using mixed lactones, a slight reduction in the growth rate was noted at a level of 32.7 mg/kg bw/day .[WHO/FAO; Joint Expert

Committee on Food Additives; FAO Nutrition Meetings Report Series 44a: Gamma-Nonalactone (104-61-0) (1967). Available from, as of July 31, 2015: http://inchem.org/pages/jecfa.html] **PEER REVIEWED**

Groups of 20 male and 20 female rats were fed diets containing 0, 0.1 and 0.5% of nonalactone for 2 years without any adverse effects being noted. [WHO/FAO; Joint Expert Committee on Food Additives; FAO Nutrition Meetings Report Series 44a: Gamma-Nonalactone (104-61-0) (1967). Available from, as of July 31, 2015: http://inchem.org/pages/jecfa.html] **PEER REVIEWED**

... 0.1-0.5% /gamma-nonalactone/ fed to groups of 20 male and female rats in diet for 2 yr produced no effects.[Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593] **PEER REVIEWED**

As taken from HSDB, 2015

While adequate studies were not available on which to base a NOEL for the three lactones derived from saturated linear hydroxycarboxylic acids, the following NOELs in 2-year rat studies have been reported for structurally-related lactones: gamma-nonalactone(250 mg/kg bw per day), gamma-undecalactone (250 mg/kg bw per day) and gamma-butyrolactone (112 mg/kg bw per day). A 2-year study in mice with gamma-butyrolactone indicated a NOEL of 262mg/kg bw per day. The studies on gamma-nonalactoneand gamma-undecalactone were considered previously by the Committee and ADIs were established at the eleventh meeting (Annex 1, reference 14). While these studies have not been conducted according to modern standards, the results do not provide cause for concern. These NOELs provide an adequate margin of safety (>1000) for gamma-decalactone, delta-decalactone and delta-dodecalactone and, therefore, these substances would not be expected to be of safety concern.

“Rat. In a 12-week feeding study on 15 males and 15 females, using mixed lactones, a slight reduction in the growth rate was noted at a level of 32.7 mg/kg body-weight/day (Oser, 1967). A 90-day study on 15 male and 15 female rats fed 0 or 67.5 mg/kg body-weight/day showed no adverse effect (Oser et al., 1965).”

Substance	Species	Route	Duration	NOEL2	Reference
gamma-Nonalactone	rat	diet	90	>62.8(M) >72.5(F)	Oser et al., 1965

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

Subchronic and/or chronic studies have been performed using γ-lactones formed from C4, C5, C9 and C11 linear saturated hydroxycarboxylic acids (Table B3). No dose-related effects were reported when rats were maintained on diets providing an average daily intake of 49 mg γ-valerolactone/kg for 90 days (Oser et al., 1965) or 500 mg/kg for 14 wk (Hagan et al., 1967); 62.8 mg γ-nonalactone/kg for 90 days (Oser et al., 1965) or 250 mg/kg for 1.5 to 2 years (Bar and Griepentrog, 1967); 16.5 mg γ-undecalactone/kg for 90 days (Oser et al., 1965), 166 mg/kg for 130 days (Galea, 1965), or 250 mg/kg for 1.5 to 2 years (Bar, V. F. and Griepentrog, F., 1967. Where we stand concerning the evaluation of flavouring substances from the viewpoint of health. Medizin Ernahrung 8, pp. 244–251Bar and Griepentrog, 1967). No effects were observed in a 49-week study in rats or a 38-week study in dogs when a mixture of Delta-lactones (Delta-decalactone and Delta-dodecalactone) was added to the diet of test animals. The mixture provided average daily intakes of Delta-decalactone and Delta-dodecalactone of 150 mg/kg and 300 mg/kg, respectively, to rats, and 75 mg/kg and 150 mg/kg, respectively to dogs (Wilson, 1961a). No adverse effects were reported when γ-butyrolactone was administered daily by gavage for 13 wk to mice or rats at a dose level of 112 mg/kg or 131 mg/kg, respectively (NTP, 1992). The intake levels that produced no effects in subchronic studies are greater than 10,000 times the daily per capita intake (“eaters

only") from use of the respective lactone as a flavour ingredient or from exposure as a natural component of food.

As taken from Adams TB et al. Food and Chemical Toxicology. Volume 36, Issue 4, 6 April 1998, Pages 249-278.

"Increased liver weights (no accompanying histopathological changes) observed in 90 day gavage study with rats."

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use - Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - NOEL

POD Value - 360.0

POD Owner - COSMOS TTC (NON-CANCER)

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

NOEL/LEL Owner - MUNRO

Original NOEL - 360.0 mg/kg bw/day

Original LEL - Not established

Critical Effects - NO EFFECTS

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

Read-across justification:

"γ-Hexalactone (CAS # 695-06-7) was used as a read-across analog for the target material γ-nonalactone (CAS # 104-61-0) for the repeated dose toxicity endpoints.

- The target substance and the read-across analog are structurally similar and belong to a class of γ-lactones.
- The key difference between the target substance and the read-across analog is the target substance has an amyl substitution on the 5 position while the read-across analog has an ethyl substitution on the same position. This structural difference is toxicologically insignificant.
- Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.

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

"There are insufficient repeated dose toxicity data on γ-nonalactone. Read-across material γ-caprolactone (CAS # 695-06-7; see Section V) has sufficient repeated dose toxicity data. In a subchronic toxicity study (GLP and OECD 407 compliant) performed on Crl:CD (Sprague Dawley) IGS BR rats, γ-caprolactone was administered through oral gavage at dose levels of 0 (vehicle control: deionized water), 30, 100, 300, or 1000 mg/kg/day for a period of 28 days. No treatment-related adverse effects were reported up to highest tested dose level. Based on the absence of systemic toxic effects, a NOAEL of 1000 mg/kg/day was selected for the repeated dose toxicity endpoint (ECHA, 2013). A default safety factor of 3 was used when deriving the NOAEL from an OECD 407 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. The derived NOAEL for the repeated dose toxicity data is 1000/3 or 333.3 mg/kg/day."As taken from Api AM et al. 2019. Food and Chemical Toxicology. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf

5.3. Reproduction toxicity

A mixture composed primarily of aliphatic carboxylic acids and lactones was added to the diet of a limited number of rats (four males, three females) at a level calculated to result in the average daily intake of 100 mg γ-butyrolactone/kg and 32 mg/kg each for Delta-octalactone, γ-nonalactone, γ-decalactone, γ-undecalactone and γ-dodecalactone for 4 to 6 months (Wilson, 1961b). Five succeeding generations were maintained on the same dietary regimen. Histopathological examinations of all treated animals revealed no effects. Although the validity of the results of the study are of limited value based on the small number of animals in the study (D. Fassett, unpublished report to RIFM, 1961), the results are consistent with those in feeding studies for individual lactone components reported above (i.e. γ-butyrolactone, γ-nonalactone, γ-decalactone).

As taken from Adams TB et al. Food and Chemical Toxicology. Volume 36, Issue 4, 6 April 1998, Pages 249-278.

Read-across justification:

"γ-Hexalactone (CAS # 695-06-7) was used as a read-across analog for the target material γ-nonalactone (CAS # 104-61-0) for the developmental toxicity endpoints.

- The target substance and the read-across analog are structurally similar and belong to a class of γ-lactones.
- The key difference between the target substance and the read-across analog is the target substance has an amyl substitution on the 5 position while the read-across analog has an ethyl substitution on the same position. This structural difference is toxicologically insignificant.
- Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.

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

"There are insufficient developmental toxicity data on γ-nonalactone. Read-across material γ-caprolactone (CAS # 695-06-7; see Section V) has sufficient developmental toxicity data. In a developmental toxicity study (GLP and OECD 414 compliant) performed on Crl:CD (Sprague Dawley) IGS BR rats (25/sex/dose), γcaprolactone was administered through oral gavage at dose levels of 0 (vehicle control: deionized water), 100, 300, or 1000 mg/kg/day for a period of 14 days during gestation from days 6–19. No treatmentrelated changes were reported for dams in clinical signs, body weights, gravid uterine weight, feed consumption, and necropsy examination. A significant decrease in fetal body weight was reported in the high-dose group; however, the decrease in body weight was within the historical control range. At 300 mg/kg/day, external malformations including meningocele were reported in 1 fetus, visceral malformations including malpositioned descending aorta were reported in another fetus, and a skeletal malformation (a vertebral centra anomaly: the right half of lumbar centrum number 2 was absent and the right half of lumbar centrum no. 1 was malpositioned) was reported in 1 fetus. However, these changes were reported in only 3 of 365 fetuses examined at this dose level and were not present at any other dose level. Other soft tissue and skeletal malformations and variants were reported in a single fetus, but they did not occur in a dose-related manner. In addition, the skeletal variants reported in all treated groups were within the historical control data and therefore not considered to be treatmentrelated. The NOAEL for maternal and developmental toxicity was considered to be 1000 mg/kg/day, as no treatment-related adverse effects were reported up to the highest dose level tested (ECHA, 2013).

There are insufficient reproductive toxicity data on γ-nonalactone or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to γ-nonalactone (8.2 µg/kg/day) is below the TTC (30 µg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use."

As taken from Api AM et al. 2019. Food and Chemical Toxicology. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf

5.4. Mutagenicity

Endpoint	Test Object	Concentration/Dose	Result	Reference	Comments
Ames test	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	37500 ug/plate	Negative	(Heck et al. 1989)	Abstract only, study cannot be validated
Mammalian	Mouse lymphoma L5178y TK	1000 ug/ml 600 ug/ml	Negative Positive	(Heck et al. 1989)	Abstract only, study cannot be validated
Unscheduled DNA synthesis	Rat hepatocytes	500 ug	Negative	(Heck et al. 1989)	Abstract only, study cannot be validated
Mutation assay	E. coli WP uvrA	0.2-1.6 mg/plate	Negative	(Yoo, 198)	Methods in Japanese, tables only in English. Study cannot be validated
Rec-assay	B. subtilis M45&H17	20 ul/disk (20000 ug/disk)	Positive	(Yoo, 198)	Methods in Japanese, tables only in English. Study cannot be validated

As taken from Flavouring Group Evaluation 10 Revision3 (FGE.10 Rev3), the EFSA Journal (2012) 10(3), 2563 available at http://www.efsa.europa.eu/en/efsajournal/doc/2563.pdf

In vitro					
Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**
Human leucocytes	0.7 mM [ca. 109 mg/l]. 4/50 abnormal metaphases (presence of chromosome aberrations)/spreads counted. Said to be significant compared to [undisclosed] control values. Limited study	Chromo-some damage	without	+ve	Withers, 1966
Mouse lymphoma	Positive results obtained in the presence of activation. [The test concentrations are not given in this brief abstract, although a review citing this abstract (JECFA, 1998) includes the values 400 and 1000 µg/ml]. Another review, (which claims mutation in the absence rather than the presence of activation) notes that the positive results may be artefact, due to changes in the culture pH or osmolality (Adams et al.	Mutation	with and without S9	+ve	Heck et al. 1989 JECFA, 1998 Adams et al. 1998

	1998).]				
Salmonella typhimurium strains TA98, TA100, 1535, TA1537, TA1538	Ames test. The test concentrations are not given in this brief abstract, although a review citing this abstract refers to 37.5 mg/plate (JECFA, 1998).	Mutation	with and without S9	-ve	Heck et al. 1989 JECFA, 1998
Rat hepatocytes	UDS. [The test concentrations are not given in this brief abstract, although a review citing this abstract refers to 500 µg/ml (JECFA, 1998).]	DNA damage (indicativetest)	not applicable	-ve	Heck et al. 1989 JECFA, 1998
Bacillus subtilis	Rec assay, 20 µl/disk.	DNA damage	without	+ve	Yoo, 1986
Escherichia coli	Rec assay ≤1.6 mg/plate	various DNA damage	without	-ve	Yoo, 1986
Various	STRUCTURALLY RELATED LACTONES Reviews also reports a mixture of positive and negative findings in many studies on 7 related materials, although numerically negatives greatly outweigh positives.	Various		not reported in review	JECFA, 1998 Adams et al. 1998

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which gamma-nonalactone was part of the test mixture include EMTEMT000217 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

gamma-Nonalactone was tested in a Bacterial reverse mutation assay (Ames test) using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 and Escherichia coli WP2 uvrA, both in the presence and absence of metabolic activation. Under the test conditions in this study, gamma-nonalactone was not mutagenic (CD-ROM 3, JTI Submission, 2002).

The mutagenicity of gamma-nonalactone was assessed in the following test systems: gene mutation in human leucocytes (dose of 0.7 mM → positive), in mouse lymphoma L5178y (dose of 1000 ug/mL → negative) and in TK+/- (dose of 400 ug/mL → positive). A rec-assay in Bacillus

subtilis (20 uL/disk → positive) and an UDS assay in rat hepatocytes (dose of 500 ug/mL → negative) were also conducted. In summary, gamma-nonalactone was shown to possess a weak mutagenicity.

In vivo Genotoxicity

An in vivo mammalian erythrocyte micronucleus test was conducted to evaluate the potential of gamma-nonalactone (CAS No. 104-61-0), a widely used fragrance material, to induce micronuclei in polychromatic erythrocytes (PCEs) in the bone marrow of the mouse. The study was conducted in compliance with GLP and OECD Test Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Male and female NMRI mice (5/sex/dose) were administered gamma-nonalactone orally at dose levels of 500, 1000 or 2000 mg/kg body weight in corn oil at a dose volume of 10 ml/kg body weight and sacrificed at 24 or 48 hours after dosing. Control groups received the vehicle alone or cyclophosphamide (40 mg/kg body weight) as a positive control. PCEs were collected at 24 and 48 hours after administration and examined microscopically for the presence of micronuclei. At least 2000 PCEs per animal were scored for micronuclei. The number of PCEs was not substantially decreased in gamma-nonalactone treated groups compared to the mean value of PCEs of the vehicle control group indicating that gamma-nonalactone did not produce any cytotoxic effects in the bone marrow. There was no significant increase in the incidence of micronucleated PCEs in gamma-nonalactone treated groups relative to their respective vehicle controls in either male or female mice, regardless of dose level or bone marrow collection time. The mean values of micronuclei observed in the gamma-nonalactone treated groups were below or near vehicle control values. Cyclophosphamide induced a statistically significant increase in micronucleated PCEs in both male and female mice. It was concluded that under the conditions of the test, gamma-nonalactone was not genotoxic (Letizia et al. 2010. Abstract taken from the Toxicologist 114(1), 237).

Other data are available for structurally related lactones:

“The only in vivo genotoxicity study with an aliphatic lactone other than γ-butyrolactone was a single intraperitoneal injection of 2000 mg γ-undecalactone/kg to male ddy mice. No increase in micronucleated polychromatic erythrocytes in $B6C3F_1$ mouse bone marrow cells was observed (Hayashi, M., Kishi, M., Sofuni, T. and Ishidate Jr, M., 1988. Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology 26, pp. 487–500)”.

“Based on the results of the battery [i.e. AMS(−), UDS (−), SCE(−), ABS(+/−) and MLA (+/−)] of in vitro genotoxicity tests and the evidence that neither γ-butyrolactone nor γ-undecalactone were genotoxic in vivo in the mouse micronucleus assay or sex-linked lethal assay, it is concluded that the group of aliphatic lactones formed from straight- and branched-chain aliphatic hydroxycarboxylic acids is not genotoxic”. As taken from Adams T. B., Greer D. B., Doull J., Munro I. C., Newberne P., Portoghese P. S., Smith R. L., Wagner B. M., Weil C. S., Woods L. A. and Ford R. A. Food and Chemical Toxicology. Volume 36, Issue 4, 6 April 1998, Pages 249-278

“The Ministry of Health, Labour and Welfare has carried out genotoxicity tests for food additives used in Japan in cooperation with the Japan Food Additives Association since 1979. Hayashi et al. summarized these data and published a list of 337 designated additives (Shitei-tenkabutsu in Japanese) with genotoxicity test data in 2000. Thereafter, 29 items were eliminated, and 146 items were newly added. Currently, 454 designated additives are allowed to be used as food additives in Japan. This report, based on the Hayashi report, covers the addition of newly derived genotoxicity test data. Routinely, the bacterial reverse mutation test (Ames test), mammalian cell chromosomal aberration test, and in vivo rodent bone marrow micronucleus test have been used for the evaluation of genotoxicity of food additives. In addition to the data from these tests being updated in this report, it newly includes results of transgenic rodent somatic and germ cell gene mutation assays (TGR assays), incorporated in the Organisation for Economic Co-operation and Development (OECD) test guidelines after 2000. We re-evaluated the genotoxicity of 13 designated food additives considering their TGR data.” As taken from Yamada M and Honma M. 2018. Genes

Environ. 40, 27. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30619512 "A mutagen".

"γ-Nonalactone was assessed in the BlueScreen assay and found negative for both cytotoxicity (reduced the relative cell density to less than 80%) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a screening assay that assesses genotoxic stress through human derived gene expression. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material. The mutagenic activity of γ-nonalactone has been evaluated in a bacterial reverse mutation assay conducted similar to OECD TG 471 using the standard plate incorporation. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with γnonalactone in dimethyl sulfoxide (DMSO) at concentrations up to 37500 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (Heck et al., 1989). Under the conditions of the study, γ-nonalactone was not mutagenic in the Ames test. Since this assay deviated from current OECD 471 guidelines for the Ames test, additional weight of evidence was made with negative results in a mammalian cell gene mutation assay conducted according to GLP regulations and OECD 476 guidelines. Mouse lymphoma L5178Y cells were treated with γ-nonalactone in acetone at concentrations up to 1562 µg/mL for 3 h or 24 h in the absence of metabolic activation or 3 h in the presence of S9. No increases in the frequency of mutant colonies were observed with any concentration of the test item, either with or without metabolic activation (ECHA, 2013). Under the conditions of the study, γ-nonalactone was not mutagenic to mammalian cells in vitro. The clastogenic activity of γ-nonalactone was evaluated in an in vivo micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in corn oil via oral gavage to groups of male and female NMRI mice. Doses of 500, 1000, or 2000 mg/kg body weight were administered for 24 or 48 h. Mice from each dose level were euthanized at 24 h. Additional samples were taken at the high dose only at 48 h. Bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2009). Under the conditions of the study, γ-nonalactone was considered to be not clastogenic in the in vivo micronucleus test. Based on the data available, γ-nonalactone does not present a concern for genotoxic potential."

As taken from Api AM et al. 2019. Food and Chemical Toxicology. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf

"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 P-H et al. 2020. J. Appl. Toxicol. 40(11), 1566-1587. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32662109/

"γ-Undecalactone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog γ-nonalactone (CAS # 104-61-0) show that γ-undecalactone is not expected to be genotoxic. Data on read-across material γ-hexalactone (CAS # 695-06-7) provide a calculated MOE > 100 for the repeated dose toxicity and developmental toxicity endpoints. Based on the existing data and read-across materials 4-hydroxy-3-methyloctanoic acid lactone (CAS # 39212-23-2) and (±) 3-methyl-γ-decalactone (CAS # 67663-01-8), γ-undecalactone does not present a concern for skin sensitization. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; γ-undecalactone is not expected to be phototoxic/photoallergenic. The reproductive and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to γ-undecalactone is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). The environmental endpoints were evaluated; γ-undecalactone was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1." As taken from Api AM et al. 2020. Food and Chemical Toxicology 138, 111101. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-67-6_0.pdf

5.5. Cytotoxicity

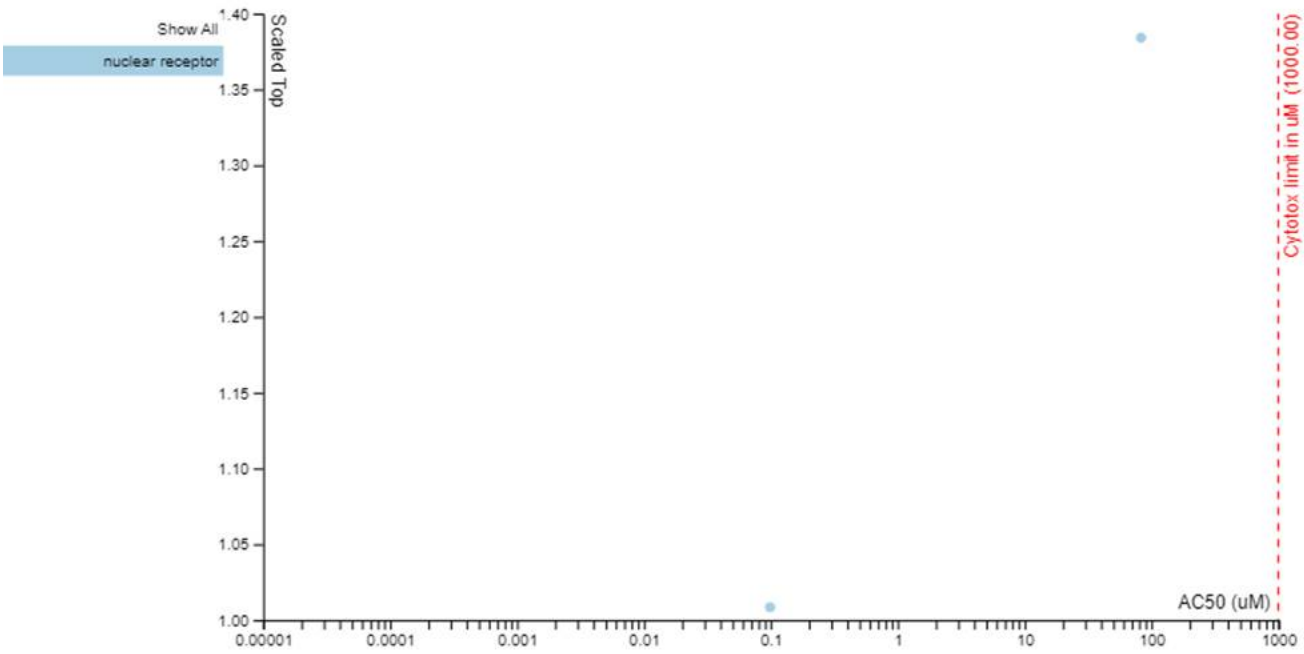
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated gamma-nonanolactone 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 gamma-nonanolactone 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

"Groups of rats (10/sex/dose level) were given a diet containing either gamma-nonalactone or gamma-undecalactone at a level of 0, 1000 or 5000 mg/kg diet for 2 years. Animals were observed throughout the study period. No adverse effects were reported. It was noted, however, that the study protocol and the presentation of the results was less than the standard required of current studies. However, the results of the study did not give rise to any safety concerns (Bär & Greipentrog, 1967)."

"Groups of 20 male and 20 female rats were fed diets containing 0, 0.1 and 0.5 per cent. of nonalactone for 2 years without any adverse effects being noted(Baer & Griepentrog,1967)." As taken from WHO Food Additives Series 40, available at http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm

Species	**Test conditions**	**Evidence of carcinogenicity**	**Reference**
Rat (10 per sex per	Diet (1000, 5000 ppm diet) 24 months. No adverse effects reported in this limited German study. An	-ve	Bar & Griepentrog,

dose)	expert review, which cites this study, notes that the protocol and presentation of results would not meet current standards (JECFA, 1998).	[limited study]	1967; JECFA, 1998
Rat Mouse (50 per species per sex per dose)	STRUCTURALLY RELATED LACTONES A more comprehensive NTP study of 1992 on gamma-butyrolactone is reported in an expert review. Oral administration for 2 years (≤ 525 or 450 mg/kg bw/day for mice and rats, respectively) gave equivocal evidence of carcinogenic activity in male mice based on marginally increased adrenal tumours and hyperplasia in the low dose group. Study sensitivity was reduced in the high dose group due to high mortality associated with fighting.	Equivocal	NTP, 1992

5.7. Irritation/immunotoxicity

Human Toxicity Excerpts:

A skin irritiant. [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 979] **PEER REVIEWED**

A maximization test was carried out on 25 volunteers. gamma-Nonalactone was tested at a concentration of 10% in petrolatum and produced no sensitization reactions. Tested at 10% in petrolatum, gamma-nonalactone produced no irritation after a 48 hr closed-patch test on human subjects. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593] **PEER REVIEWED**

Non-Human Toxicity Values:

Gamma-nonalactone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was slightly irritating. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 593] **PEER REVIEWED** As taken from HSDB, 2015

A recently developed model for allergic contact dermatitis (ACD) is applied to 148 pesticides to assess both allergens and nonallergens. Those reported to be moderate to strong irritants, but scoring as probable non-allergens, are classified as immediate type primary irritants. Although there is no certainty in the absence of animal testing, the performance of the model with respect to non-irritating pesticides is exceptional and those classified as ACD are considered as highly probable allergens based on previous validation experience. To the credit of the modern pesticide industry, only 13 of the 148 pesticides were identified as probable ACD problems with 25 others showing some form of direct irritation. As the seriousness of dermal irritation in the handling of pesticides becomes more clearly recognized, it can be expected that early animal testing will allow few of these problems to reach the future market without appropriate handling precautions.

As taken from Magee Philip S., Classical and Three-Dimensional QSAR in Agrochemistry, Chapter 9, pp 120–129. Chapter DOI: 10.1021/bk-1995-0606.ch009, ACS Symposium Series, Vol. 606 ISBN13: 9780841233218eISBN: 9780841215313 .Publication Date (Print): May 05, 1995

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reaction Severity	Reference

Standard Draize test	Administration onto the skin	Rodent - rabbit	100 mg/24H	Severe	CTOIDG Cosmetics and Toiletries. (Allured Pub. Corp., POB 318, Wheaton, IL 60189) V.91- 1976-
TDLo - Lowest published toxic dose	Ocular	Rodent - rabbit	100 pph	Sense Organs and Special Senses (Eye) - conjunctive irritation	TOVEFN Toksikologicheskii Vestnik. (18-20 Vadkovskii per. Moscow, 101479, Russia) History Unknown Volume(issue)/page/year: (4),50,2003

As taken from RTECS, 2007.

“Not irritating to skin of rabbits or humans”.

“Not sensitizing to skin of guinea pigs or humans”.

Read-across justification:

“(±) 3-Methyl-γ-decalactone (CAS # 67663-01-8) was used as a read-across analog for the target material γ-nonalactone (CAS # 104-61-0) for the skin sensitization endpoint.

- The target substance and the read-across analog are structurally similar and belong to a class of γ-lactones.
- The key difference between the target substance and the read-across analog is that the target substance has a pentyl substitution at the 5 position while the read-across analog has a pentyl substitution at the 5 position and a methyl substitution at 4 the position. This structural difference is toxicologically insignificant.
- Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
- Differences are predicted for Jmax, which estimates skin absorption. Jmax for the target substance corresponds to skin absorption ≤80, and Jmax for the read-across analog corresponds to skin absorption ≤40. While percentage skin absorption estimated from Jmax indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
- The target substance and the read-across analog have acylation alerts. Based on the limited data on the target and data on the read-across analog, it is confirmed that the substances do not present a concern for skin sensitization. Therefore, the predictions are superseded by data.
- The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

4-Hydroxy-3-methyloctanoic acid lactone (CAS # 39212-23-2) was used as a read-across analog for the target material γ-nonalactone (CAS # 104-61-0) for the skin sensitization endpoint.

- The key difference between the target substance and the read-across analog is that the target substance has a pentyl substitution at the 5 position while the read-across analog has a butyl substitution at the 5 position and a methyl substitution at the 4 position. This structural difference is toxicologically insignificant.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material."

"Limited skin sensitization studies are available for γ-nonalactone. Based on the existing data and readacross materials 4-hydroxy-3-methyloctanoic acid lactone (CAS # 39212-23-2; see Section V) and (±) 3-methyl-γ-decalactone (CAS # 67663-01-8; see Section V), γ-nonalactone does not present a concern for skin sensitization under current, declared levels of use. The chemical structures of these materials indicate that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v4.1). No predictive in chemico or in vitro skin sensitization studies are available on γ-nonalactone or read-across materials 4- hydroxy-3-methyloctanoic acid lactone and (±) 3-methyl-γdecalactone in the literature. In guinea pig maximization tests, readacross materials 4-hydroxy-3-methyloctanoic acid lactone and (±) 3-methyl-γ-decalactone did not present reactions indicative of sensitization up to 10% and 20% respectively (RIFM, 1988a; RIFM, 2002). In human maximization tests, no skin sensitization reactions were observed with γ-nonalactone (RIFM, 1976; RIFM, 1972). Based on weight of evidence (WoE) from structural analysis, human and animal studies, and read-across materials 4-hydroxy-3-methyloctanoic acid lactone and (±) 3-methyl-γ-decalactone, γ-nonalactone does not present a concern for skin sensitization under current, declared levels of use.

There are no phototoxicity studies available for γ-nonalactone in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of absorbance, γ-nonalactone does not present a concern for phototoxicity or photoallergenicity.

UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol−1 · cm−1 (Henry et al., 2009)."

As taken from Api AM et al. 2019. Food and Chemical Toxicology. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing gamma-Nonalactone 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 gamma-Nonalactone 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	4	JTI KB Study Report(s)
In vitro cytotoxicity	4	JTI KB Study Report(s)

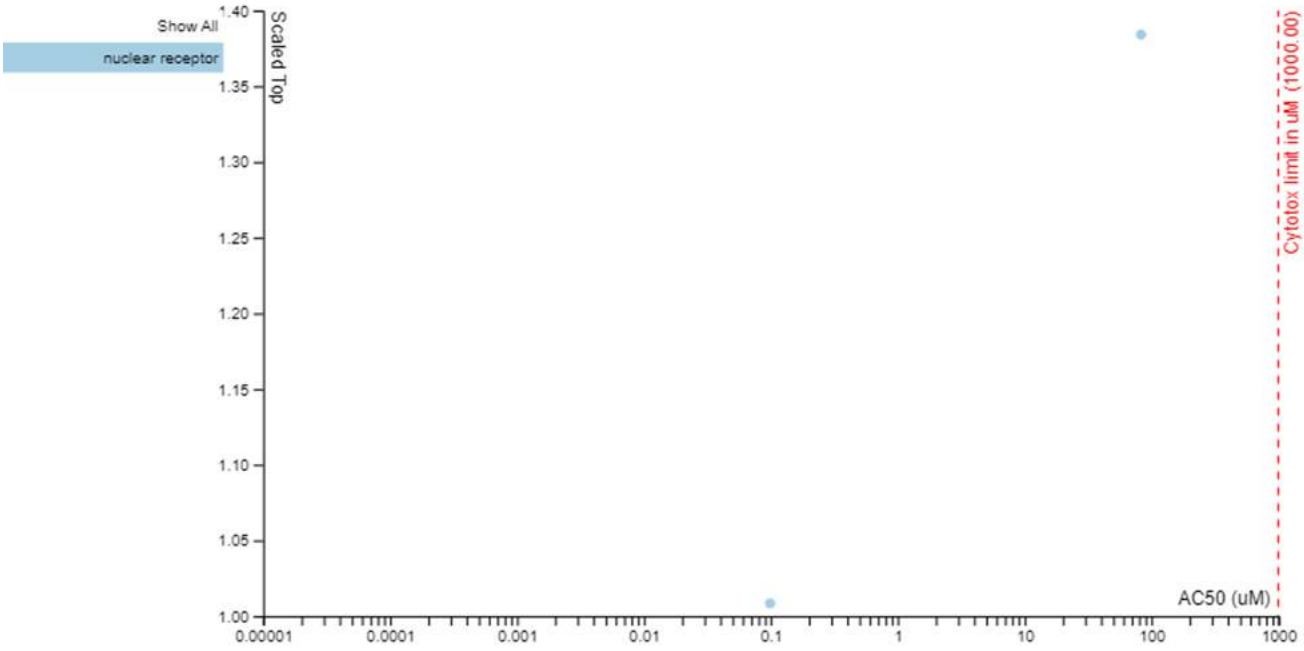
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated gamma-nonanolactone 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 gamma-nonanolactone 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

“The reproductive and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to γ-valerolactone is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively).”

As taken from Api AM et al. 2019. Food and Chemical Toxicology. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

”Neurotoxin: acute solvent syndrome”.

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

“Causes … abnormal liver and kidney function tests in high-dose animal studies”.

As taken from Haz-Map, 2020.

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 gamma-Nonalactone 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 gamma-Nonalactone. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	6	Carmines, 2002 & Rustemeier et al. 2002
	140	Baker et al. 2004a
	1.3 13 221 340	JTI KB Study Report(s)
	39	Roemer et al., 2014
In vitro genotoxicity	6	Carmines, 2002 & Roemer et al. 2002
	140	Baker et al. 2004c
	1.3	Renne et al. 2006

	13 166 340	JTI KB Study Report(s)
	62.8	fGLH Study Report (2010)
	39	Roemer et al., 2014
In vitro cytotoxicity	6	Carmines, 2002 & Roemer et al. 2002
	140	Baker et al. 2004c
	13 166 340	JTI KB Study Report(s)
	62.8	fGLH Study Report (2010)
	39	Roemer et al., 2014
Inhalation study	0.7	Gaworski et al. 1998
	6	Carmines, 2002 & Vanscheeuwijck et al. 2002
	140	Baker et al. 2004c
	1.3	Renne et al. 2006
	1.3 13 166 340	JTI KB Study Report(s)
	39	Schramke et al., 2014
Skin painting	0.7	Gaworski et al. 1999
	1.3 13	JTI KB Study Report(s)
In vivo genotoxicity	39 340	Schramke et al., 2014 JTI KB Study Report(s)

Transfer studies:

In a pyrolysis study, 100% of gamma-nonalactone (“nonan-4-olide”) added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing gamma-Nonalactone 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 gamma-Nonalactone 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	4	JTI KB Study Report(s)
In vitro cytotoxicity	4	JTI KB Study Report(s)

Aerosol from heated tobacco stick(s) containing gamma-Nonalactone 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	0.044	Labstat International Inc. (2020a)
In vitro genotoxicity	0.044	Labstat International Inc. (2020b)
In vitro cytotoxicity	0.044	Labstat International Inc. (2020b)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing gamma-Nonalactone 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)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following information:

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

Bond Method :ou	4.23E-004 atm-m3/mole (4.29E+001 Pa-m3/mole)
Group Method:	1.69E-004 atm-m3/mole (1.71E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.020E-006 atm-m3/mole (2.046E-001 Pa-m3/mole) VP: 0.0118 mm Hg (source: MPBPVP)

	WS: 1.2E+003 mg/L (source: WSKOWWIN)

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

Log Kow used:	2.08 (KowWin est)
Log Kaw used:	-1.762 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	3.842
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.9558 0.9988 3.2925 (days-weeks) 4.1204 (days) 0.8411 0.9271 0.5140
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):	1.59 Pa (0.0119 mm Hg)
Log Koa (Koawin est):	3.842
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.89E-006 1.71E-009

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	6.83E-005
Mackay model:	0.000151
Octanol/air (Koa) model:	1.36E-007

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

OVERALL OH Rate Constant =	9.6930 E-12 cm3/molecule-sec
Half-Life =	1.103 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	13.242 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.00011 (Junge-Pankow, Mackay avg) 1.36E-007 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	120 L/kg (MCI method)
Log Koc:	2.079 (MCI method)
Koc :	102.3 L/kg (Kow method)
Log Koc:	2.010 (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: 0.000169 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	5.606 hours
Half-Life from Model Lake:	166 hours (6.915 days)

Removal In Wastewater Treatment:

Total removal:	9.82 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	2.10 percent
Total to Air:	7.63 percent

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)

Air	4.88	26.5	1000
Water	26.5	208	1000
Soil	68.5	416	1000
Sediment	0.149	1.87e+003	0

Persistence Time: 247 hr

Environmental Fate:

Environmental fate data are not required to support the low-volume, outdoor, residential uses of the furanones. However, environmental fate data generally are required to support aquatic nonfood uses such as the mosquito larvicide use. A quantitative environmental fate assessment cannot be made for the furanones at this time because no environmental fate data have been submitted for review. The furanones are classified as lactones, and some open literature data are available on physiochemical properties of lactones and their possible effect on the environment. The reported data indicate lactones may not be stable in alkaline environments.

As taken from EPA R.E.D FACTS available at http://www.epa.gov/oppsrrd1/REDs/factsheets/3138fact.pdf

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2(3H)-Furanone, dihydro-5-pentyl- (CAS RN 104-61-0) 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	0.9271
TOPKAT probability of biodegradation	0.911
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	1.103

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

10.2. Aquatic toxicity

Acute toxicity data indicate that the formulated product is practically non-toxic on an acute oral and subacute dietary basis to birds, mammals, and freshwater fish. The product is slightly toxic to freshwater invertebrates.

As taken from EPA R.E.D FACTS available at http://www.epa.gov/oppsrrd1/REDs/factsheets/3138fact.pdf

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2(3H)-Furanone, dihydro-5-pentyl- (CAS RN 104-61-0) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	51.3
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	51.3
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	21.427
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	289.4524
Toxicity to fish (LC50 in mg/l) as predicted by Aster	28.798244
Toxicity to fish (LC50 in mg/l) as predicted by PNN	409.34513
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	29.3
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	104.943
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	9.74E-001

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

Toxicity to Aquatic Animals

(1) Freshwater Fish

To establish the toxicity of a pesticide to freshwater fish, the minimum data required on the technical grade of the active ingredient are two freshwater fish toxicity studies. One study should use a coldwater species (preferably the rainbow trout), and the other should use a warmwater species (preferably the bluegill sunfish). Data that have been submitted for these two tests have been with a formulated product, not the technical grade material. The following table shows the results of these tests.

Fresh Fish Acute Toxicity Findings					
Species	**% A.I.a**	**LC40 ppm a.i.**	**MRID No.**	**Toxicity Category**	**Fulfills Guideline Requirements**
Rainbow trout	4.015% 079701 0.024% 122301 0.049% 122302	569b	109343	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent
Fathead minnow	4.015% 079701 0.024% 122301 0.049% 122302	1490b	109344	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent

a Active ingredient are listed by their Shaughnessey numbers

b Result is based on the moving average method

The results of the 96-hour acute toxicity studies indicate that the formulated product is practically nontoxic to fish (MRID 109343-109344). However, since this product contains only a small percent of active ingredient, it cannot be concluded from these results that furanones are practically nontoxic to fish. Assuming that all of the toxicity of the formulated product was caused by the furanones, the estimated acute LC50 of the furanones is >0.42 ppm a.i. for the rainbow trout and >1.1 ppm a.i. for the fathead minnow. These results tell little about the toxicity of the furanones as active ingredients. Furanones could fall into any toxicity classification except very highly toxic. (MRID No.109343) Because the tests were conducted with formulated product rather than technical grade active ingredients, they do not fulfill the guideline requirements for furanones. However, these data are considered adequate for assessing the risk of the use of the Doo- Not product. They may also be adequate for other low-use products that contain furanones in identical concentrations.

(2) Freshwater Invertebrates

The minimum testing required to assess the hazard of a pesticide to freshwater invertebrates is a freshwater aquatic invertebrate toxicity test, preferably using first instar Daphnia magna or early instar amphipods, stoneflies, mayflies, or midges. Data that have been submitted for a test with Daphnia magna have been with a formulated product, not the technical grade material. The table below gives the results of these tests.

Fresh Invertebrate Toxicity Findings					
Species	**% A.I.a**	**LC40 ppm a.i.**	**MRID No.**	**Toxicity Category**	**Fulfills Guideline Requirements**
Daphnia magna	4.015% 079701 0.024% 122301 0.049% 122302	17b	109343	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent

There is sufficient information to characterize the formulated product containing furanones as slightly toxic to aquatic invertebrates (MRID 109345). However, since this product contains only a small percent of active ingredient, it cannot be inferred from these results that furanones are slightly toxic to freshwater invertebrates. Assuming that all of the toxicity of the formulated product was caused by the furanones, the estimated acute LC50 of the furanones is >0.012 ppm a.i. for Daphnia magna. These results probably overestimate the toxicity of furanone because much of the toxicity of the formulated product could be the result of the other active ingredient, limonene, or of inert ingredients. These results tell little about the actual toxicity of the furanones as active ingredients. Furanones may fall into any of five toxicity classifications (MRID 109345).

Because the tests were conducted with formulated product rather than technical grade active ingredients, they do not fulfill the guideline requirements for furanones. However, these data are considered adequate for assessing the risk of the use of the Doo- Not product. They may also be adequate for other low-use products that contain furanones in identical concentrations. A life-cycle test with a freshwater invertebrate (guideline 72-4b) is needed for the mosquito larvicide use of furanones because it involves direct application to water and because some of the acute risk quotients for freshwater invertebrates are greater than 0.01. This guideline requirement is not fulfilled.

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 104-61-0:

Values used to Generate ECOSAR Profile:

Log Kow: 2.076 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1201 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations:

Ester

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	
Esters :	Fish	96-hr	LC50	18.854	
Esters :	Daphnid	48-hr	LC50	38.875	
Esters :	Green Algae	96-hr	EC50	16.347	
Esters :	Fish		ChV	1.396	
Esters :	Daphnid		ChV	26.006	
Esters :	Green Algae		ChV	4.295	
Esters :	Fish (SW)	96-hr	LC50	28.549	
Esters :	Mysid	96-hr	LC50	27.046	
Esters :	Fish (SW)		ChV	4.149	
Esters :	Mysid (SW)		ChV	1704.303 *	
Esters :	Green Algae		ChV	12.790	
Neutral Organic SAR :	Fish	96-hr	LC50	109.650	
(Baseline Toxicity) :	Daphnid	48-hr	LC50	62.699	
	Green Algae	96-hr	EC50	48.082	
	Fish		ChV	10.807	
	Daphnid		ChV	6.235	
	Green Algae		ChV	109.650	

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.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Acute toxicity data indicate that the formulated product is practically non-toxic on an acute oral and subacute dietary basis to birds, mammals, and freshwater fish. The product is slightly toxic to freshwater invertebrates.

As taken from EPA R.E.D FACTS available at http://www.epa.gov/oppsrrd1/REDs/factsheets/3138fact.pdf

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 104-61-0:

Values used to Generate ECOSAR Profile:

Log Kow: 2.076 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1201 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations:

Ester

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	1924.955 *

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.

Toxicity to Terrestrial Animals

(1) Birds, Acute and Subacute

To establish the toxicity of furanones to birds, the following tests are required using the technical grade material: one avian single-dose oral (LD50) study on one species (preferably mallard or bobwhite quail); two subacute dietary studies (LC50) on one species of waterfowl (preferably the mallard duck) and one species of upland game bird (preferably bobwhite quail). Data that have been submitted for these three tests have been with a formulated product, not the technical grade material. The following tables show the results of these tests.

Fresh Fish Acute Toxicity Findings					
Species	**% A.I.a**	**LC40 ppm a.i.**	**MRID No.**	**Toxicity Category**	**Fulfills Guideline Requirements**
Nothern Bobwhite	4.015% 079701 0.024% 122301 0.049% 122302	>2000	109342 Bottoms, J 1982	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent

a Active ingredient are listed by their Shaughnessey numbers

Fresh Fish Acute Toxicity Findings					
Species	**% A.I.a**	**LC40 ppm a.i.**	**MRID No.**	**Toxicity Category**	**Fulfills Guideline Requirements**

Nothern Bobwhite	4.015% 079701 0.024% 122301 0.049% 122302	>5000	109342 Bottoms, J 1982	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent
Mallard	4.015% 079701 0.024% 122301 0.049% 122302	>5000	109341 Bottoms, J 1982	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent

These results indicate that the formulated product Doo-Not Dog Repellent is practically nontoxic to avian species on an acute oral and subacute dietary basis (MRID 109340-109341). However, since this product contains only a small percent of active ingredient, it cannot be inferred from these results that furanones are practically nontoxic. If the furanones caused all of the toxicity of the formulated product, an estimated acute oral LD50 of the furanones for avian species would be >1.46 mg ai/kg, and an estimated subacute dietary LC50 would be >3.65 ppm ai. These results tell little about the actual toxicity of the furanones as active ingredients. Furanones may fall into any of five toxicity classifications.

(2) Mammals

Wild mammal testing is required on a case-by-case basis, depending on the results of the lower tier studies such as acute and subacute testing, intended use pattern, and pertinent environmental fate characteristics.

17 The oral rat LD50 for Doo-Not Dog & Cat Repellent (Reg. No. 45987-1) is >5 g/kg (Accession No. 245190). This result indicates that this formulated product is practically nontoxic, but it tells little about the toxicity of furanones as active ingredients. If all of the toxicity of the formulated product was caused by the furanones, an estimated acute oral LD50 of the furanones for avian species would be >3.65 mg ai/kg. Furanones thus may fall into any of five toxicity classifications.

As taken from EPA. 1996. Re-registration Eligibility Decision (RED) available at http://archive.epa.gov/pesticides/reregistration/web/pdf/3138fact.pdf

“New Zealand flower thrips, Thrips obscuratus (Thysanoptera: Thripidae), are attracted to ripening fruits, especially peaches. Volatiles from unripe and ripe peach fruits were collected and analyzed by gas chromatography/mass spectrometry. Six lactones were found only in ripe peach volatiles: γ-heptalactone, γ-octalactone, γ-nonalactone, 6-pentyl-2H-pyran-2-one, γ-decalactone, and δ-decalactone. When these compounds were tested individually in field-trapping experiments, three of them (γ-octalactone, γ-nonalactone, and 6-pentyl-2H-pyran-2-one) attracted New Zealand flower thrips. In another field-trapping experiment, aimed at testing various combinations of the three active compounds, no synergistic effects were found among all combinations tested; no combination caught more thrips than 6-pentyl-2H-pyran-2-one, alone. A further field-trapping experiment was conducted to determine the dose (10, 100, and 500 mg) of 6-pentyl-2H-pyran-2-one that gave the greatest catch of T. obscuratus, while also comparing it against another attractant, ethyl nicotinate, for T. obscuratus. The greatest catches in traps baited with either attractant were at loadings of 500 mg. At both 10 and 500 mg, traps baited with 6-pentyl-2H-pyran-2-one caught more T. obscuratus than those baited with the same amounts of ethyl nicotinate. 6-Pentyl-2H-pyran-2-one is a potent attractant for New Zealand flower thrips and, therefore, could be used for monitoring and control of New Zealand flower thrips. Work is underway developing

monitoring and control options utilizing 6-pentyl-2H-pyran-2-one for this important pest". As taken from El-Sayed AM et al. 2014. J. Chem. Ecol. 40(1), 50-5. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24435661

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.037 (BCF = 10.88 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.4791 days (HL = 0.03319 days)
Log BCF Arnot-Gobas method (upper trophic):	0.756 (BCF = 5.701)
Log BAF Arnot-Gobas method (upper trophic):	0.756 (BAF = 5.701)
log Kow used:	2.08 (estimated)

Ecological Effects Risk Assessment:

The furanones, when used as mosquito larvicides, are applied directly to water. The public health larvicidal use of furanones applied at 4.10 lbs/acre (A) to 6 inches of water results in exceedances of the level of concern (LOC) for freshwater invertebrates. The LOC for endangered species is exceeded when the product is applied at 1.64 lbs/A in water 6 inches deep or less, or when it is applied at 4.10 lbs/A in water 1 foot deep or less.

This assessment is based on testing done on a formulated product and tells little about the actual toxicity of the furanones as active ingredients. Chronic invertebrate toxicity data and basic environmental fata data would improve the Agency's understanding and assessment of the potential risk posed by the use of the furanones in mosquito larvicides.

The volume of products containing furanones that are used annually as mosquito larvicides is low, and the percentage of furanone in the products is also low. The low volume and low percent of furanone support the conclusion that widespread adverse impacts are not likely to result from the mosquito larvicide use if continued at the amounts currently produced and used.

It should be recognized that the risk posed to aquatic invertebrates is common to most, if not all, products registered for mosquito larvicide use. It is the nature of these products to be harmful to aquatic invertebrates because the target species is itself an aquatic invertebrate. The mosquito larvicide product containing furanones would cause less harm to aquatic ecosystems than many other products because its risk is limited to aquatic invertebrates, whereas others pose a risk to fish and birds, as well.

As taken from EPA R.E.D FACTS available at http://www.epa.gov/oppsrrd1/REDs/factsheets/3138fact.pdf

The Ecological Categorization Results from the Canadian Domestic Substances List state that 2(3H)-Furanone, dihydro-5-pentyl- is not bioaccumulative in the environment:

Log Kow predicted by KowWin	2.08
Log BAF T2MTL predicted by Gobas	0.931646881356691
Log BCF 5% T2LTL predicted by Gobas	0.85001686069244

Log BCF Max predicted by OASIS	1.73560750539549
Log BCF predicted by BCFWIN	0.899

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

11. References

- Adams T.B. et al. (1998). Fd Chem. Toxic. 36, 249.
- Api AM et al. (2019). RIFM fragrance ingredient safety assessment, γ-nonalactone, CAS Registry Number 104-61-0. Food and Chemical Toxicology. Epub ahead of print. DOI: 0.1016/j.fct.2019.110905. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-61-0.pdf
- Api AM et al. (2020). RIFM fragrance ingredient safety assessment, γ-undecalactone, CAS Registry Number 104-67-6. Food and Chemical Toxicology 138, 111101. DOI: 10.1016/j.fct.2019.111101. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/104-67-6_0.pdfBaker R and Bishop L. (2004). The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrolysis 71, 223–311.
- 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.
- 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.
- Bar F. & Griepentrog F. (1967). Medizin Ernahr. 8, 244.
- 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.
- CD-ROM 1 & 3, JTI Submission, 2002ChemIDplus.Accessed June 2021. Available at https://chem.nlm.nih.gov/chemidplus/
- ChemSpider. Record for 2781 (CAS RN 104-61-0). Accessed June 2021. Available at http://www.chemspider.com/Chemical-Structure.7424.html
- CIR (2019). Cosmetic Ingredient Review. Safety Assessment of Vanilla-Derived Ingredients as Used in Cosmetics. Draft Tentative Report for Panel Review. Release date 15 November 2019. Available at https://cir-safety.org/sites/default/files/Vanilla_0.pdf
- COE (2000). Chemically-defined flavouring substances. Council of Europe. Partial Agreement in the Social and Public Health Field, Strasbourg. 4th Edition, revised.
- CosIng. Cosmetic substances and ingredients database. Record for gamma-nonalactone (CAS RN 104-61-0). Undated, accessed June 2021. Available at https://ec.europa.eu/growth/tools-databases/cosing/
- COSMOS Database (undated). Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety. Record for nonano-1,4-lactone (CAS RN 104-61-0). Accessed June 2021. Available at https://ng.cosmosdb.eu/
- CPID (undated). Consumer Product Information Database. Record for dihydro-5-pentyl-2(3H)-furanone (CAS RN 104-61-0). Accessed June 2021. 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 (2022a). European Chemicals Agency. Information on Chemicals. Record for nonan-4-olide. Last updated 1 February 2022. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances
- ECHA (2022b). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 15 June 2022. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR (undated). Record for 2,3(H)-furanone, di-hydro-5-pentyl- (CAS RN 104-61-0). Accessed May 2017. (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). EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); 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. Question No EFSA-Q-2011-01010, EFSA-Q-2010-01554, adopted on 2 February 2012. EFSA Journal 10(3), 2563 http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2563/epdf
- El-Sayed AM et al. (2014). 6-Pentyl-2H-pyran-2-one: A Potent Peach-Derived Kairomone for New Zealand Flower Thrips, Thrips obscuratus. J. Chem. Ecol. 40(1), 50-5. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24435661
- EPA (1996). Re-registration Eligibility Decision (RED): Furanone. Available at http://archive.epa.gov/pesticides/reregistration/web/pdf/3138fact.pdf
- EPISuite (2017). Record for 2(3H)-furanone, dihydro-5-pentyl- (CAS RN 104-61-0). 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 2,3(H)-furanone, di-hydro-5-pentyl- (CAS RN 104-61-0). Accessed May 2017. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download at
- European Commission (2012). Database of food flavourings. Record for nonano-1,4-lactone Last modified 17 September 2012. Accessed June 2021. Available at https://webgate.ec.europa.eu/foods_system/
- FAO (undated). Food and Agriculture Organization of the United Nations. Online Edition: “Specifications for Flavourings”. Record for gamma-nonalactone. Undated, accessed June 2021. Available at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/details/en/c/44/
- FAO Nutrition Meetings Report Series 44a, available at http://www.inchem.org/documents/jecfa/jecmono/v44aje29.htm
- FDA (2022a). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 17 May 2022. Accessed June 2021. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2022b). US Food and Drug Administration.Electronic Code of Federal Regulations (eCFR) Title 21. Current as of 13 June 2022. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- fGLH Study Report (2010).
- 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 localization, stoichiometry, specificity, distinction from esterases. J. Biol. Chem. 241, 4835-4841.
- Fl@vouring substances database (Flavis). EUROPA food flavouring website. Accessed March 2020. Available at: http://ec.europa.eu/food/food/chemicalsafety/flavouring/database/dsp_search.cfm

- Flavouring Group Evaluation 10 Revision1 (FGE.10 Rev1), the EFSA Journal (2009) 934, 66-114; available at http://www.efsa.europa.eu/fr/scdocs/doc/934.pdf
- Gaworski C.L. et al.(1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposures in rats. Inhalation Toxicology, 10:357-381.
- Gaworski C.L. et al.(1999). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology 139, 1-17.
- GESTIS (undated). GESTIS Substance Database. Record for nonan-4-olide (CAS RN 104-61-0). Accessed June 2021. Available at https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp
- Hall RL and Oser BL (1965). Recent Progress in the Consideration of Flavoring Ingredients Under the Food Additives Amendment. III. GRAS Substances. Food Technology 253, 151. Available at https://www.femaflavor.org/sites/default/files/3. GRAS Substances(2001-3124)_0.pdf
- Hayashi M et al. (1988). Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology 26, pp. 487–500.
- Haz-Map (2020). Record for gamma-nonalactone (CAS RN 104-61-0). Last updated 29 July 2020. Accessed June 2021. Available at https://haz-map.com/
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Record for gamma-nonalactone (CAS RN 104-61-0). Last updated 4 May 2021. Accessed June 2021. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=2499&lang=eng
- Heck J.D. et al.(1989). Toxicologist 9, 257.
- HSDB (2015). Record for dihydro-5-pentyl-2(3H)-furanone. Hazardous Substances Databank Number: 5360. Last Revision Date: 23 December 2015. Accessed June 2021. Available at: https://www.toxinfo.io/
- Hung P-H 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. IFRATransparency List. Accessed June 2021. Available at: https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
- JECFA (1998). Safety evaluation of certain food additives and contaminants. Prepared by the forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Fd Add. Ser. 40, 1.
- JTI KB Study Report (s).
- JTI Study Report (s).
- Labstat International Inc. (2020a) Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2020b) 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. (2021) Characterization of E-cigarette Aerosol. Analytical Test Report.
- 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.
- Letizia C et al. (2010). An in vivo mouse micronucleus test with gamma-nonalactone. Toxicologist 114(1), 237.

- Lloyd R A et al.(1976). Flue-cured tobacco flavour. 1. Essence and essential oil components. Tobacco Science, 20, 40-48.
- Ma X-K et al. (2019). Analysis of the Volatile Components in Selaginella doederleinii by Headspace Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry.Molecules 25(1), 115. DOI:10.3390/molecules25010115. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31892247/
- Magee Philip S. Classical and Three-Dimensional QSAR in Agrochemistry, Chapter 9, pp 120–129. Chapter DOI: 10.1021/bk-1995-0606.ch009, ACS Symposium Series, Vol. 606 ISBN13: 9780841233218eISBN: 9780841215313 .Publication Date (Print): May 05, 1995
- NTP (1992). gamma-Butyrolactone (CAS No. 96-48-0) in F344/N rats and B6C3F1 mice (gavage studies). TR 406 (cited in Adams et al. 1998).
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Record for dihydro-5-pentyl-2(3H)-furanone (CAS RN 104-61-0). Date added to inventory: 1 December 2006. Accessed March 2020. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/14971
- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). 2(3H)-Furanone, dihydro-5-pentyl-. Accessed May 2017. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Oser B et al. (1965). Tests on flavouring matters. Fd Cosm Tox, 3, 563-569.
- PubChem (2021). Record for gamma-nonalactone (CAS RN 104-61-0). Created 26 March 2005. Last modified 19 June 2021. Available at https://pubchem.ncbi.nlm.nih.gov/compound/7710
- Purkis SW, Mueller C & Intorp M (2011). The fate of ingredients in and impact on cigarette smoke. Food and Chemical Toxicology 49, 3238-3248.
- Renne R et al. (2006). Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats. Inhalation Toxicology, 18:685-706, 2006.
- Roemer E et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology, 40, 105-111.
- 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.
- ROTH, R.H., AND GIARMAN, N.J. (1966). gamma-Butyrolactone and gamma-hydroxybutyric acid — I. Distribution and metabolism. Biochem. Pharmacol. 15, 1333-1348.
- RTECS (2007). Registry of Toxic Effects of Chemical Substances. Record for 2(3H)-furanone, dihydro-5-pentyl-(104-61-0). Last updated February 2007. Accessed June 2021.
- Rustemeier K et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- 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.
- Stedman, R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207.
- US EPA (2020). Safer Chemical Ingredients List. Last updated 26 October 2020. Accessed June 2021. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA 2012 CDR list (Chemical Data Reporting Rule). Accessed June 2021. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA InertFinder Database (2021). Last updated 10 June 2021. Accessed June 2021. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA Office of Pesticide Programs (2021). Records for 2(3H)-furanone, dihydro-5-pentyl- and “Use code no. 122301”. Last updated 24 June 2021. Accessed June 2021. Available at https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1:0::NO:1

- US EPA ToxCast. Accessed June 2021. Available via US EPA CompTox Chemistry Dashboard at https://comptox.epa.gov/dashboard
- US EPA TSCA inventory. Accessed June 2021. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- Vanscheeuwijck P.M. et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes.Part 4: subchronic inhalation toxicity. Food and Chemical Toxicology 40, 113-131
- WHO (2021). Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Record for gamma-nonalactone. Accessed June 2021. Available at https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=4338
- WHO Food Additives Series 40, available at http://www.inchem.org/documents/jecfa/jecmono/v040je12.htm
- Wiedmer C and Buettner A (2019). Identification and characterisation of odorants in a squishy toy using gas chromatography-mass spectrometry/olfactometry after thermal extraction. Anal. Bioanal. Chem. 411(17), 3757-3761. DOI: 10.1007/s00216-019-01860-9. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31079176/
- Withers R.F.J. (1966). Mechanisms of Mutation and Inducing Factors. Landa Z. (Ed.). The action of some lactones and related compounds on human chromosomes, 359.
- Yamada M and Honma M (2018). Summarized data of genotoxicity tests for designated food additives in Japan. Genes Environ. 40, 27. DOI: 10.1186/s41021-018-0115-2. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30619512
- Yoo Y.S. (1986). J. Osaka Cy med.Cent. 34, 267.
- Zeng Z et al. (2008). Molecules.2009 Aug 10;14(8):2927-34.

12. Other information

- Chou HC et al. (2018). Late-Season Shiraz Berry Dehydration That Alters Composition and Sensory Traits of Wine. J. Agric. Food Chem. 66(29), 7750-7757. DOI: 10.1021/acs.jafc.8b01646. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29962206
- Geffroy O et al. (2018). Modulating analytical characteristics of thermovinified Carignan musts and the volatile composition of the resulting wines through the heating temperature. Food Chem. 257, 7-14. DOI: 10.1016/j.foodchem.2018.02.153. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29622232
- Lee SM et al. (2018). Investigation on the formations of volatile compounds, fatty acids, and γ-lactones in white and brown rice during fermentation. Food Chem. 269, 347-354. DOI: 10.1016/j.foodchem.2018.07.037. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30100445
- Siebert TE et al. (2018). Investigation of 'stone fruit' aroma in Chardonnay, Viognier and botrytis Semillon wines.Food Chem. 256, 286-296. DOI: 10.1016/j.foodchem.2018.02.115. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29606450

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Flavouring Group Evaluation 10, Revision 1 (FGE10 Rev1) [1]

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

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC)

(EFSA-Q-2003-153B)

Adopted on 31 January 2008

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked 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 Scientific Panel is asked to evaluate 58 flavouring substances in the Flavouring Group Evaluation 10, Revision 1 (FGE.10Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 58 flavouring substances belong to chemical groups 9, 13 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The 58 flavouring substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-five of the 58 flavouring substances possess one or more chiral centres and six can exist as geometrical isomers due to the presence and the position of a double bond. For eight of these substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168] the stereoisomeric composition/composition of mixture has not been specified.

Fifty-one of the flavouring substances belong to structural class I, six of the substances belong to structural class II and one substance belongs to structural class III according to the decision tree approach presented by Cramer et al. (1978).

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Flavouring Group Evaluation 10 Revision1 (FGE.10 Rev1). 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.

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Forty-eight 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 Intake (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 Flavour 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 the 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.

According to the default MSDI approach, the 58 flavouring substances in this group to which the Procedure has been applied have intakes in Europe from 0.0012 to 12 microgram/*capita*/day, which are below the thresholds of concern value for structural class I, II and III substances of 1800, 540 and 90 microgram/person/day, respectively.

The combined estimated daily *per capita* intake as flavourings of the 51 candidate substances assigned to structural class I is 42 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.

On the basis of the data available it is concluded that there is no indication that the flavouring substances in the present Flavouring Group Evaluation possess genotoxic potential. However, 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.

It can be anticipated that, at the estimated levels of intake as flavouring substances, 55 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 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 the 58 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 51 flavouring substances in structural class I range from 1600 to 5100 microgram/person/day. For 49 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 one substance from structural class III the mTAMDI is 4100, which is above the threshold of 90 microgram/person/day.

Thus, for 55 flavouring substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For one substance [FL-no: 06.135] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000. Two of the 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.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for 50 flavouring substances. For eight substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168] information on composition of mixture and/or stereoisomerism is missing. For two substances [FL-no: 06.088 and 06.095] identity tests are missing. Thus, the final evaluation of the materials of commerce cannot be performed for these eight substances pending further information.

For the remaining 50 flavouring substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.090, 06.097, 06.102, 07.169, 08.053, 08.082, 08.090, 08.103, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 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, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058 and 10.068] the Panel concluded that they would present no safety concern at their 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.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 2006/252/EC (EC, 2006). 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, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The Flavouring Group Evaluation (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 positive 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, 1996).

HISTORY OF THE EVALUATION

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		58

The present revision of FGE.10, FGE.10Rev1, includes 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] has been made available since the first FGE. Furthermore, substance [FL-no: 10.043] which can be metabolised to an alpha,beta-unsaturated ketone has been withdrawn from this FGE.10Rev1 to be evaluated together with other alpha,beta-unsaturated ketones in FGE.217 (EFSA, 2008b).

TERMS OF REFERENCE

The European Food Safety Authority EFSA is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 10 revision 1

1.1. Description

The present Flavouring Group Evaluation 10, Revision 1 (FGE.10Rev1), 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), deals with 58 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 58 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 Manufacturers Association- (FEMA-) numbers, structure and specifications. A summary of their evaluation status is given in Table 2a.

Twelve 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, 10.068 and 10.168]; thirty-one 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 and 09.916]; 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 beta-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 three candidate substances are aliphatic dicarboxylic acids [FL-no: 08.082, 08.053 and 08.103].

The 58 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.

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

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-five 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 - 10.040, 10.045, 10.048, 10.049, 10.052, 10.058, 10.068 and 10.168]. In six of these cases [FL-no: 06.088, 06.095, 06.135, 09.916, 10.040 and 10.168], the stereoisomeric composition has not been specified.

Due to the presence and the position of a double bond, six of the 58 substances can exist as geometrical isomers [FL-no: 09.350, 09.351, 09.565, 10.038, 10.039 and 10.040]. For three of the substances [FL-no: 09.565, 10.038 and 10.040] the stereoisomeric composition / composition of mixture has not been specified.

1.3. Natural Occurrence in Food

Forty-eight of the 58 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), juice, butter, meat, cheese, 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. These reports include:

- Octane-1,3-diol [FL-no: 02.198]: up to 21 mg/kg in apple and up to 95.1 mg/kg in apple juice.
- Hexadecano-1,5-lactone [FL-no: 10.049]: up to 10.6 mg/kg in butter and up to 1.3 mg/kg in heated lamb and mutton fat.
- Hexadecano-1,4-lactone [FL-no: 10.048]: up to 16.7 mg/kg in heated butter.
- 2-Butoxyethan-1-ol [FL-no: 02.242]: 0.02 mg/kg in mozzarella cheese.
- Hexadecano-1,16-lactone [FL-no: 10.047]: 0.0145 mg/kg in skimmed milk powder.
- Heptano-1,5-lactone [FL-no: 10.045]: up to 0.4 mg/kg in green tea.
- 1-Hydroxypropan-2-one [FL-no: 07.169]: up to 4 mg/kg in coffee.
- 2-Ethyl-4-methyl-1,3-dioxolane [FL-no: 06.088]: up to 2 mg/kg in port wine.
- 4-methyl-2-propyl-1,3-dioxolane [FL-no: 06.095]: up to 2 mg/kg in port wine.
- 1,1,3-Triethoxypropane [FL-no: 06.097]: up to 3 mg/kg in pear brandy and less than 0.8 mg/kg in wisky.
- 2-Isobutyl-4-methyl-1,3-dioxolane [FL-no: 06.135]: up to 2 mg/kg in port wine.
- Nonanedioic acid [FL-no: 08.103]: up to1.5 mg/kg in beer.
- Propyl lactate [FL-no: 09.815]: trace amount in white wine.
- Isobutyl lactate [FL-no: 09.590]: 20 mg/kg in port wine.

- Ethyl 3-hydroxyoctanoate [FL-no: 09.916]: up to 0.05 mg/kg in papaya, 0.02 mg/kg in orange juice and 0.03 mg/kg in grapefruit juice.

Accoring to TNO 10 of the substances have not been reported in any food items. These 10 substances are: 2-hexyl-5-hydroxy-1,3-dioxane [FL-no: 06.102], methyl lactate [FL-no: 09.644], ethyl butyryl lactate [FL-no: 09.502], pentadecano-1,14-lactone [FL-no: 10.068], ethyl 2-acetylbutyrate [FL-no: 09.824], iso-propyl 4-oxopentanoate [FL-no: 09.833], ethyl 3-acetohexanoate [FL-no: 09.832], methyl 5-hydroxydecanoate [FL-no: 09.633], di(2-methylbutyl) malate [FL-no: 09.874] and dec-8-eno-1,5-lactone [FL-no: 10.040] (TNO, 2000).

2. Specifications

Purity criteria for the 58 substances have been provided by the Flavour Industry (EFFA, 2003c) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for 56 of the 58 substances, for two substances [FL-no: 06.088 and 06.095] identity tests are missing.

Furthermore, information on geometrical stereoisomerism/chirality is needed for eight of the candidate substances (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, 1999).

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, 1999).

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 in 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 Maximised Survey-derived Daily Intake (MSDI) (SCF, 1999) 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[2] (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, 1999).

The total annual volumes of production of the 58 candidate substances from use as flavouring substances in Europe has been reported to be approximately 325 kg (EFFA, 2000c; EFFA, 2003d). For the 75 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 58 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a).

Nearly 90 % of the total annual volumes of production for the candidate substances is accounted for by five of these flavourings: sec-butyl lactate [FL-no: 09.333], diethyl maleate [FL-no: 09.351], ethyl 2-acetoxypropionate [FL-no: 09.360], isobutyl lactate [FL-no: 09.590] and isopentyl lactate [FL-no: 09.601].

The estimated daily *per capita* intake of sec-butyl lactate from use as a flavouring substance is 3.7 microgram, that of diethyl maleate is 12 microgram, that of ethyl 2-acetoxypropionate is 4.9 microgram, that of isobutyl lactate is 3.7 microgram, and that of isopentyl lactate is 7.2 microgram. The daily *per capita* intakes for each of the remaining substances are less than 1.2 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 57 of the 58 candidate substances information on food categories and normal and maximum use levels[3,4,5] were submitted by the Flavour Industry (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s;

2 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.
3 "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i)
4 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).
5 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).

EFFA, 2004ag; EFFA, 2007a; Flavour Industry 2006a). For one substance [FL-no: 06.135] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000.

The 57 candidate substances 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.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All except [FL-no: 06.135]
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 06.135]
Category 3	Edible ices, including sherbet and sorbet	All except [FL-no: 06.135]
Category 4.1	Processed fruits	All except [FL-no: 06.135]
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All except [FL-no: 06.135]
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 06.135]
Category 7	Bakery wares	All except [FL-no: 06.135]
Category 8	Meat and meat products, including poultry and game	All except [FL-no: 06.135]
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 06.135, 08.090]
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 06.095, 06.135, 09.644]
Category 13	Foodstuffs intended for particular nutritional uses	All except [FL-no: 06.095, 06.135, 09.644]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 06.135]
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 06.135]
Category 15	Ready-to-eat savouries	All except [FL-no: 06.135]
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 - 15	All except [FL-no: 06.135]

According to the Flavour Industry the normal use levels for the 58 candidate substances are in the range of 1 – 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a). Information on use levels has not been provided for [FL-no: 06.135].

The mTAMDI values for the 57 candidate substances, for which use levels have been reported, from structural class I range from 1600 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 candidate substance from structural class III the mTAMDI is 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

A more detailed description of the metabolism of the candidate substances in this FGE is given in Annex III.

In general, lactones are formed by acid-catalysed intramolecular cyclisation of gamma- or delta-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.

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).

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto 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 & 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 innocous 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.

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 [Dick el al., 1988; Ashley et al., 1994; 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 structually 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; 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.

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.

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 this 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 this FGE.10, Revision 1.

For the safety evaluation of the 58 candidate substances from chemical groups 9, 13 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 58 substances are summarised in Table 2.

Step 1

Fifty-one 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; 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. Therefore, these substances are evaluated via the B-side of the Procedure. The evaluation of the remaining 55 candidate substances proceeds via the A-side of the Procedure.

Step A3

Step A3 applies to 50 candidate substances from structural class I [FL-no: 02.132, 02.198, 05.149, 07.169, 08.053, 08.082, 08.090, 08.103, 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, 10.038, 10.039, 10.040, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 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 50 candidate substances, which have been assigned to structural class I, have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 12 microgram. The four candidate substances from structural class II have MSDIs ranging from 0.0061 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 55 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 JECFA has established an acceptable daily intake (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 $25x10^6$ -fold below the ADI value. 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/day) the margin of safety would be $5x10^8$, 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 structural similarities to 2-butoxyethan-1-ol, a NOAEL could not be established in subchronic/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 for chronic exposure of 0.5 mg/kg body weight (bw)/day (EPA, 1999).

In the EU-RAR (2007), a Human equivalent LOAEL of 9.5 mg/kg bw/d 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-triethoxypropane [FL no: 06.097] an MSDI of 0.0012 microgram/*capita*/day can be calculated (see Table 6.1). The Reference dose 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 58 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 51 candidate substances in structural class I range from 1600 to 5100 microgram/person/day. For 49 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 one substance from structural class III the mTAMDI is 4100, which is above the threshold of 90 microgram/person/day.

Thus, for 55 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For one substance [FL-no: 06.135] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000 (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

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

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)
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
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
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
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
10.068	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

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)
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

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 Flavouring Group Evaluation 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 is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d), the combined estimated daily *per capita* intake as flavourings of the 51 candidate flavouring substances assigned to structural class I is 42 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d), the combined estimated daily *per capita* intake as flavourings of the six candidate flavouring 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 12 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 5 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 39 candidate substances are structurally related to 32 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 38

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 15 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 >5000 mg/kg 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/day and approximately 30 g/kg bw/day are reported (Annex IV, Table IV.1).

Forty-eight (out of 81) 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 are reported (Annex IV, Table IV.1).

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute/subchronic/chronic toxicity data are available for five candidate substances, 2-butoxyethanol [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 25 supporting substances of the present Flavouring Group Evaluation (JECFA, 1998a; JECFA, 2000c) (Annex IV, Table IV.2).

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 inhalation, oral or dermal exposure to 2-butoxyethan-1-ol; in which the haematotoxic action is produced by the metabolite butoxyacetic acid. 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). LOAELs of 69 and 82 mg/kg bw/day (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 subpopulations, 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, 31, 62.5 and 125 ppm and B6C3F1 mice were exposed to 0, 62.5, 125 and 250 ppm 2-butoxyethan-1-ol; (NTP, 1993a). 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[6] [FL-no: 05.149] (50, 250, 1000 ppm 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 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 (Annex IV, Table IV.5).

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) (Annex IV, Table IV.3).

For 2-butoxyethan-1-ol [FL-no: 02.242] no effects on fertility were observed 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 & Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Offspring of mothers using >1000 mg 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/day. Thus, 600 mg/day is considered as NOAEL for the teratogenic effects of valproic acid in humans.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 11 of the 58 candidate substances. These 11 substances are pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168],

[6] *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.*

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] and glutaric acid [FL-no: 08.082]. There were genotoxicity data on 26 supporting substances (Annex IV, Table IV.4 and IV.5).

In vitro

Pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168] and methyl acetoacetate [FL-no:09.634] were reported to be negative in microbial mutagenicity assays.

1-Hydroxypropan-2-one [FL-no: 07.169] was positive in Ames tests using strains TA100 and TA104 in the presence and absence of S9 metabolic activation (Yamaguchi, 1982; ; Garst et al., 1983; Yamaguchi & Nakagawa, 1983; Marnett et al., 1985a). 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 other *in vitro* endpoints or 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 & 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 & 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 28 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, 5). Results of *in vivo* bone marrow micronucleus assays 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) 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 this 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.

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.

9. Conclusions

The 58 candidate substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-five of the 58 candidate substances possess one or more chiral centres and six can exist as geometrical isomers due to the presence and the position of a double bond. For eight of these substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168] the stereoisomeric composition/composition of mixture has not been specified.

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

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

According to the default MSDI approach, the 58 flavouring substances in this group to which the Procedure has been applied have intakes in Europe from 0.0012 to 12 microgram*/capita/*day, which are below the thresholds of concern value for structural class I, II and III substances of 1800, 540 and 90 microgram/person/day, respectively.

The combined estimated daily *per capita* intake as flavourings of the 51 candidate substances assigned to structural class I is 42 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 12 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 5 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 39 candidate substances are structurally related to 32 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 38 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

On the basis of the data available it is concluded that there is no indication that the candidate substances in the present Flavouring Group Evaluation possess genotoxic potential. However, 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.

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 structually 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; 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 the 58 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 51 candidate substances in structural class I range from 1600 to 5100 microgram/person/day. For 49 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 one substance from structural class III the mTAMDI is 4100, which is above the threshold of 90 microgram/person/day.

Thus, for 55 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For one substance [FL-no: 06.135] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000. The two candidate substances [FL-no: 05.149 and 07.169], which have mTAMDI intake estimates below the threshold of concern for structural class I, are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 58 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for 50 flavouring substances. For eight substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168] information on composition of mixture and/or stereoisomerism is missing. For two substances [FL-no: 06.088 and 06.095] an identity test is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for eight substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168] pending further information.

For the remaining 50 candidate substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.090, 06.097, 06.102, 07.169, 08.053, 08.082, 08.090, 08.103, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 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, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058 and 10.068] the Panel concluded that they would present no safety concern at the level of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 10 REVISION 1

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

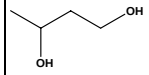
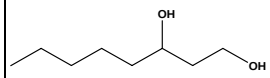
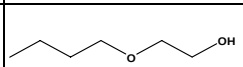
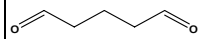
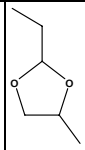
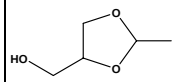
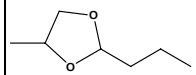
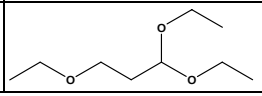
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
02.132	Butane-1,3-diol		107-88-0	Liquid $C_4H_{10}O_2$ 90.12	Soluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	188 MS 95 %	1.430-1.436 1.005-1.011	
06.088	2-Ethyl-4-methyl-1,3-dioxolane 6)		4359-46-0	Liquid $C_6H_{12}O_2$ 116.16	Soluble 1 ml in 1 ml	116 95 %	1.402-1.408 0.916-0.922	ID 7).
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		3674-21-3	Liquid $C_5H_{10}O_3$ 118.13	Practically insoluble or insoluble 1 ml in 1 ml	187 MS 95 %	1.440-1.446 1.120-1.126	Racemate. CASrn in Register to be changed to 3773-93-1 (EFFA). CASrn in Register refers to the (2R, 4S) enantiomer.
06.095	4-Methyl-2-propyl-1,3-dioxolane 6)		4352-99-2	Liquid $C_7H_{14}O_2$ 130.19	Soluble 1 ml in 1 ml	143 95 %	1.409-1.415 0.907-0.913	ID 7).
06.097	1,1,3-Triethoxypropane		10075 7789-92-6	Liquid $C_9H_{20}O_3$ 176.26	Practically insoluble or insoluble 1 ml in 1 ml	185 MS 95 %	1.403-1.409 0.890-0.896	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

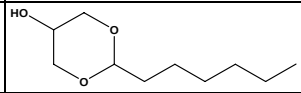
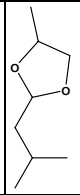
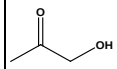
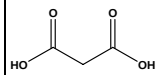
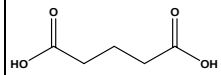
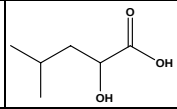
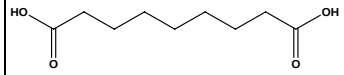
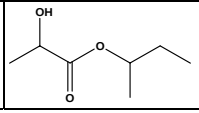
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		2016 1708-36-7	Solid $C_{10}H_{20}O_3$ 188.22	Practically insoluble or insoluble 1 ml in 1 ml	255 44 MS 95 %	n.a. n.a.	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane 6)		18433-93-7	Liquid $C_8H_{16}O_2$ 144.21		150 MS 96 %	n.a. 0.895	
07.169	1-Hydroxypropan-2-one		11101 116-09-6	Liquid $C_3H_6O_2$ 74.08	Soluble 1 ml in 1 ml	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 1 ml in 1 ml	264 135 MS 95 %	n.a. n.a.	
08.082	Glutaric acid		110-94-1	Solid $C_5H_8O_4$ 132.12	Soluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	225 (13 hPa) 107 MS 95 %	n.a. n.a.	
09.333	sec-Butyl lactate		18449-60-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble 1 ml in 1 ml	172 MS 95 %	1.414-1.420 0.970-0.976	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

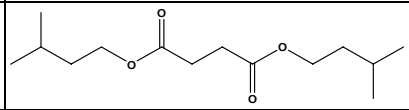
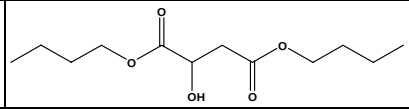
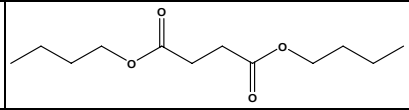
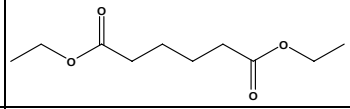
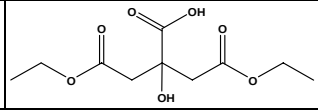
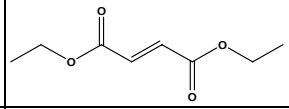
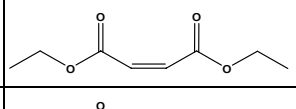
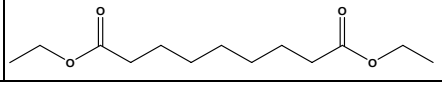
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.345	Di-isopentyl succinate		10555 818-04-2	Liquid $C_{14}H_{26}O_4$ 258.36	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	170 (16 hPa) 82 MS 95 %	n.a. n.a.	CASrn in Register to be changed to 6280-99-5 (racemate).
09.347	Dibutyl succinate		141-03-7	Liquid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	290 NMR 95 %	1.432-1.438 0.970-0.976	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

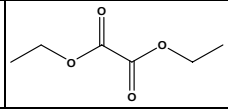
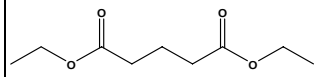
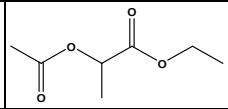
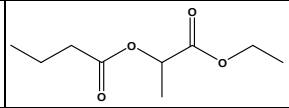
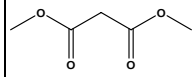
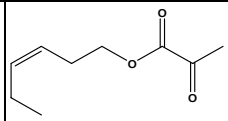
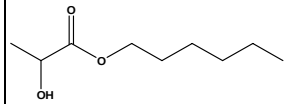
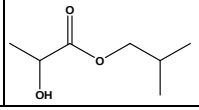
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.353	Diethyl oxalate		95-92-1	Liquid $C_6H_{10}O_4$ 146.14	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	181 MS 95 %	1.411-1.417 1.150-1.156	
09.565 1846	Hex-3-enyl 2-oxopropionate 6)		3934 10684 68133-76-6	Liquid $C_9H_{14}O_3$ 170.21	Practically insoluble or insoluble 1 ml in 1 ml	76 (0.7 hPa) IR NMR 98 %	1.437-1.445 0.982-0.990	
09.580	Hexyl lactate		20279-51-0	Liquid $C_9H_{18}O_3$ 174.24	Slightly soluble 1 ml in 1 ml	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 1 ml in 1 ml	182 MS 95 %	1.415-1.421 0.968-0.974	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

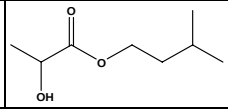
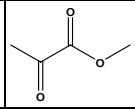
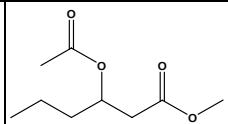
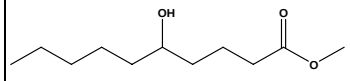
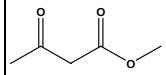
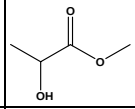
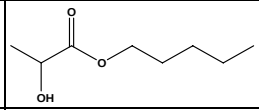
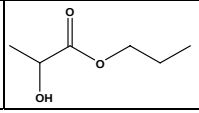
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.601	Isopentyl lactate		10720 19329-89-6	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	206 MS 95 %	1.423-1.429 0.965-0.971	Racemate.
09.815	Propyl lactate		616-09-1	Liquid $C_6H_{12}O_3$ 132.16	Sparingly soluble 1 ml in 1 ml	170 MS 95 %	1.414-1.420 1.000-1.006	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

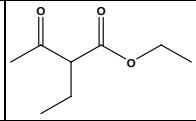
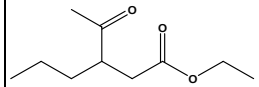
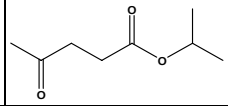
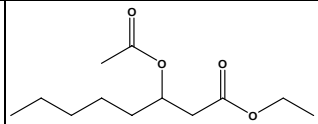
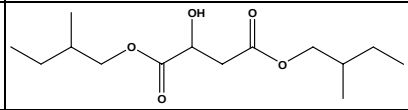
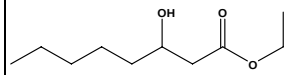
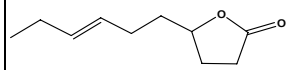
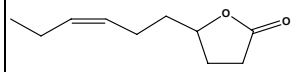
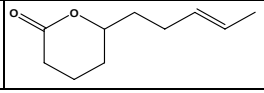
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.824	Ethyl 2-acetylbutyrate		607-97-6	Liquid $C_8H_{14}O_3$ 158.20	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	335 74 NMR 95 %	n.a. n.a.	Racemate. CASrn to be introduced 253596-99-5.
09.916	Ethyl 3-hydroxyoctanoate 6)		10603 7367-90-0	Liquid $C_{10}H_{20}O_3$ 188.27	Practically insoluble or insoluble 1 ml in 1 ml	118 (12 hPa) MS 95 %	1.421-1.427 0.973-0.979	
10.038	Dec-7-eno-1,4-lactone		67114-38-9	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble 1 ml in 1 ml	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate, Configuration of double bond not 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 1 ml in 1 ml	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate.
10.040	Dec-8-eno-1,5-lactone 6)		32764-98-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble 1 ml in 1 ml	157 (15 hPa) MS 95 %	1.462-1.468 0.972-0.978	Configuration of double bond not specified.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 1

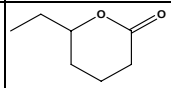
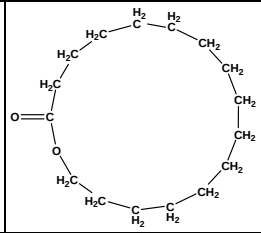
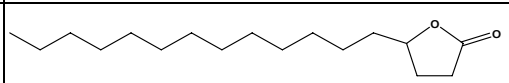
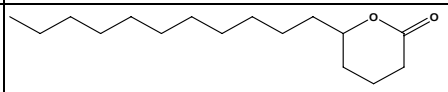
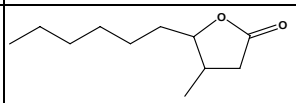
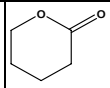
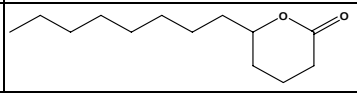
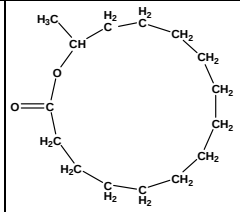
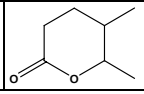
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
10.045	Heptano-1,5-lactone		10660 3301-90-4	Liquid $C_7H_{12}O_2$ 128.17	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1

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
10.068	Pentadecano-1,14-lactone		32539-85-8	Liquid $C_{15}H_{28}O_2$ 240.38	Practically insoluble or insoluble 1 ml in 1 ml	108 (0.1 hPa) MS 95 %	1.466-1.472 0.942-0.948	Racemate.
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one 6)		4141 10413-18-0	Liquid $C_7H_{12}O_2$ 128.17	Slightly soluble 1 ml in 1 ml	60 MS 98 %	1.452-1.458 1.019-1.025	

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)

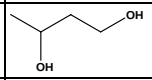
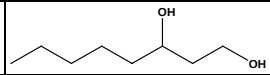
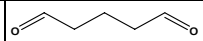
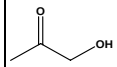
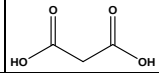
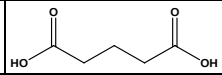
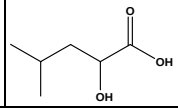
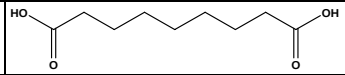
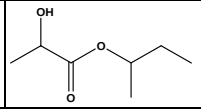
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	
02.198	Octane-1,3-diol		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)	
09.333	sec-Butyl lactate		3.7	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)

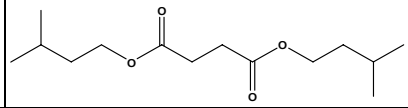
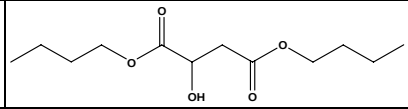
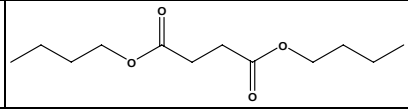
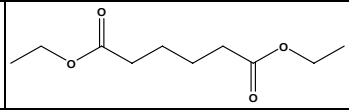
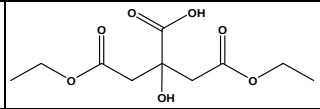
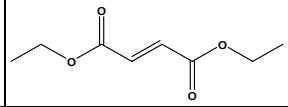
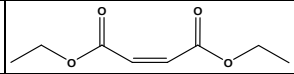
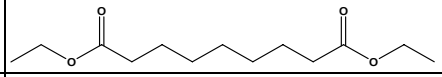
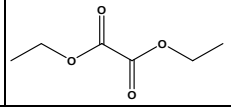
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.345	Di-isopentyl succinate		0.037	Class I A3: Intake below threshold	4)	6)	
09.346	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)	
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)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

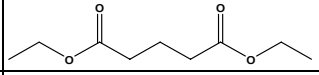
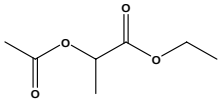
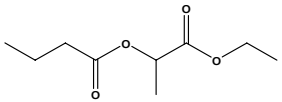
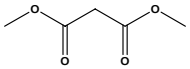
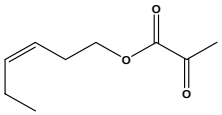
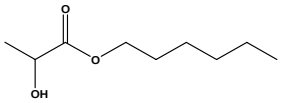
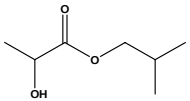
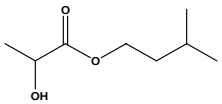
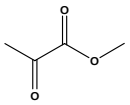
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.354	Diethyl pentanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.360	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)	7)	
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)

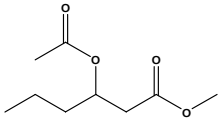
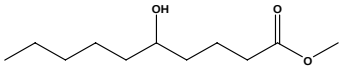
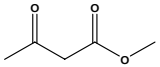
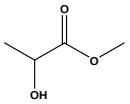
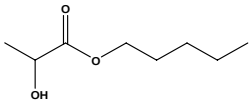
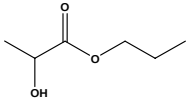
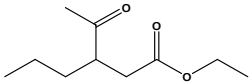
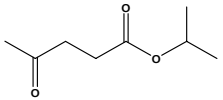
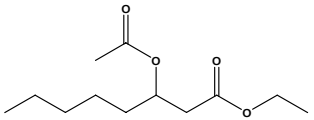
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
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)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

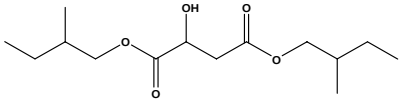
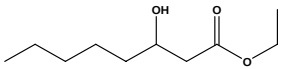
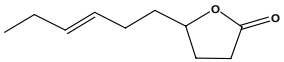
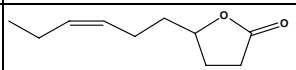
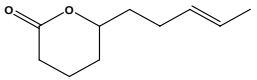
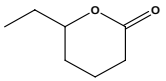
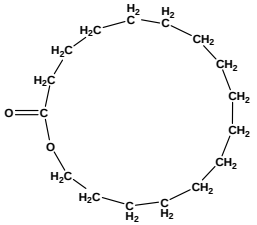
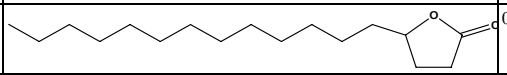
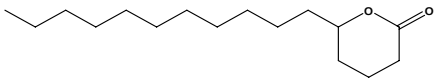
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.874	Di(2-methylbutyl) malate		0.015	Class I A3: Intake below threshold	4)	6)	
09.916	Ethyl 3-hydroxyoctanoate		0.011	Class I A3: Intake below threshold	4)	7)	
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)	
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)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

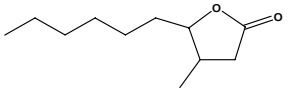
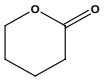
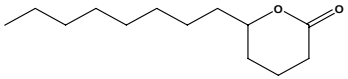
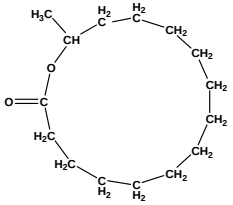
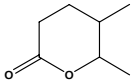
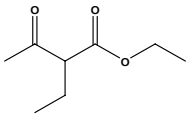
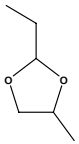
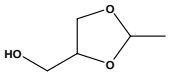
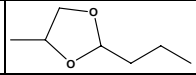
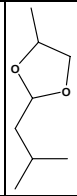
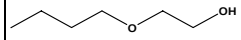
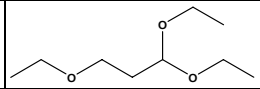
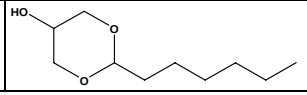
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.052	3-Methylnonano-1,4-lactone		0.61	Class I A3: Intake below threshold	4)	6)	
10.055	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)	
10.068	Pentadecano-1,14-lactone		0.9	Class I A3: Intake below threshold	4)	6)	
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		1.2	Class I A3: Intake below threshold	4)	7)	
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)	7)	
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		0.012	Class II 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
06.095	4-Methyl-2-propyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	7)	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		1.2	Class II A3: Intake below threshold	4)	7)	
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)	

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, Class II = 540, 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.*

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

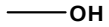
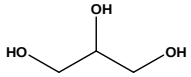
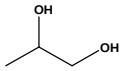
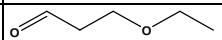
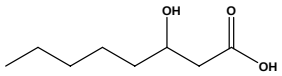
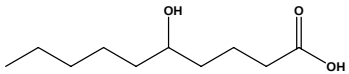
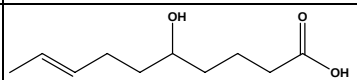
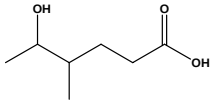
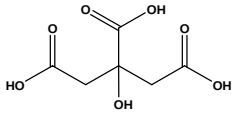
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of "flavouring agent"		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

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

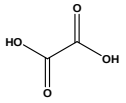
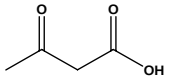
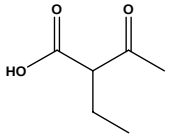
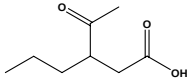
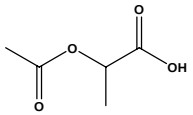
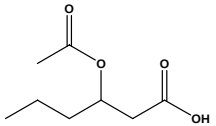
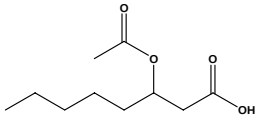
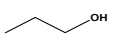
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Oxalic acid		Not evaluated as flavouring substance		Not in EU-Register
	Acetoacetic acid		Not evaluated as flavouring substance		Not in EU-Register
	2-Acetylbutyric 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
02.002	Propan-1-ol 82		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

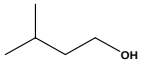
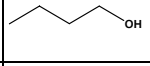
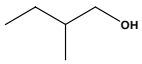
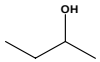
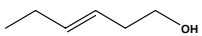
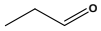
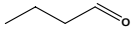
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.076	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 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 below threshold	
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	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

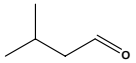
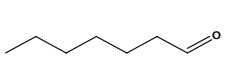
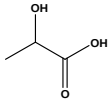
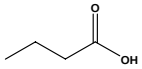
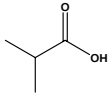
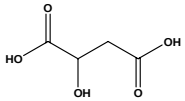
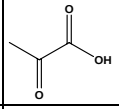
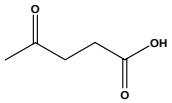
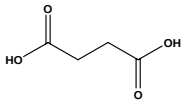
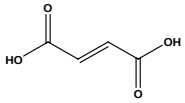
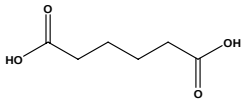
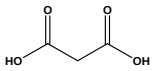
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
05.006	3-Methylbutanal 258		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.031	Heptanal 95		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.004	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	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
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	

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
08.025	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	

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, Class II = 540, 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

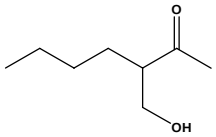
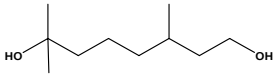
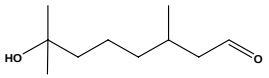
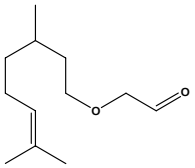
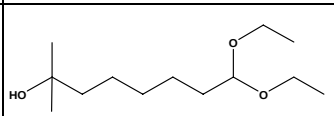
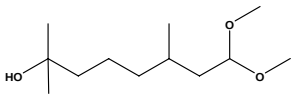
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2001c)	7	No safety concern d) Category B	Not 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.

Table 3: Supporting Substances Summary

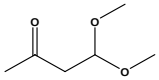
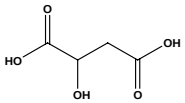
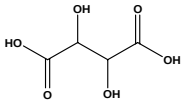
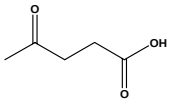
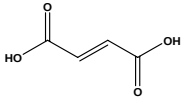
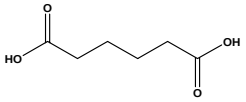
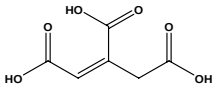
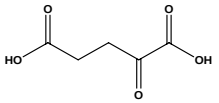
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
06.038	4,4-Dimethoxybutan-2-one		3381 10029 5436-21-5	593 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	
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 (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)	

Table 3: Supporting Substances Summary

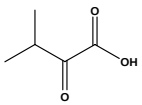
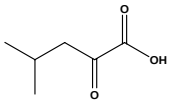
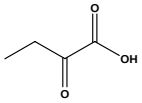
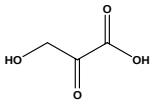
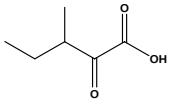
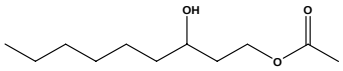
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.051	3-Methyl-2-oxobutyric acid		3869 2262 759-05-7	631 JECFA specification (JECFA, 1999c)	0.012	No safety concern a) 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)	
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: Substances 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).

Table 3: Supporting Substances Summary

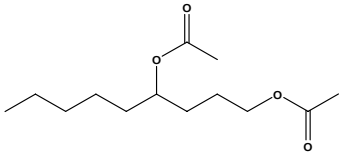
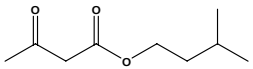
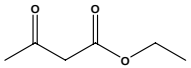
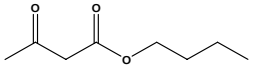
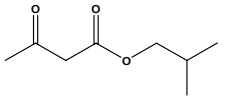
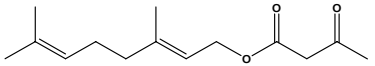
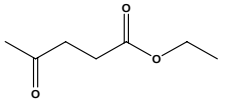
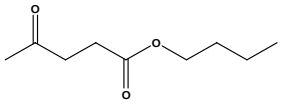
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.280	Nonane-1,4-diyl diacetate		3579 11927 67715-81-5	609 JECFA specification (JECFA, 2002d)	0.037	No safety concern a)	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)	
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)	

Table 3: Supporting Substances Summary

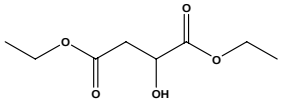
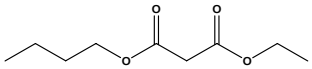
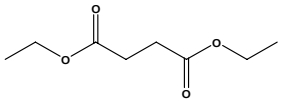
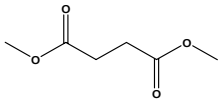
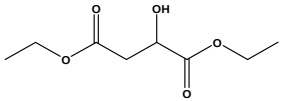
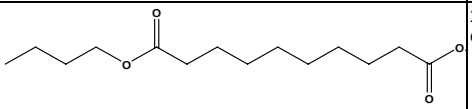
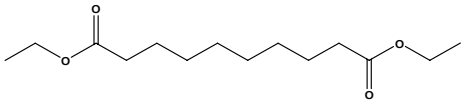
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.439	Diethyl malate		2374 382 7554-12-3	620 JECFA specification (JECFA, 2000d)	3.7	No safety concern a) Deleted b)	JECFA evaluated diethyl malate.CASrn in Register refers to the racemate Deleted:Substances 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)	
09.475	Diethyl sebacate		2376 623 110-40-7	624 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

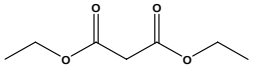
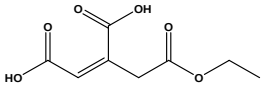
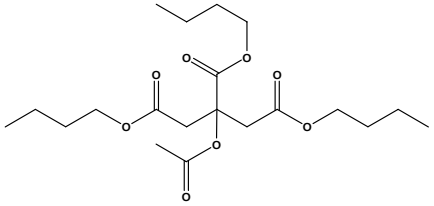
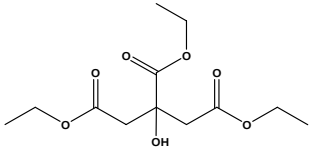
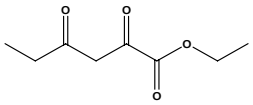
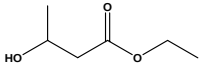
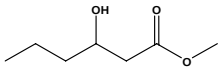
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.490	Diethyl malonate		2375 2106 105-53-3	614 JECFA specification (JECFA, 2002d)	650	No safety concern a) Category A b)	
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.

Table 3: Supporting Substances Summary

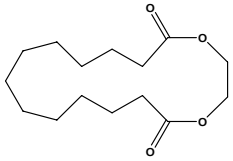
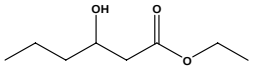
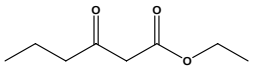
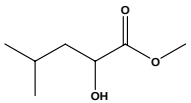
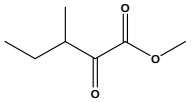
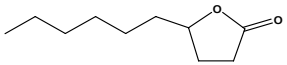
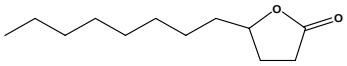
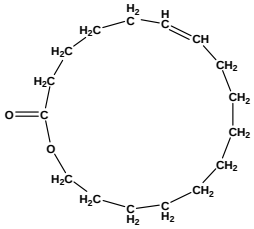
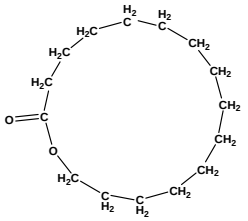
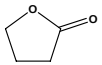
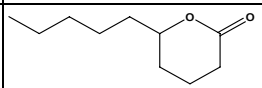
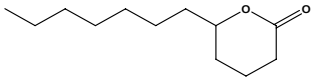
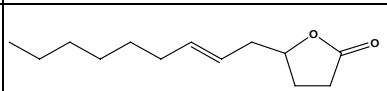
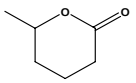
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.533	Ethyl brassylate		3543 10571 105-95-3	626 JECFA specification (JECFA, 2002d)	3.0	No safety concern a)	
09.535	Ethyl 3-hydroxyhexanoate		3545 11764 2305-25-1	601 JECFA specification (JECFA, 2002d)	60	No safety concern a)	JECFA evaluated ethyl 3-hydroxyhexanoate (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).

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
10.003	Hexadec-6-eno-1,16-lactone		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.
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.

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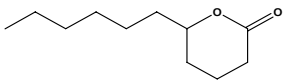
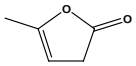
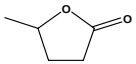
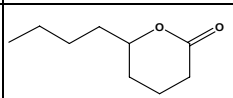
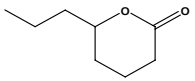
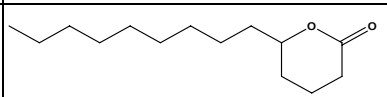
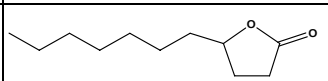
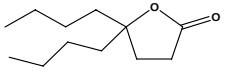
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.011	Undecano-1,5-lactone		3294 688 710-04-3	234 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category 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.
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).

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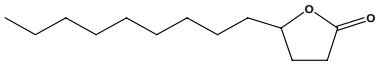
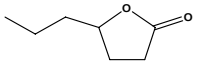
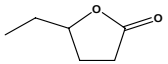
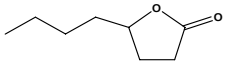
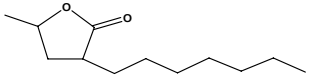
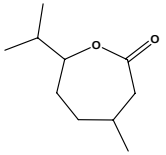
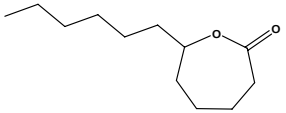
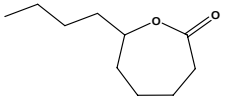
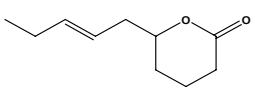
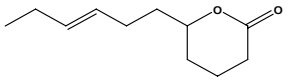
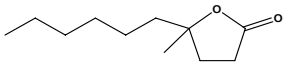
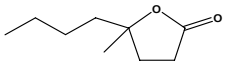
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.019	Dodecano-1,4-lactone		2400 2240 2305-05-7	235 JECFA specification (JECFA, 1998b)	190	No safety concern c) Category 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 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.

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
10.033	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 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).

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, 1999), 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 estimated 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[7] (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[8] (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.

7 "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).
8 "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).

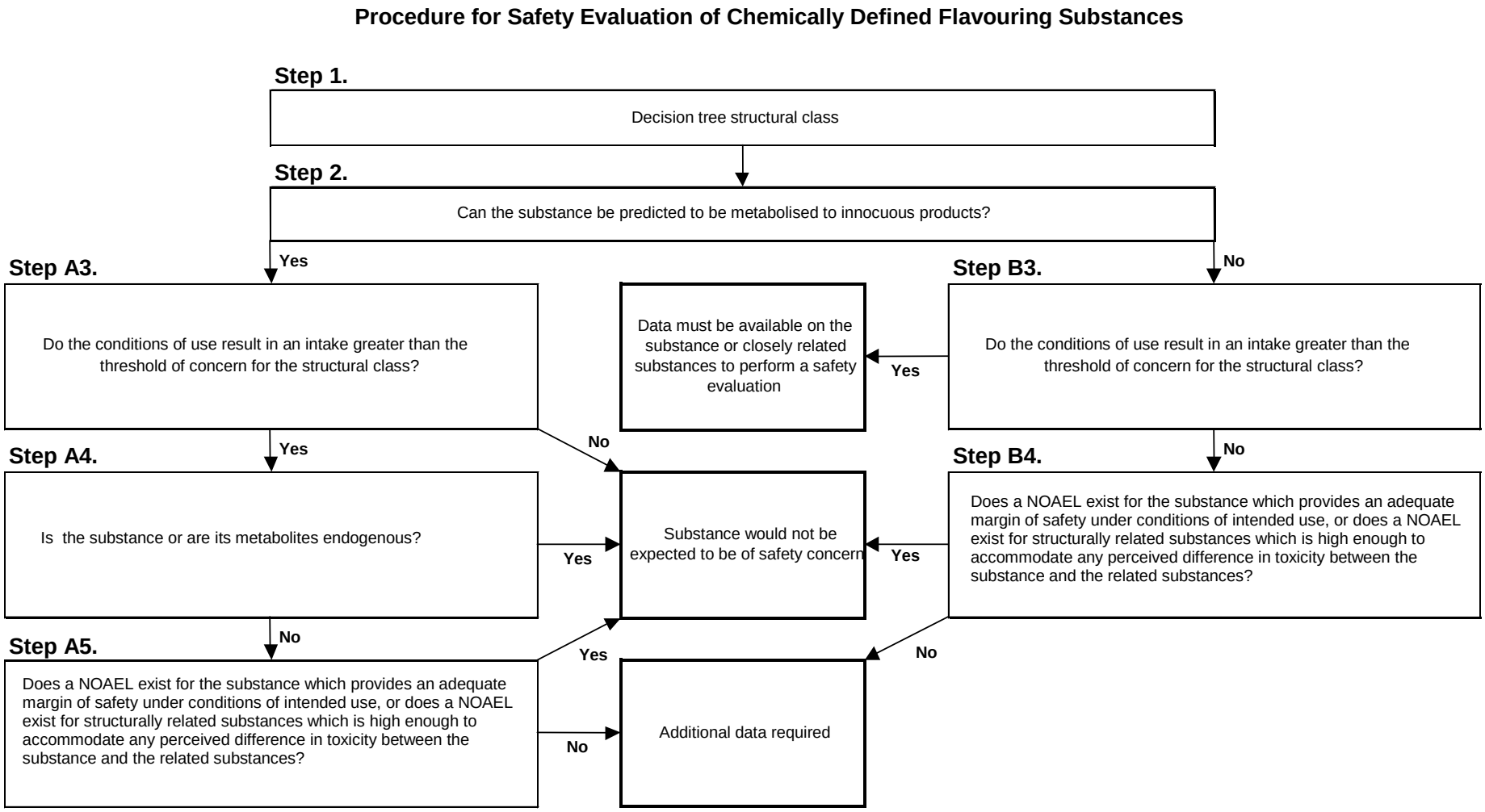


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 Industy for 57 of the 58 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).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.10

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 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.198	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.242	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
05.149	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
06.088	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
06.090	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
06.095	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	- -	- -	5 25	10 50	20 100	5 25
06.097	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
06.102	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	52 5	10 50	3 15	10 50	15 75	5 25
07.169	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
08.053	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.10

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**
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.082	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
08.090	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	- -	- -	- -	5 25	10 50	5 25	10 50	15 75	5 25
08.103	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
09.333	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.345	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.346	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.347	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.348	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.349	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.350	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.351	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.352	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.353	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.354	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.360	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.502	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.558	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.565	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.580	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 200	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.590	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.601	10 50	5 75	10 50	7 35	- -	20 100	15 75	15 75	2 10	2 10	- -	- -	5 25	10 50	5 50	20 100	20 100	5 25
09.626	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.629	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.633	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.634	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.644	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	- -	- -	5 25	10 50	10 50	5 25
09.683	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.815	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.824	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.832	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.833	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.862	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.874	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.916	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.038	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.039	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.040	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.10

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	25	50	100	25
10.045	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.047	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.048	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.049	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.052	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.055	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.058	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.068	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
10.168	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25

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
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 present 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 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	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	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	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
15	Ready-to-eat savouries			Exception b
16	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 57 flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a). The mTAMDI values are only given for highest reported normal use.

Table II.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
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

Table II.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)
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
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
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
10.068	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

ANNEX III: METABOLISM

Absorption, Distribution, Metabolism and Elimination

III.1. Aliphatic Lactones

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 gamma- or delta-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 favored (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 & Ballotti, 1970).

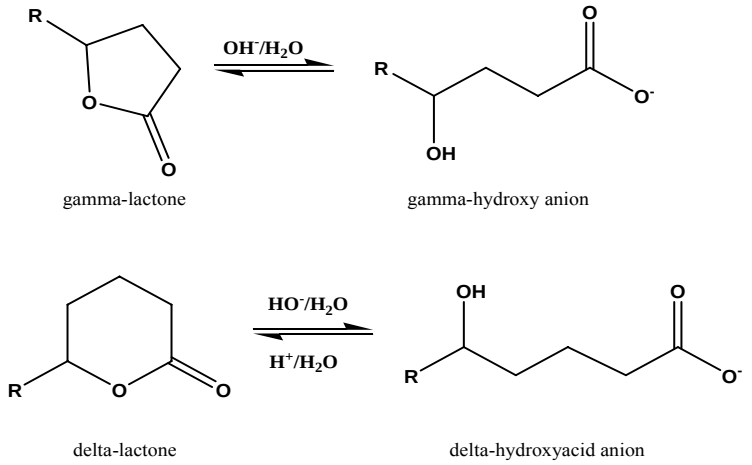


Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion.*

III.1.2. Hydrolysis of Aliphatic Lactones

Ten candidate substances [FL-no: 10.038, 10.039, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058 and 10.068] 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 central nervous system depression, attributed to its alcaline 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 & Giarman, 1966), intraperitoneally or orally (Guidotti & 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 & Giarman, 1966; Guidotti & 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 & 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 & 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 (Guidotti & Ballotti, 1970; Lettieri & Fung, 1978; Arena & Fung, 1980). 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 & 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 & 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 & 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 & Giarman, 1965). Oxidation of gamma-butyrolactone to succinate by alcohol dehydrogenase and succinic semialdehyde dehydrogenase occurs primarily in the liver (Jakoby & Scott, 1959); succinate then participates in the citric acid cycle (Walkenstein et al., 1964; Möhler et al., 1976; Lee, 1977; Doherty & Roth, 1978). 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 been previously 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 & Voet, 1990).

The principal metabolic pathways utilised 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 (Williams, 1959; Voet & Voet, 1990) 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 sulfate 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, 1959).

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, 1959). Larger alkyl substituents (>C2) located at the alpha- or beta-position inhibit metabolism to CO_2 (Deisinger et al., 1994; Deuel, 1957; Albro, 1975), 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

Twenty-nine candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 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 and 09.874]. They are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto 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 (Heymann, 1980; Anders, 1989). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals, these enzymes occur in most tissues throughout the body (Heymann, 1980; Anders, 1989) 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 & 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 not 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 & 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. Greater than 87 % of the administered radioactivity was eliminated within 24 hours after dosing. At least nine urinary metabolites (59–70 %) were detected. Five 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

Two candidate substances [FL-no: 06.090 and 06.097] 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 [FL-no: 06.100] was >99 % hydrolysed *in vitro* to the terpenoid hydroxycitronellal and ethanol 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 biotransformation and excreted.

III.2.3. Alpha-hydroxy- and Alpha-ketoacids 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, 09.580, 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 (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 & Voet, 1990).

III.2.4. Beta-keto- and Beta-hydroxyacids and Their Esters

One candidate substance [FL-no: 08.053] is a beta-ketoacids. In addition, five candidate substances [FL-no: 09.346, 09.558, 09.634, 09.824, 09.862 and 09.874] are precursor of acetoacetic acid or its beta-hydroxy or aldehyde precursor. The latter two 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 completely metabolised. At elevated endogenous levels, beta-ketoacids may undergo non-enzymatic decarboxylation, which, for acetoacetic acid, yields acetone and CO_2 (Voet & 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 & Li, 1980) and excreted as the glucuronic acid conjugate (Williams, 1959).

Products of partial beta-oxidation or glucuronic acid conjugation have been also 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, 08.082, 08.053, 08.103, 09.345, 09.346, 09.347, 09.348, 09.349, 09.350, 09.351, 09.352, 09.353, 09.354, 09.558 and 09.874] 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, 09.351 and 09.874]) and citric acid (from [FL-no: 09.349]) are components of the tricarboxylic acid cycle (Voet & 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 & 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 & 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 resulted 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 (Feldman & Weiner, 1972; Bosron & Li, 1980; Levi & Hodgson, 1989). The resulting carboxylic acids would be metabolised in the fatty acid pathway and tricarboxylic acid cycle (Voet & 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. efter acetone exposure, after fasting or in relation to certain diseases) the propan-1,2-diol route is the dominating pathway.

In the first step acetone is oxidised to 1-hydroxypropan-1,2-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.

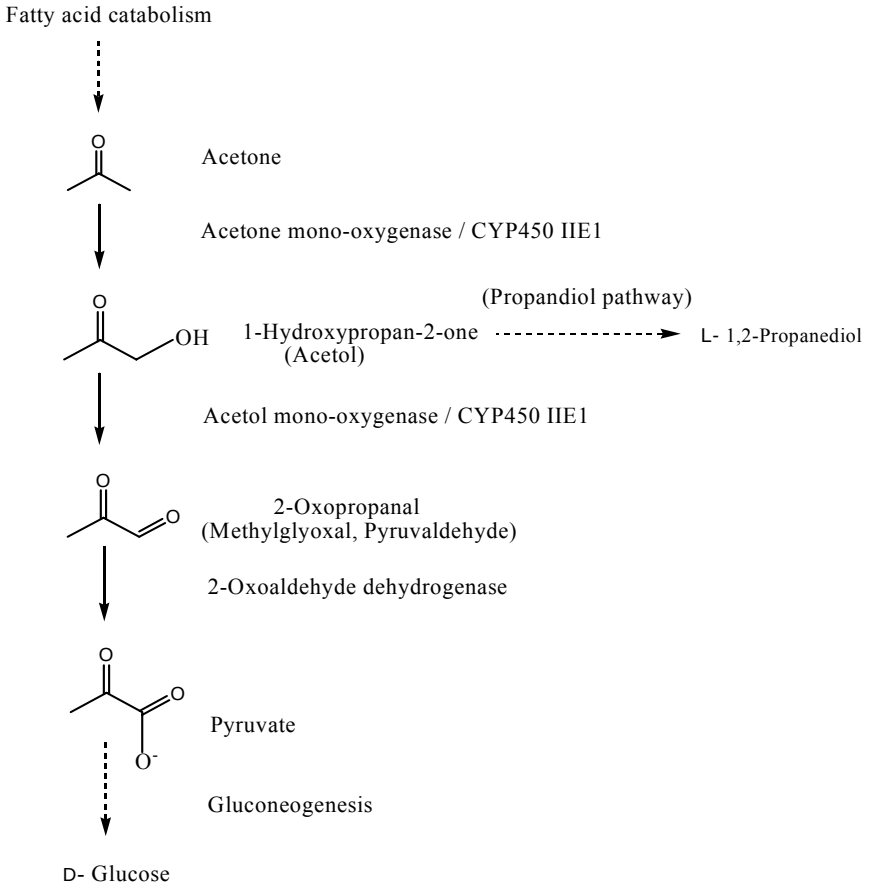


Figure III.2. *Acetone metabolism (methylglyoxal pathway).*

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 that indicate 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 (Ghanayem et al., 1987a; Ghanayem et al., 1987b; Ghanayem et al., 1987c; Medinsky et al., 1990; Corley et al., 1994).

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 2-3% 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 in the 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 (4-5 weeks old) and adult (9-13 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 silphate) (Ghanayem et al., 1987b).

2-Butoxyethan-1-ol [FL-no: 02.242] was administered to male F344/N rats (11-12 weeks old) at concentrations in drinking water of 290, 860 and 2590 ppm over a 24-hour period. Butoxyethanol was administered as 2-butoxy[U-^{14}C]ethanol, and exhaled air, urine and faeces were collected over a 72-hour 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 8-10 % 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 also were 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 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 the 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.

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 & 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 micromols/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 Petersen, 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 infusion over one hour. In the first hour after the cessation of the intravenous 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 gamma- or delta-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 then are 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 (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy- or keto-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 & 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 innocous 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.

Two candidate substances [FL-no: 06.090 and 06.097] 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 structually 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; 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 15 candidate substances of the present Flavouring Group Evaluation of 58 substances from chemical groups 9, 13 and 30 and for 48 supporting substances evaluated by JECFA at the 49th and 53rd meetings. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

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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)
Isobutyl lactate [09.590]	Rat	NR	Oral	> 2000	(Riebeek, 1989)
(butyro-1,4-lactone [10.006])	Mouse	NR	Gavage	1245	(Schafer & 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, 1972a)
	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, 1977d)
(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)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat	M, F	Gavage	1600 – 5000	(Piccirillo & Hartman, 1980a)
(Mixture of 5-Hydroxy-2-decenoic acid delta-lactone, 5-Hydroxy-2-dodecenoic acid delta-lactone, and 5-Hydroxy-2-tetradecenoic acid delta–lactone)	Rat	M, F	Gavage	3363	(Reagan & Becci, 1984a)
(Dodec-6-eno-1,4-lactone [10.009])	Rat	M, F	Oral	> 5000	(Watanabe & Morimoto, 1990a)
(Hexadec-6-eno-1,16-lactone [10.003])	Rat	NR	Oral	> 5000	(Wohl, 1974a)
(3,7-Dimethyloctano-1,6-lactone [10.027])	Rat	M, F	Gavage	> 5000	(Lewis & Palanker, 1979a)
(5-Hexyl-5-methyldihydrofuran-2(3H)-one [10.051])	Rat	NR	Oral	> 5000	(Moreno, 1976j)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Citronellyl oxyacetaldehyde [05.079])	Rat	NR	Oral	> 5000	(Moreno, 1973d)
1-Hydroxypropan-2-one [07.169]	Rat	NR	Oral	2200[2]	(Smyth & 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 & 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)
(Ethyl 2,4-dioxohexanoate [09.514])	Rat	M, F	Gavage	6450	(Wolven & Levenstein, 1969)
2-Butoxyethan-1-ol [02.242]	Rat	M	Gavage	1480	(Smyth et al., 1941)
	Rat	NR	Oral	1174	(BASF, 1956)
	Rat	NR	Oral	620	(Rowe & Wolf, 1982)
	Rat	M, F	Oral	2800	(Carpenter et al., 1956)
	Rat	M	Gavage	2680	(Myers & Homan, 1980)
	Rat	NR	Oral	470	(Wolf, 1959)
	Rat	M	Gavage	1190 – 2800	(Weil & 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 & 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 & Moldovan, 1973a)
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, 1976a)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
	Rat	NR	Oral	5331	(Merck Index, 1992)
(Diethyl malonate [09.490])	Rat	NR	Oral	14900	(Smyth et al., 1969a)
	Mouse	NR	Gavage	5400	(Wolven & 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)
Glutaraldehyde [05.149]	Rat	NR	Gavage	252	(Stonehill et al., 1963)
	Rat	M	Gavage	733[7]	(Ballantyne & Myers, 2001)
	Rat	M	Gavage	2380[8]	(Smyth et al., 1962)
	Rat	M	Gavage	540[9]	(Striegel & 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 & 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, 1953a)
	Mouse	NR	Gavage	> 32000	(Lawrence et al., 1974)
(Triethyl citrate [09.512])	Rat	NR	Gavage	7000[4]	(Finkelstein & Gold, 1959)
(Tributyl acetylcitrate [09.511])	Rat	NR	Gavage	> 30000[12]	(Finkelstein & Gold, 1959)
(3-Hydroxy-2-oxopropionic acid [08.086])	Rat	NR	Oral	2000	(Hoechst, 1995)

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 LD50 not reported.Value reported as approximate LD50.*

[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 toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 and for 23 supporting substances evaluated by JECFA at the 49th and 53rd meetings. No carcinogenicity data are available. The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No/Group	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)	a)
(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)	a)
(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)	a)
	Rat; M, F 1/20	Diet	2 years	50[2]	(Bär & Griepentrog, 1967)	a)
(Decano-1,4-lactone [10.017])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
(Decano-1,5-lactone [10.007])	Rat; M, F 1/NR	Diet	49 weeks	150[2]	(Fassett, 1961)	a)
	Dog; M, F 1/NR	Diet	38 weeks	250[2]	(Fassett, 1961)	a)
(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)	a)
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär & Griepentrog, 1967)	a)
(Undecano-1,4-lactone [10.002]) contimued	Rat; M, F NR[4]	Diet	90	14.1[2]	(Shillinger, 1950)	a)

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No/Group	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
(Dodecano-1,4-lactone [10.019])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
(Dodecano-1,5-lactone [10.008])	Rat; M, F 1/NR	Diet	49 weeks	300[2]	(Fassett, 1961)	a)
	Dog; M, F 1/NR	Diet	38 weeks	150[2]	(Fassett, 1961)	a)
(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)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat; M, F 1/30	Diet	90	12.1[2]	(Cox et al., 1974h)	a)
(5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [10.023])	Rat; M, F 1/30	Diet	90	M: 1.29[2] ; F: 1.47[2]	(Posternak et al., 1969)	a)
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Rat; M, F 6/8-16	Diet	13 - 52 weeks	46[2]	(Munday & Kirkby, 1973; Munday & Kirkby, 1971a)	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)
Butane-1,3-diol [02.132]	Rat; M 15/10	Diet	30 weeks	200000 ppm (10000 mg/kg/day)	(Miller & 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

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No/Group	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; M, F 3/60	Diet	2 years	100000 ppm (5000 mg/kg/day)	(Scala & 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 & 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 2/3	Diet	16	1000[2]	(Tischer et al., 1942)	a)
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Rat; M, F 1/20 1/60	Diet	2 years	250[2]	(Bär & Griepentrog, 1967)	a)
Malonic acid [08.053]	Rat; M, F 3/140	Diet	2 years	10[9]	(Hogan & 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 & 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 & 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)	

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No/Group	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	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 significnat 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 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, 1953a)	a)
	Rat; M 5/16	Diet	2 years	6250[2]	(Smith, 1953a)	a)
(Triethyl citrate [09.512])	Rat; M, F 3/7	Diet	2 months	4000[2]	(Finkelstein & Gold, 1959)	a)
	Cat; NR 1/6	Gavage	2 months	< 285	(Finkelstein & Gold, 1959)	a)
(Tributyl acetylcitrate [09.511])	Rat; M, F 2/4	Diet	2 months	5000[2]	(Finkelstein & Gold, 1959)	a)
	Cat; NR 2/4	Gavage	2 months	< 5700	(Finkelstein & Gold, 1959)	a)

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 from chemical 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. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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 Foetal: 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 Foetal: 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] Foetal: 1180[7]	(Hardin et al., 1987; Schuler et al., 1984; Smith, 1983)	FGE.10 refers to (EPA, 1999; 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; Foetal: 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) Foetal: 250 ppm (24.3 mg/kg/day) Reproductive: > 1000 ppm (84.5mg/kg/day)	(Neeper-Bradley & Ballantyne, 2000)	
	Rat; F	Drinking water	3/25	Developmental toxicity: Gestation days 6 – 16	Maternal: 50 ppm (5 mg/kg/day); Foetal: 750 ppm(68 mg/kg/day)[10]	(Hellwig, 1991a)	
	Rat; F	Gavage	3/21 – 26	Developmental toxicity: Gestation days 6 – 15	Maternal: 50; Foetal: 100	(Ema et al., 1992)	
	Mouse; F	Oral	3/NR	Developmental toxicity: Gestation days 7 – 12	Embryotoxicity: 30; Foetal: 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; Foetal: 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, 1974)	
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)	

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 eight candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 and for 23 supporting substances evaluated by JECFA at the 49th and 53rd meetings. Supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

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 & Dean, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/ml	Negative[1]	(Baker & Bonin, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Rowland & Severn, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500µg/plate	Negative[1]	(Simmon & Shephard, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537	NR	Negative[1]	(Nagao & Takahashi, 1981)	
	Ames test	*S. typhimurium* TA98, TA100,	1000 mg	Negative[1]	(Ichinotsubo et al., 1981a)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 10000 µg/plate	Negative[3]	(Richold & 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 & 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

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*	NR	Negative[1]	(Green, 1981)	
	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 & Parry, 1981)	
	Cell growth inhibition	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp & 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 & 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

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
(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 600 µg/ml	Negative[2] Positive[3]	(Heck et al., 1989)	Abstract only, study cannot be validated
	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 & 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	
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Formation of 32P-labelled DNA fragment (test on isolated DNA).	*p53* tumour suppression gene	1mM (128 µg/ml)	Negative[7]	(Yamashita et al., 1998)	
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 & 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 & 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)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Ames test; preincubation protocol	*S. typhimurium* TA97, TA102	0.1-10 mg/plate (10 – 10000 µg/plate)	Negative[1]	(Fujita & Sasaki, 1987)	
	Rec-assay	*B. subtilis*, H17, M45	20 µg/disk	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*; H17, M45	20µl/disk (20000 µg/disk)	Positive	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
	Rec-assay	*E. coli*; WP2 *uvrA*	200-1600µg/plate	Positive[8]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
	Rec-assay	*B. subtilis*; H17, M45	10-20µl/ml (10-20 µg/ml)	Negative[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated
	Rec-assay	*B. subtilis*; H17, M45	10-20µl/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 & 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 & 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
2-Butoxyethan-1-ol [02.242] continued	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 & 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 & Au, 1995)	Non-cytotoxic concentration range. Reliable study, conclusion comprehensible
	Sister chromatid exchange	Chinese hamster ovary cells	0.007 - 0.25 %	Negative[1]	(Slesinski & Weil, 1980)	Reliable study, conclusion comprehensible

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	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 currunt 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 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 h) approx. at 90mM, 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 currunt OECD guidelines
	Unscheduled DNA synthesis	Rat hepatocytes	0.1 – 100 x 10^{-3}%	positive[1, 11]	(Slesinski & 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 & 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)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	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 & Al-Lami, 1988)	
Diethyl maleate [09.351]	Forward mutation assay	Mouse lymphocytes L5178Y TK+/-	2.250 – 9.750 x 10^{-3} mol/l (387 - 1679 µg/ml)	Positive[1]	(Wangenheim & 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 TA102, TA104 and TA 102 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

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	15.4 µg/plate[2, 15] 51.6 µg/plate[3]	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be 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 two laboratories aimed to develop sensitive test protocol. 1-16 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)	
(Ethylene 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 & 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, 2004)	Test performed both in the incorporation and preincubation assays

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 and for eight supporting substances evaluated by JECFA at the 49th and 53rd meetings. Supporting substances are listed in brackets.

TABLE IV.5: GENOTOXICITY STUDIES (*IN VIVO*)

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 LD50	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 & 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
2-Butoxyethan-1-ol [02.242] continued	*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.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
	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 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 h 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.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(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 h after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not 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 h 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: 1375mg/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 & 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: 0,55ml/kg (males) of a 5 % solution	Negative	(Putman, 1987)	Time points of investigation: single dose: 8,12 hours. Repeated dose: 12 hours. 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 & 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
Glutaraldehyde [05.149] continued	*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

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
	In vivo SLRL test	*D. melanogaster*	three day exposure via diet	3500 ppm	Negative	(Zimmering et al., 1989)	Study in compliance with OECD477
	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 OECD477
(Adipic acid [08.026])	*In vivo* chromosomal nondisjunction	*D. melanogaster*		4000 ppm	Negative	(Ramel & 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.
(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 h 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.*

REFERENCES:

Aeschbacher, H.U., Wolleb, U., Loliger, J., Spadone, J.C., Liardon, R., 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem. Toxicol. 27(4), 227-232.

Al-Ani, F.Y., Al-Lami, S.K., 1988. Absence of mutagenic activity of acidity regulators in the Ames *Salmonella*/microsome test. Mutat. Res. 206, 467-470.

Albro, P.W., 1975. The metabolism of 2-ethylhexanol in rats. Xenobiotica 5(10), 625-636.

Aldridge, W.N., 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, M.W., 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson, D.H., Caldwell, J., Paulson, G.D. (Eds.). Intermediary xenobiotic metabolism in animals. Taylor and Francis, New York, pp. 81-97.

Andersen, P.H., Jensen, N.J. ,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., Fung, H.L., 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, D.L., Bonin, M.A., Cardinali, F.L., McCraw, J.M., Wooten, J.V., 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, R.S.U., Bonin, A.M., 1981. Study of 42 coded compounds with the *Salmonella*/mammalian microsome assay. Prog. Mutat. Res. 1, 249-260.

Ballantyne, B., Myers, R.C., 2001. Related articles: The acute toxicity and primary irritancy of glutaraldehyde solutions. Vet. Hum. Toxicol. 43(4), 193-202.

Barnhart, J.L., 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, M.E., 1984. Agents affecting the male reproductive system: Effects of structure on activity. Drug Metab. Rev. 15, 941-996.

Besrat, A., Polan, C.E., Henderson, L.M., 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., 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. (In German)

Bosron, W.F., Li, T.K., 1980. Alcohol dehydrogenase. In: Jakoby, W.B. (Ed.). Enzymatic Basis of Detoxification vol. 1. Academic Press, New York, 231-248.

Boyland, E., Chasseaud, L.F., 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, J.C., Brown, G.L., Caldwell, J.A., Drobeck, H.P., 1984. Teratology and mutagenicity studies with glutaric acid. Teratology 29(2), 19A.

Brauninger, R.M., 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, T.M., Dean, B.J., 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.

Bär, F., 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. (In German)

Carpenter, C.P., Pozzani, U.C., Weil, C.S., Nair, J.H., Keck, G.A., Smyth, H.F., 1956. The toxicity of butyl cellosolve solvent. Arch. Ind. Health 14, 114-131.

Chiewchanwit, T., Au, W.W., 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, W.M., Purchase, R., Ford, G.P., Creasy, D.M., Brantom, P.G., Gangolli, S.D., 1992. A 28-day feeding study with ethyl acetoacetate in rats. Food Chem. Toxicol. 30(7), 567-573.

Corley, R.A., Bormett, G.A., Ghanyem, B.I., 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.

Cox, G.E., Bailey, D.E., Morgareidge, K., 1974h. 90-day feeding study in rats with compound 14807 (5-hydroxy-2,4-decadienoic acid-lactone). Lab. no. 2107o. December 30, 1974. Unpublished report submitted by EFFA to SCF.

Cramer, G.M., Ford, R.A., Hall, R.L., 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., 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, B.J., 1981. Activity of 27 coded compounds in the RL1 chromosome assay. Prog. Mutat. Res. 1, 570-579.

Deichmann, W., Hirose, B.R., Witherup, S., 1945. Observation on the effect of gamma-valerolactone upon experimental animals. J. Ind. Hyg. Toxicol. 27(9), 263-268.

Deisinger, P.J., Boatman, R.J., Guest, D., 1994. Metabolism of 2-ethylhexanol administered orally and dermally to the female Fischer 344 rat. Xenobiotica 24(5), 429-440.

Deuel Jr., H.J., 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, R.B., Brown, W.D., Setzer, J.V., Taylor, B.J., Shukla, R., 1988. Effects of short duration exposures to acetone and methyl ethyl ketone. Toxicol Lett. 43, 31-49.

Dillon, D., Combes, R., 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, J.D., Roth, R.H., 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, 1996. 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, 2006. Commission Decision 2006/252/EC of 27 March 2006 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 29.3.2006, L 91, 48.

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, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

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.eu.int/science/afc/afc_meetings/502_en.html

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 25th 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, M.C., Marande, A.M., Poirot, O., Terzetti, F., 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., 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://europa.eu.int/comm/eurostat/newcronos/queen/display.do?screen=detail&language=en&product=YES&root=YES/yearlies/c/ca/caa/caa10000. August 13, 2004.

Exon, J.H., Mather, G.G., Bussiere, J.L., Olson, D.P., Talcott, P.A., 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, R.I., Weiner, H., 1972. Horse liver aldehyde dehydrogenase. I. Purification and characterization. J. Biol. Chem. 247(1), 260-266.

Fey, E.G., White, H.A., Rabin, B.R., 1981. Development of the degranulation test system. Prog. Mutat. Res. 1, 236-244.

Finkelstein, M., 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, W.N., Bessman, S.P., 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, O.G., Nelson, A.A., 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.

Florin, I., Rutberg, L., Curvall, M., Enzell, C.R., 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

Foulger, J.H., 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, J.M., Valencia, R., Zimmering, S., 1994. Chemical mutagenesis testing in *Drosophil*a. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ. Mol. Mutag. 23, 208-227.

Frankenfeld, J.W. Mohan, R.R., Squibb, R.L., 1975. Preservation of grain with aliphatic 1,3-diols and their esters. J. Agric. Food Chem. 23, 418-425.

Fujita, H., 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)

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., 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., Pickering, C., 1981. Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay. Prog. Mutat. Res. 1, 280-284.

Garst, J., Stapleton, P., 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, B.I., Blair, P.C., Thompson, M.B., Maronpot, R.R., Matthews, H.B., 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, B.I., Burka, L.T., Matthews, H.B., 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, B.I., Burka, L.T., Sanders, J.M., Matthews, H.B., 1987c. Metabolism and disposition of ethylene glycol monobutyl ether (2-butoxyethanol) in rats. Drug Metab. Disposition 15(4), 478-484.

Gollapudi, B.B., Barber, E.D., Lawlor, T.E., Lewis, S.A., 1996. Re-examination of the mutagenicity of ethylene glycol monobutyl ether to *Salmonella* tester strain TA97a. Mutat. Res. 370(1), 61-64.

Green, M.H.L, 1981. A differential killing test using an improved repair-deficient strain of *Eschericia coli*. Prog. Mutat. Res. 1, 184-194.

Guidotti, A., Ballotti, P.L., 1970. Relationship between pharmacological effects and blood and brain levels of gamma-butyrolactone and gamma-hydroxybutyrate. Biochem. Pharmacol. 19, 884-894.

Gulati, D.K., Hommel, L., Poonacha, K.B, Russell, V., Russell, S., Lamb, J.C., 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, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A., Brouwer, J.B., 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, B.D., Schuler, R.L., Burg, J.B., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J., Smith, K.N., 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., Zeiger, E., 1983. *Salmonella* mutagenicity test results for 250 chemicals. Environ. Mutag. 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, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Heindel, J.J., Gulati, D.K., Russell, V.S., Reel, J.R., Lawton, A.D., Lamb, J.C., 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., Vainio, H., 1980. Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals. Arch. Toxicol. 46, 277- 285.

Henrich, R.T., McMahon, J.M., 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, F.G., Cox, G.E., Bailey, D.E., Parent, R.A., Becci, P.J., 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, W.B. (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

Hiser, M.F., Markley, B.J., Reitz, R.H., Nieusma, J.L., 1992. Metabolism and disposition of acetyl tributyl citrate in male Sprague-Dawley rats. Toxicologist 12, 161.

Hoechst, 1995. Material safety data sheet. 3-hydroxy-2-oxopropionic acid. Data submitted by EFFA to SCF.

Hoflack, J.C., Lambolez, L., Elias, Z., Vasseur, P., 1995. Mutagenicity of ethylene glycol ethers and of their metabolites in *Salmonella typhimurium* his-. Mutat. Res. 341(4), 281-287.

Hogan, G.K., Rinehart, W.E., 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, D.B., 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, H.J., Holland, E.G., Hazleton, L.W., 1957. Safety of adipic acid as compared with citric and tartaric acids. J. Agric. Food Chem. 5, 759-762.

Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain. A.J., Bridges, J.W., 1981. Fluctuation test with S9 and hepatocyte activation. Prog. Mutat. Res. 1, 361-370.

Humbert, R. Adler, D.A., Disteche, C.M., Hassett, C., Omleoinski, C.J., Purlong, C.E., 1993. The molecular basis of the human serum paraoxonase activity polymorphism. Nature Genetics 3, 73-76.

Ichinotsubo, D., Mower, H., Mandel, M., 1981a. Testing of a series of paired compounds (carcinogen and noncarcinogenic structural analog) by DNA repair-deficient *E. coli* strains. Prog. Mutat. Res. 1, 195-198.

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, M.Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., 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., 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, W.B., Scott, E.M., 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. 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 Commitee 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 Commitee 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 Commitee 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 Commitee 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 Commitee 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 Commitee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., Fitzhugh, O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Johanson, G., Wallén, M., Nordqvist, M.B., 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., Teranishi, T., 1999. Congeital malformations due to antiepileptic drugs. Epilepsy Res. 33, 145-158.

Kaphalia, B.S., Ghanayem, B.I., Ansari, G.A.S., 1996. Nonoxidative metabolism of 2-butoxyethanol via fatty acid conjugation in Fischer 344 rats. J. Toxicol. Environ. Health 49(5), 463-479.

Kassinova, G.V., Kavaltsova, S.V., Marfin, S.V., Zakhrov, I.A., 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, J.A., Salamone, M.F., 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., 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., 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.

Kopf, R., Loeser, A., Meyer, G., 1950. Untersuchungen über die Pharmakologie und Toxikologie mehrwertiger Alkohole (1,3-butylenglykol). Arch. Exp. Pathol. Pharmakol. 210, 346-360. (In German)

Krasavage, W.J., 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, H.A., Salvin, E., Johnson, W.A., 1938. The formation of citric and alpha-ketoglutaric acids in the mammalian body. Biochem. J. 32, 113-117.

Kronevi, T., Holmberg, B., Arvidsson, S., 1988. Teratogenicity test of gamma-butyrolactone in the Sprague-Dawley rat. Pharmacol. Toxicol. 62, 57-58.

Krop, S., Gold, H., Paterno, C.A., 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, Y.S., Ishibashi, T., 1984a. [Rec-assay of food additives]. Nippon. Koshu. Eisei. Zasshi 31(6), 277-281. (In Japanese)

Kuroda, M., Yoshida, D., 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, W.H., Malik, M., 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, C.R., 1977. Evidence for the beta-oxidation of orally administered 4-hydroxybutyrate in humans. Biochem. Med. 17, 284-291.

Leegwater, D.C., 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, J.T., Fung, H.L., 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., 1976a. Acute oral toxicity (rats-5 gms./kg. body weight dose). Dermal toxicity (rabbits-5 gms./kg. body weight dose). Ethyl caprate, Assay no. 62990; Ethyl isovalerate, Assay no. 62992. Leberco Laboratories, Inc. May 18, 1976. Unpublished report submitted by EFFA to SCF.

Levey, S., Lasichak, A.G., Brimi, R., Orten, J.M., Smyth, C.J., Smith, A.H., 1946. A study to determine the toxicity of fumaric acid. J. Am. Pharm. Assoc. 35, 298-304.

Levi, P.E., Hodgson, E., 1989. Metabolites resulting from oxidative and reductive processes. In: Hutson, D. H., Caldwell, J., Paulson, G.D. (Eds.). Intermediary Xenobiotic Metabolism in Animals. Taylor and Francis, London, pp. 119-138.

Levin, D.E., Hollstein, M., Christman, M.F., Schwiers, E.A., Ames, B.N., 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, C.A., Palanker, A.L., 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., LeTalaer, J.Y., 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, D.J., 1981. *Salmonella*/microsome tests on 42 coded chemicals. Prog. Mutat. Res. 1, 285-297.

Mankes, R.F., Renak, V., Fieseher, J., 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, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N., 1985a. Naturally-occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat. Res. 148, 25-34.

Marshall, L.M., Orten, J.M., Smith, A.H., 1949. The determination of fumaric acid in animal tissues by partition chromatography. J. Biol. Chem. 179, 1127-1139.

Martin, C.N., McDermid, A.C., 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., Sugimura, T., 1981. Reverse mutation test on 42 coded compounds with *E. coli* WP2 system. Prog. Mutat. Res. 1, 387-395.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Caspary, W.J., 1988b. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay II: 18 coded chemicals. Environ. Mol. Mutag. 11, 91-118.

McKelvey, J.A., Garman, R.H., Anuszkiewicz, C.M., Tallant, M.J., 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, M.A., Singh, G., Bechtold, W.E., Bond, J.A., Sabourin, P.J., Birnbaum. L.S., Henderson, R.F., 1990. Disposition of three glycol ethers administered in drinking water to male F344/N rats. Toxicol. Appl. Pharmacol. 102(3), 443-455.

Mehlman, M.A., Tobin, R.B., Hahn, H.K.J., Kleager, L., Tate, R.L., 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, S.A., Dymsza, H.A., 1967. Utilization by the rat of 1,3-butanediol as a synthetic source of dietary energy. J. Nutr. 91, 79-88.

Mingrone, G., Greco, A.V., Nazzaro-Porro, M., Passi, S., 1983. Toxicity of azelaic acid. Drugs Exp. Clin. Res. 9(6), 447-455.

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P., Spalding, J.W., 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.

Moran, E.J., Easterday, D.D., Oser, B.L., 1980. Acute oral toxicity of selected flavor chemicals. Drug Chem. Toxicol. 3(3), 249-258.

Moreno, O.M., 1972a. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Citonellyl butyrate. MB Research Laboratories, Inc. Project no. MB 72-5x. November 1, 1972. Unpublished data submitted by EFFA to SCF.

Moreno, O.M., 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, O.M., 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, O.M., 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, O.M., 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, O.M., 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, O.M., 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, O.M., 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, O.M., 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, O.M., 1977d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Acetyl butyryl, project no. MB 77-1744, August 18, 1977. Acetyl methyl carbinol, project no. MB 77-1691, June 20, 1977. Acetyl propionyl, MB 76-1445, January 25, 1997. MB Research Laboratories, Inc. Unpublished data submitted by EFFA to SCF.

Moreno, O.M., 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, O.M., 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, O.M., 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, O.M., 1977f. 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, O.M., 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, O.M., 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, O.M., 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. Ppomalus; 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. 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.

Morgott, D.A., 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.

Munday, R., Kirkby, W.W., 1971a. Biological evaluation of a flavor cocktail. 2. 13-Week study in rats. Research report PCW 71 1624. Unpublished data submitted by EFFA to SCF.

Munday, R., Kirkby, W.W., 1973. Biological evaluation of a flavor cocktail. 3. One-year feeding study in rats. Research report PCW73 1103. Unpublished data submitted by EFFA to SCF.

Myers, R.C., Homan, E.R., 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, R.C., Carpenter, C.P., Cox, E.F., 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, R.C., Carpenter, C.P., Cox, E.F., 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.

Müller, W., Engelhart, G., Herbold, B., Jäckh, R., Jung, R., 1993. Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. Environ. Health Perspec. Suppl. 101(3), 33-36.

Möhler, H., Patel, A.J., Balázs, R., 1976. Gamma-hydroxybutyrate degradation in the brain *in vivo*: Negligible direct conversion to GABA. J. Neurochem. 27, 253-258.

Nagano, K., Nakayama, E., Adachi, H., 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., 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., Yamazaki, K., 1984. Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. Environ. Health Perspec. 57, 75-84.

Nagao, M., 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., Löscher, W., 1986., Pharmacologic evaluation of metabolites and analogs of valproic acid: Teratogenic potencies in mice. Fundam. Appl. Toxicol. 6, 669-676.

Neeper-Bradley, T.L., 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., Stookey, G., 1992. Mutagenicity of glutaraldehyde in the Ames test. J. Dent. Res. 71, 227.

Noblitt, T., Li, Y., Dunipace, A., Origel, A., 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 technincal 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., Kunita, N., 1979. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

Okamoto, K., Riccio, E.S., 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.

Oser, B.L., Carson, S., Oser, M., 1965. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 3(4), 563-569.

Osteux, R., Laturaze, J., 1954. Biological chemistry - Paper chromatography of fixed organic acids found in urine. Comp. Rend. 239, 512-513.

Packman, E.W., Abbott, D.D., Harrisson, J.W.E., 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, A.S., Nazarro-Porro, M., 1989. Azeliac acid - biochemistry and metabolism. Acta Derm. Venereol. Suppl., 143, 8-13.

Patty, F.A., 1963. Patty's Industrial Hygiene and Toxicology, vol. 2. John Wiley & Sons Inc., New York, p. 1546.

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, V.J., Hartman, W.C., 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, N.M., Linder, A., Vodoz, C.A., 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, M.J., Simmon, V.F., Mortelmanns, K.E., 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutat. Res. 260, 321-329.

Putman, D.L., 1987. Cytogenicity 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., Magnusson, J., 1979. Chemical induction of nondisjunction in *Drosophila*. Environ. Health Perspect. 31, 59-66.

Rapson, W.H., Nazar, M.A., Butzky, V.V., 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bull. Environ. Contam. Toxicol. 24, 590-596.

Reagan, E.L., Becci, P. J., 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, P.G.J., van Oostrum, E.C.M., Roverts, W.G., Koeter, H.B.W.M., 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., Jones, E., 1981. Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay. Prog. Mutat. Res. 1, 314-322.

Riebeek, W.M., 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, H.S., Hyman, J., Leifer, Z., 1981. DNA polymerase deficient assay. Prog. Mutat. Res. 1, 210-218.

Roth RH, Giarman J., 1965. Preliminary report on the metabolism of gamma-butyro-lactone and gamma-hydroxybutyric acid. Biochem. Pharmacol. 14(2), 177-178.

Roth, R.H., Giarman, N.J., 1966. Gamma-butyrolactone and gamma-hydroxybutyric acid-I. Distribution and metabolism. Biochem. Pharmacol. 15, 1333-1348.

Rowe, V.K., Wolf, M.A., 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., 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., 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, 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.

Rydén, E., Ekström, C., Hellmér, L., 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., 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., Sato, T., 1989. Evaluation of genotoxic activities of disinfectants and their metabolites by the umu test. Mutat. Res. 216(6), 373.

Salamone, M.F., Heddle, J.A., Katz, M., 1981. Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. Prog. Mutat. Res. 1, 686-697.

Samren, E.B., van-Duijn, C.M., Koch, S., Hiilesmaa, V.K., Klepel, H., Bardy, A.H., Mannagetta, G.B., Deichl, A.W., Gaily, E., Granstrom, M.L., Meinardi, H., Grobbee, D.E., Hofman, A., Janz, D., 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, J.R., 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, R.A., Paynter, O.E., 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, 1999. 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, E.W., Bowles, W.A., 1985. The acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol. 14, 111-129.

Schuler, R.L., Hardin, B.D., Niemeier, R.W., Booth, G., Hazelden, K., Piccirillo, V., 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., Bey, B., 1994. Mutagenicity of dentin bonding agents. J. Biomed. Mater. Res. 28, 1061-1067.

Sharp, D.C., Parry JM, 1981. Induction of mitotic gene conversion by 41 compounds using the yeast culture JD1. Prog. Mutat. Res. 1, 491-501.

Shelanski, M.V., Moldovan, M., 1973a. Acute oral toxicity (rats - 5 gms/kg body weight dose). Dermal toxicity (rabbits - 5 gms/kg body weight dose). Geranyl isobutyrate. Food and Drug Research Laboratories, Inc. IBL no. 12207-F. 30 January 1973. Unpublished report submitted by EFFA to SCF.

Shellenberger, T.E., 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, Y.I., 1950. [Action of some synthetic substances on animal organism]. Gig. Sanit. 3, 37-41. (In Russian)

Shimizu, H., Suzuki, Y., Takemura, N., Goto, S., Matsushita, H., 1985. The results of microbial mutation test for forty-three industrial chemicals. Jap. J. Ind. Health 27, 400-419.

Simmon, V.F., Shephard, G.F., 1981. Mutagenic activity 42 coded compounds in the *Salmonella*/microsome assay. Prog. Mutat. Res. 1, 333-342.

Simola, P.E., Krusius, F.E., 1938. The formation of alpha-ketoglutaric acid in animal metabolism. Suomen Kemistilehti 11B, 9.

Singh, A.R., Lawrence, W.H., 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, M.E., 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, T.R., Andon, B.M., Kaden, D.A., Thilly, W.G., 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, R.B., Price, C.J., Marr, M.C., Morrissey, R.E., Schwetz, B.A., 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, R.S., Weil, C.S., 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, R.S., Hengler, W.C., Guzzie, P.J., Wagner, K.J., 1983. Mutagenicity evaluation of glutaraldehyde in a battery of *in vitro* bacterial and mammalian test systems. Food Chem. Toxicol. 21(5), 621-629.

Smith, J.N., 1953a. Studies in detoxication. The glucuronic acid conjugation of hydroxyquinolines and hydroxypyridines in the rabbit. Biochem. J. 55, 156-160.

Smith, C.C., 1953b. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate and methoxyethyl oleate. Arch. Ind. Hyg. Occup. Med. 7(4), 310-318.

Smith, K.N., 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., H.F., Carpenter, C.P., 1948. Further experience with the range-finding test in the industrial toxiclogy laboratory. J. Ind. Hyg. Toxicol. 30, 63-68.

Smyth Jr., H.F., Seaton, J., Fischer, L., 1941. The single dose toxicity of some glycols and derivatives. J. Ind. Hyg. Toxicol. 23, 259-268.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., 1949. Range-finding toxicity data. List III. J. Ind. Hyg. Toxicol. 31, 60-62.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., 1951a. Range finding toxicity data: List IV. Arch. Ind. Hyg. Occup. Med. J. 4, 119-122.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., 1954. Range-finding toxicity data: List V. Arch. Ind. Hyg. Occup. Med. 10, 61-68.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., Striegel, J.A., 1962. Range-finding toxicity data: List VI. Am. Ind. Hyg. J. 23, 95-107.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., Striegel, J.A., Nycum, J.S., 1969a. Range-finding toxicity data: List VII. Am. Ind. Hyg. Assoc. J. 30(5), 470-476.

Spencer, P.S., Bischoff, M.C., Schaumburg, H.H., 1978. On the specific molecular configuration of neurotoxic aliphatic hexacarbon compounds causing central-peripheral distal axonopathy. Toxicol. Appl. Pharmacol. 44, 17-28.

St. Clair, M.B.G., Bermudez, E., Gross, E.A., Butterworth, B.E., Recio, L., 1991. Evaluation of the genotoxic potential of glutaraldehyde. Environ. Mol. Mutag. 18, 113-119.

Stonehill, A.A., Krop, S., Borick, P.M., 1963. Buffered glutaraldehyde: a new chemical sterilizing solution. Am. J. Hosp. Pharm. 20, 458-465.

Striegel, J.A., Carpenter, C.P., 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, J.A., 1981. Activity of 42 coded compounds in the BHK-21 cell transformation tests. Prog. Mutat. Res. 1, 638-646.

Summer, K.H., Rozman, K., 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, R.L., Mehlman, M.A., Tobin, R.B., 1971. Metabolic fate of 1,3-butanediol in the rat: conversion to beta-hydroxybutyrate J. Nutr. 101, 1719-1726.

Tischer, R.G., Fellers, C.R., Doyle, B.J., 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.

Topham, J.C., 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. 74, 379-387.

Trueman, R.W., 1981. Activity of 42 coded compounds in the *Salmonella* reverse mutation test. Prog. Mutat. Res. 1, 343-350.

Tsuchimoto, T., Matter, B.E., 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., Sestakova, H., 1997. Mutagenic activity of substances of plant origin. Potravin. Vedy 15(4), 271-288. (In Rumanian)

Tweats, D.J., 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., 2004. 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, J.P., Hermansky, S.J., Losco, P.E., 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., 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., Ballantyne, B., 2002. Genetic toxicology studies with glutaraldehyde. J. Appl. Toxicol. 22, 45-60.

Vergnes, J.S., Morabit, E.R., 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, J.S., Morabit, E.R., 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, E.H., Mc Ewen, J.D., Haun, C.C., Kinkead, E.R., 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., 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., Voet, J.G., 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, V.O., 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, S.S., Wiser, R., Gudmundsen, C., Kimmel, H., 1964. Metabolism of gamma -hydroxybutyric acid. Biochim. Biophys. Acta 86, 640-642.

Wang, G., Maranelli, G., Perbellini, L., Raineri, E., Brugnone, G., 1994. 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., Bolcsfoldi, G., 1988. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3(3), 193-205.

Watanabe, S., Morimoto, Y., 1990a. 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, C.S. and Wright, G.J., 1967. Intra- and interlaboratory comparative evaluation of single oral test. Toxicol. Appl. Pharmacol. 11, 378-388.

Wenzel, D.G., Koff, G.Y., 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, P.J., Lewis, S.C., Traul, K.A., 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, D.J., Gatehouse, D.G., 1990. Comparison of *Salmonella typhimurium* TA102 with *Escherichia coli* WP2 tester strains. Mutagenesis 5(3), 285-291.

Wild, D., King, M.T., Gocke, E., 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, R.T., 1959a. Detoxication mechanisms. The metabolism and Detoxification of Drugs, Toxic Substances, and Other Organic Compounds. 2nd Ed. Chapman and Hall Ltd, London, p. 318-.

Wohl, A.J., 1974a. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Amyl vinyl carbinol. Biological Science Laboratories. April 2, 1974. Unpublished data submitted by EFFA to SCF.

Wolf, M.A., 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.

Wolven, A.M., Levenstein, I., 1969. Acute oral toxicity reports on rats. Diethylmalonate. Leberco Laboratories. Assay no. 23460. July 31, 1962. Unpublished data submitted by EFFA to SCF.

Yamaguchi, T., 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.

Yamashita, N., Murata, M., Inoue, S., Hiraku, Y., Yoshinaga, T., Kawanishi, S., 1998. Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper (II). Mutat. Res. 397, 191-201.

Yingnian, Y., Yifab, D., Ming, F., 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, Y.S., 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Med. J. 34(3-4), 267-288. (In Japanese)

Yoon, J.S., Mason, J.M., Valencia, R., Woodruff, R.C., 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., Margolin, B.H., 2000. The proportions of mutagens among chemicals in commerce. Reg. Toxicol. Pharmacol. 32, 219-225.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., 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., Mortelmans, K., 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutag. 19(21), 2-141.

Zimmering, S., Mason, J.M., 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., Liebich, H.M., 1971. Profile of volatile metabolites in human urine. Clin. Chem. 17(7), 592-594.

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.

SCIENTIFIC PANEL MEMBERS

Fernando Aguilar, Herman Nybro Autrup, Susan Barlow, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Nathalie Gontard, David Michael Gott, Sandro Grilli, Rainer Gürtler, John Chr. Larsen, Jean-Charles Leblanc, Catherine Leclercq, F. Xavier Malcata, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Magdalena, Catharina Maria Rietjens, Paul P. Tobback, Fidel Toldrá.

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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.

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2 Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Leon Brimer, Laurence Castle, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Fidel Toldra, Detlef Wölfle. Correspondence: cef@efsa.europa.eu

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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.

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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

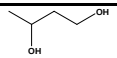
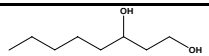
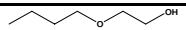
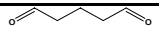
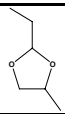
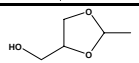
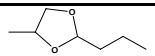
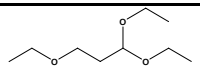
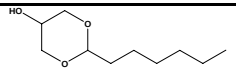
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.132	Butane-1,3-diol		 107-88-0	Liquid $C_4H_{10}O_2$ 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

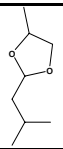
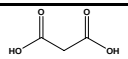
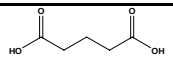
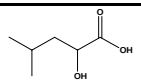
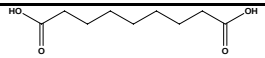
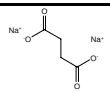
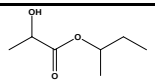
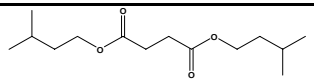
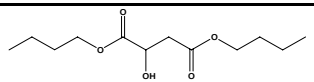
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		4378 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

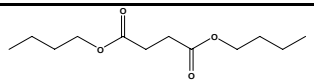
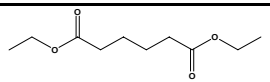
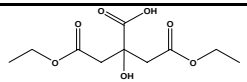
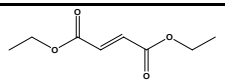
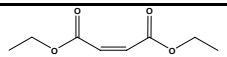
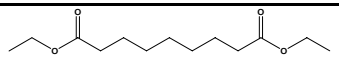
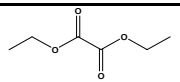
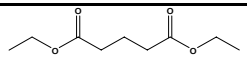
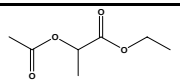
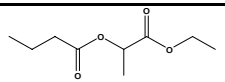
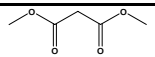
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.347	Dibutyl succinate		 141-03-7	Liquid $C_{12}H_{22}O_4$ 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

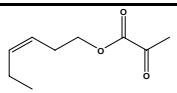
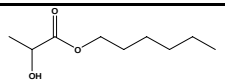
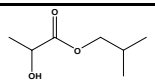
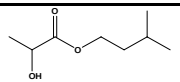
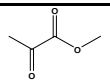
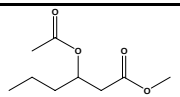
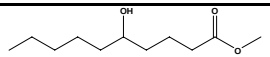
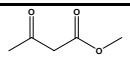
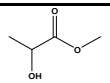
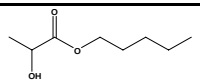
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
09.565 1846	Hex-3-enyl 2-oxopropionate		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

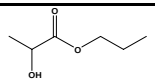
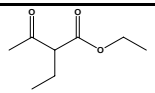
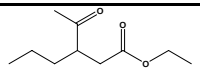
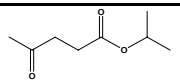
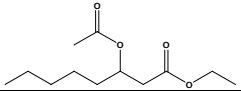
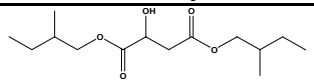
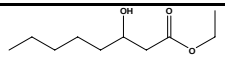
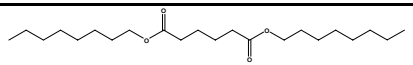
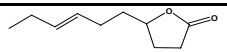
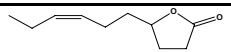
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.815	Propyl lactate		616-09-1	Liquid $C_6H_{12}O_3$ 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

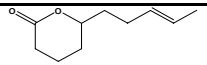
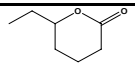
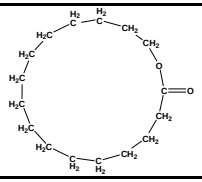
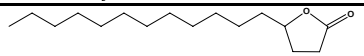
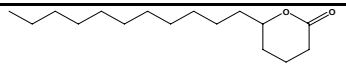
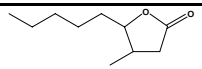
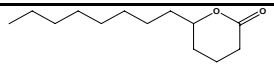
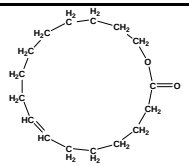
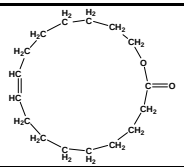
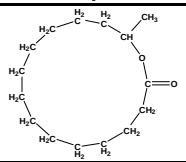
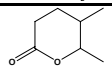
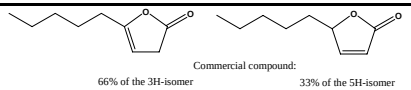
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.040	Dec-8-eno-1,5-lactone		32764-98-0	Liquid $C_{10}H_{16}O_2$ 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)

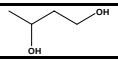
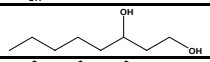
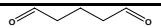
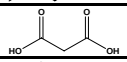
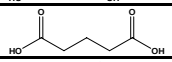
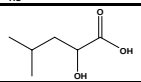
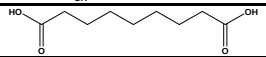
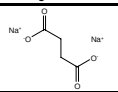
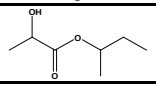
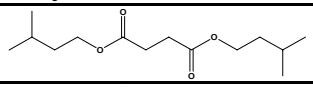
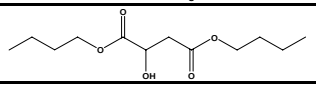
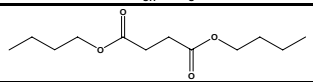
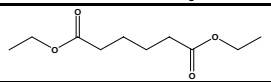
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	
02.198	Octane-1,3-diol		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)

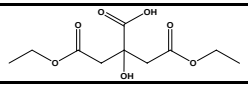
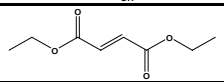
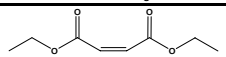
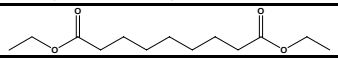
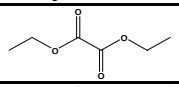
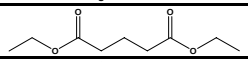
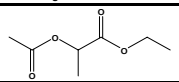
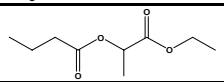
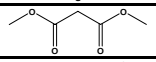
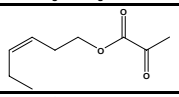
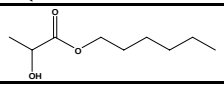
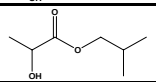
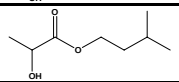
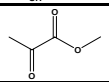
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.349	Diethyl citrate		0.12	Class I A3: Intake below threshold	4)	6)	
09.350	Diethyl 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)

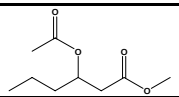
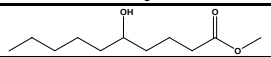
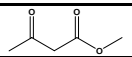
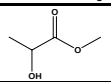
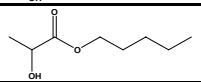
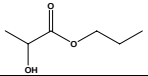
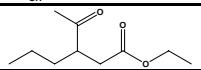
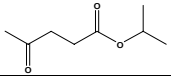
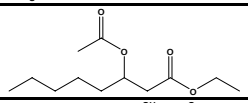
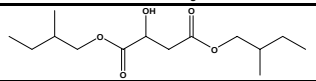
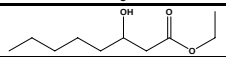
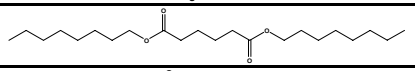
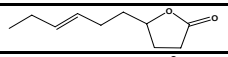
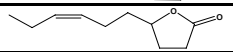
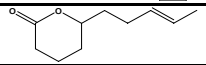
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.633	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)

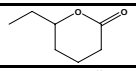
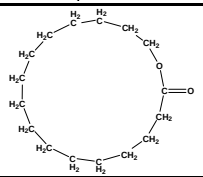
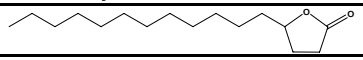
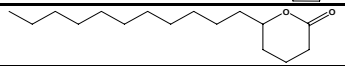
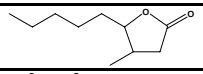
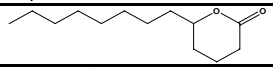
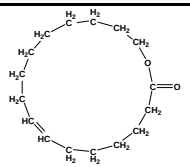
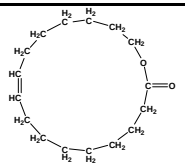
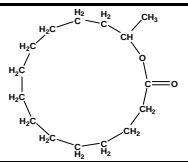
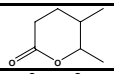
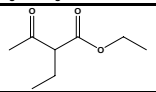
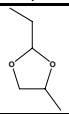
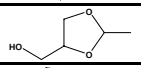
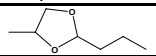
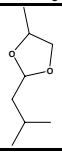
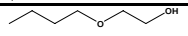
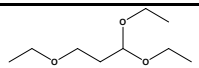
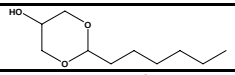
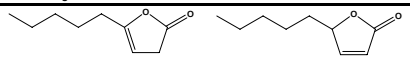
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.045	Heptano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	
10.047	Hexadecano-1,16-lactone		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

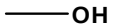
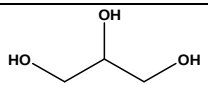
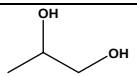
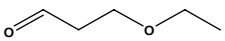
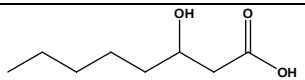
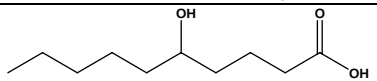
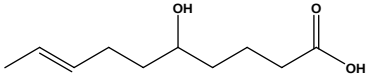
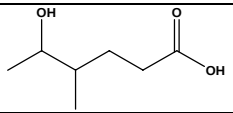
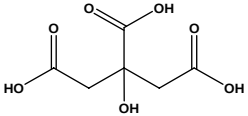
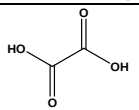
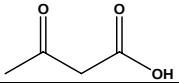
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of “flavouring agent”		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

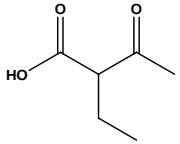
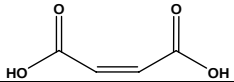
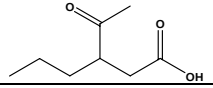
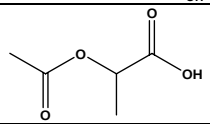
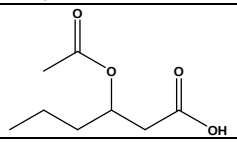
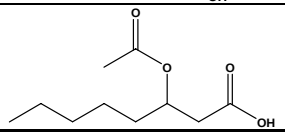
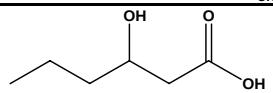
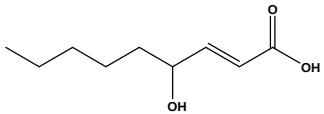
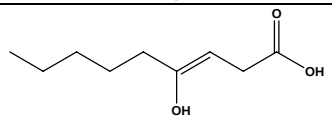
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Acetylbutyric acid		Not evaluated as flavouring substance		Not in EU-Register
	Maleic acid		Not evaluated as flavouring substance		Not in 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

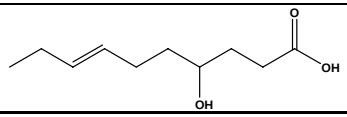
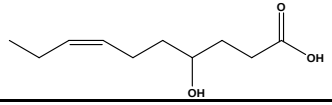
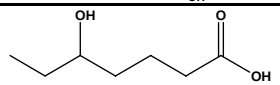
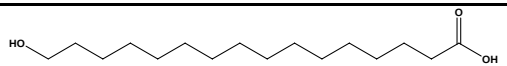
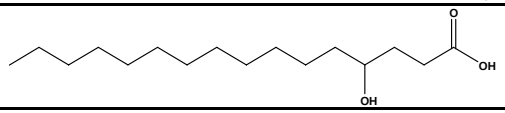
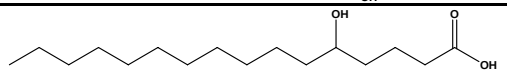
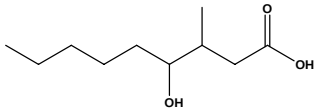
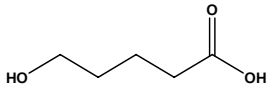
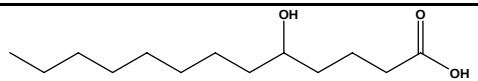
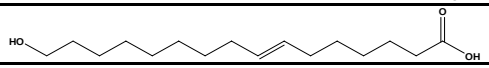
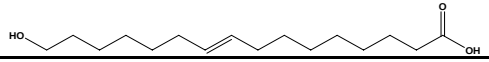
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	(E)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	(Z)-4-Hydroxydec-7-enoic acid		Not evaluated as 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

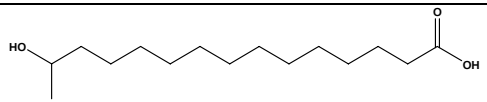
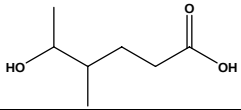
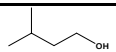
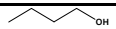
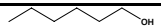
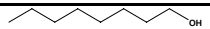
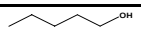
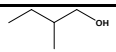
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	14-Hydroxypentadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as 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

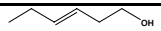
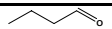
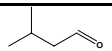
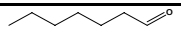
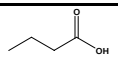
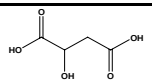
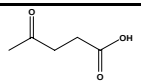
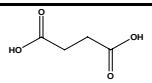
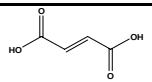
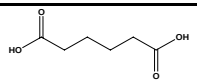
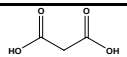
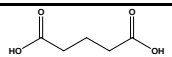
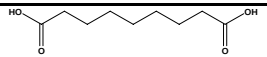
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
					used as flavouring agents.
02.079	Isopropanol 277		Category 1 a) No safety concern f)	Class I A3: Intake above threshold, A4: Endogenous	
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

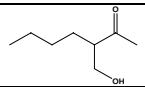
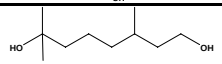
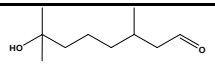
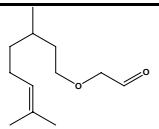
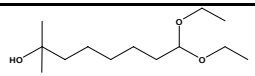
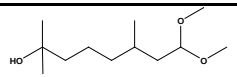
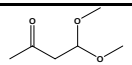
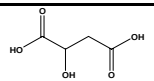
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2003b)	4.6	No safety concern d) Category B	Not 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

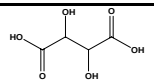
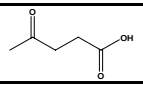
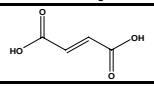
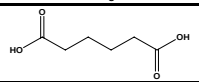
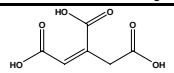
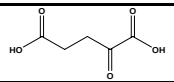
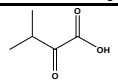
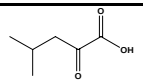
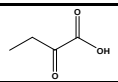
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							(JECFA, 1970a).
08.018	Tartaric acid		3044 18 133-37-9	621 JECFA specification (JECFA, 1999c)	3800	No safety concern 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

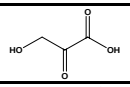
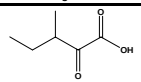
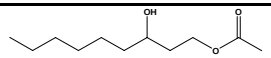
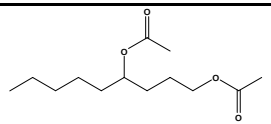
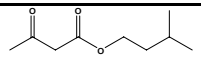
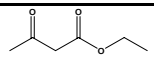
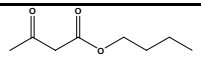
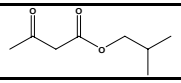
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.086	3-Hydroxy-2-oxopropionic acid		3843 1113-60-6	635 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
08.093	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

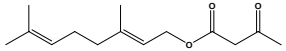
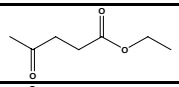
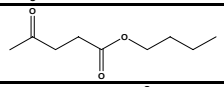
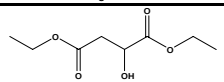
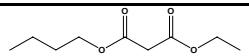
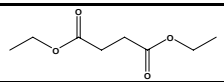
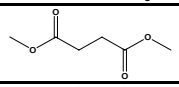
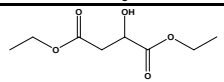
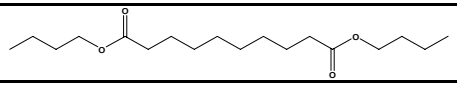
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.405	Geranyl acetoacetate		2510 243 10032-00-5	599 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B 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

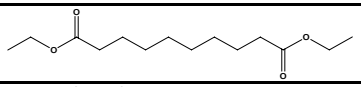
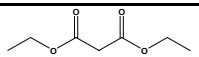
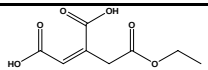
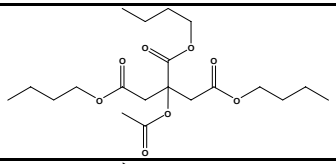
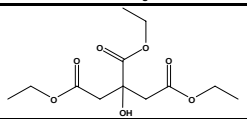
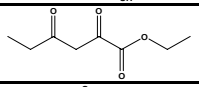
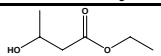
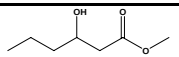
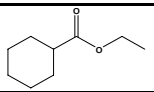
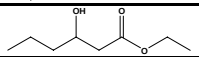
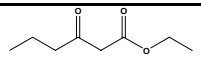
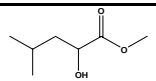
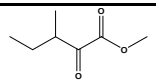
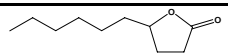
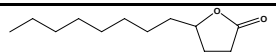
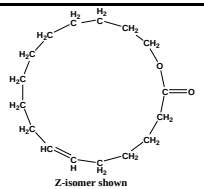
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.475	Diethyl sebacate		2376 623 110-40-7	624 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category A b)	
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.

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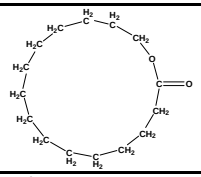
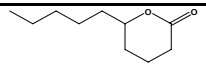
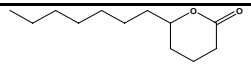
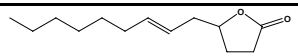
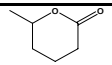
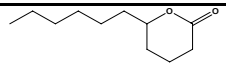
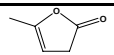
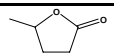
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.004	Pentadecano-1,15-lactone		2840 181 106-02-5	239 JECFA specification (JECFA, 2000d)	73	No safety concern c) Category 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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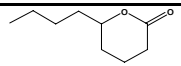
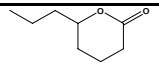
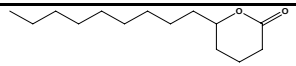
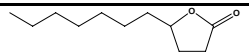
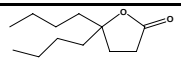
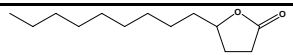
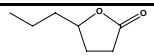
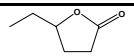
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.014	Nonano-1,5-lactone		3356 2194 3301-94-8	230 JECFA specification (JECFA, 1998b)	130	No safety concern c) Category B 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

Table 3: Supporting Substances Summary

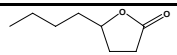
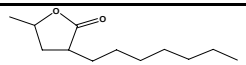
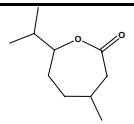
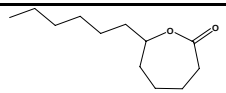
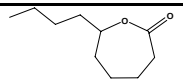
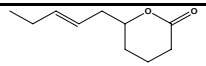
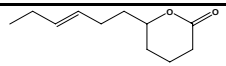
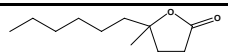
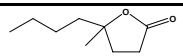
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							the racemate.
10.022	Octano-1,4-lactone		2796 2274 104-50-7	226 JECFA specification (JECFA, 2000d)	430	No safety concern 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

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
							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).

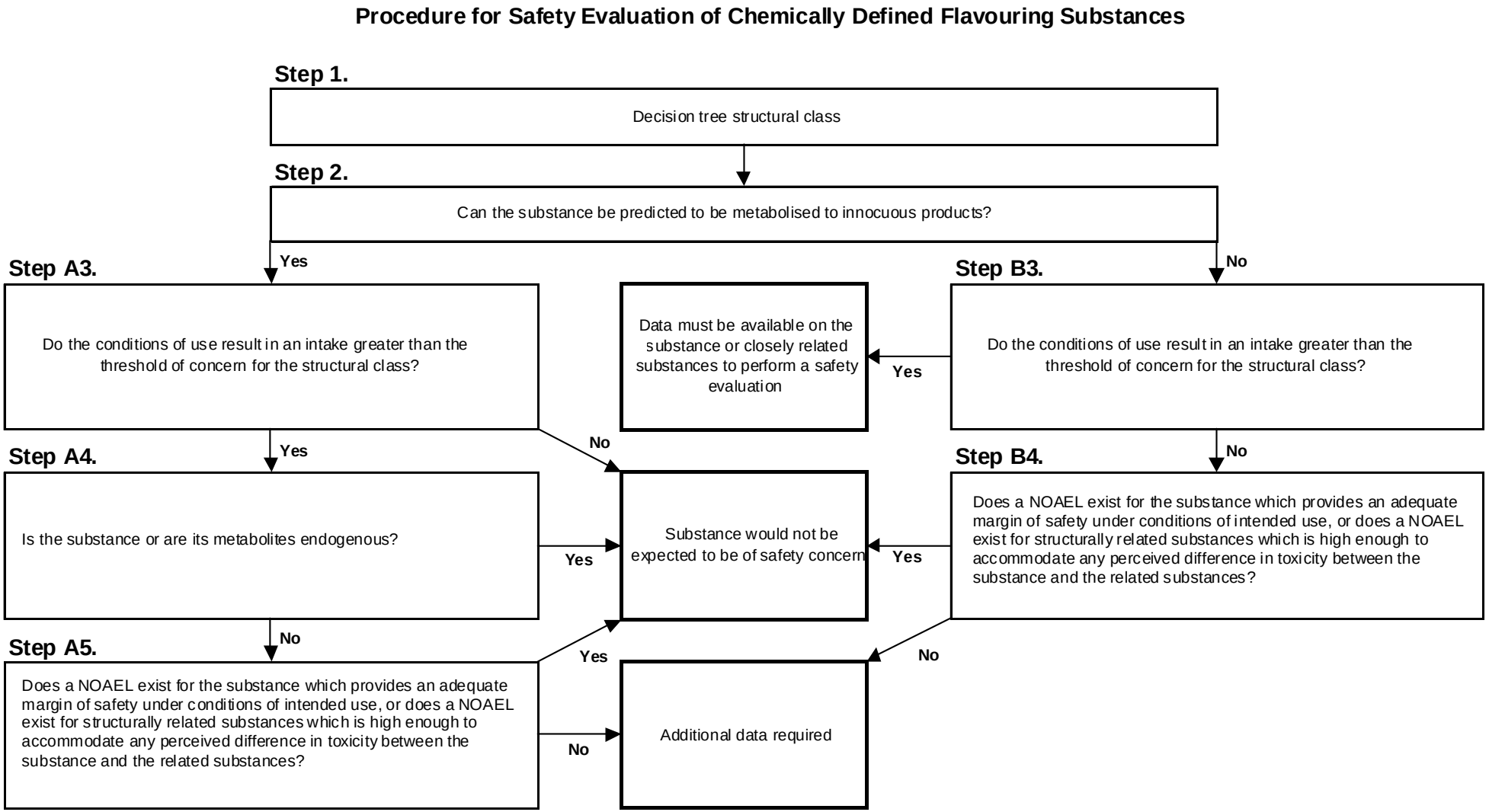

Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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).

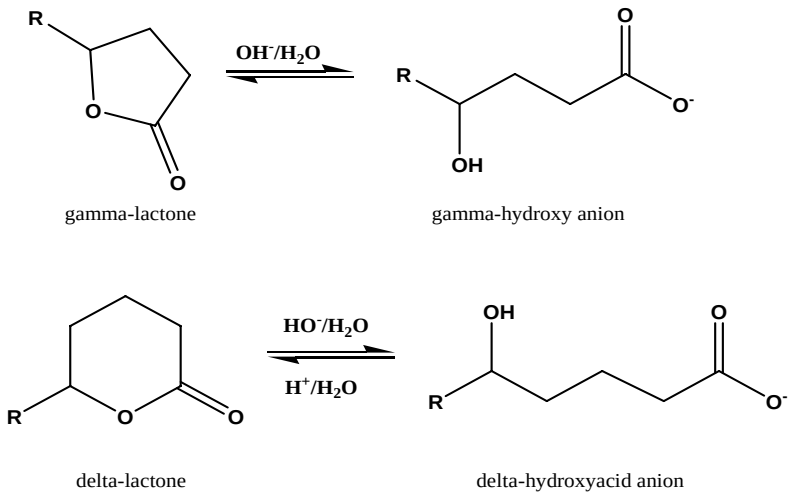

Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion*

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.

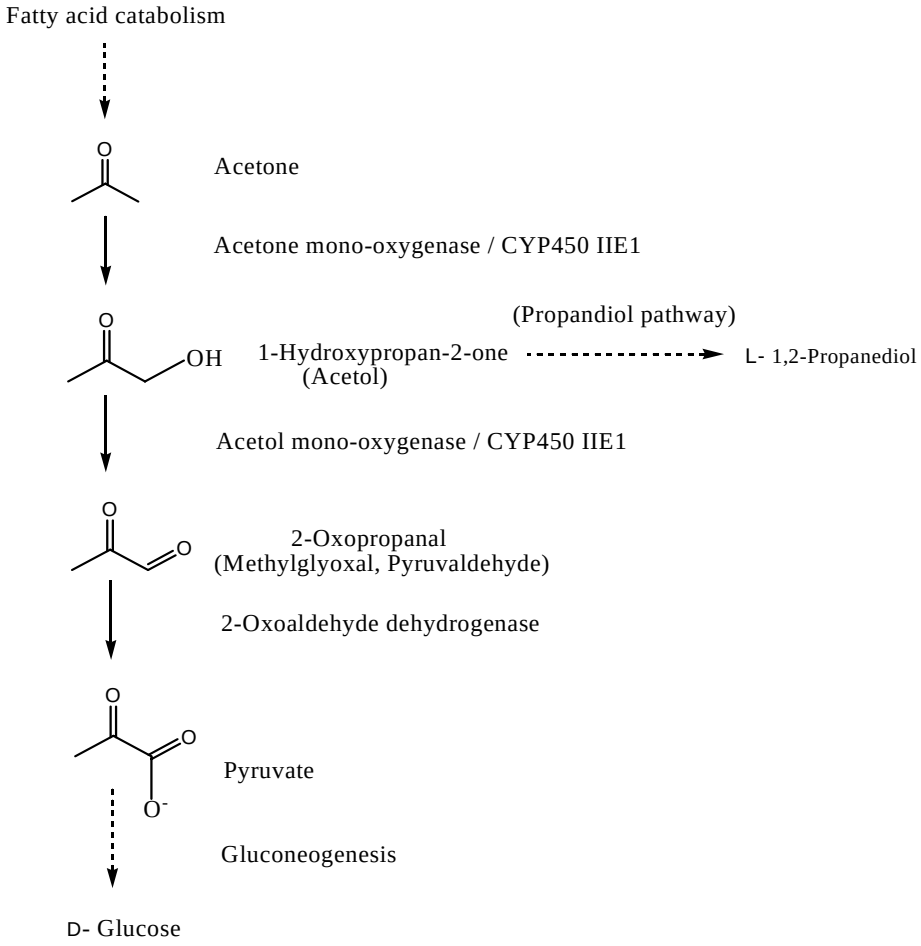

Figure III.2. *Acetone metabolism (methylglyoxal pathway)*

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)	

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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

United States Environmental Protection Agency | Prevention, Pesticides And Toxic Substances (7508W) | EPA 738-R-96-009 June 1996

Reregistration Eligibility Decision (RED)

Furanone

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

I am pleased to announce that the Environmental Protection Agency has completed its reregistration eligibility review and decisions on the pesticide chemical case Furanone which includes the active ingredients dihydro-5-pentyl-2(3H)-furanone and dihydro-5-heptyl-2(3H)-furanone. The enclosed Reregistration Eligibility Decision (RED) contains the Agency's evaluation of the data base of these chemicals, its conclusions of the potential human health and environmental risks of the current product uses, and its decisions and conditions under which these uses and products will be eligible for reregistration. The RED includes the data and labeling requirements for products for reregistration. It may also include requirements for additional data (generic) on the active ingredients to confirm the risk assessments.

To assist you with a proper response, read the enclosed document entitled "Summary of Instructions for Responding to the RED." This summary also refers to other enclosed documents which include further instructions. You must follow all instructions and submit complete and timely responses. **The first set of required responses is due 90 days from the receipt of this letter. The second set of required responses is due 8 months from the date of this letter.** Complete and timely responses will avoid the Agency taking the enforcement action of suspension against your products.

If you have questions on the product specific data requirements or wish to meet with the Agency, please contact the Special Review and Reregistration Division representative, Emily Mitchell at (703) 308-8583. Address any questions on required generic data to the Planning and Reregistration Division representative, Emily Mitchell at (703) 308-8583.

Sincerely yours,

Lois Rossi, Division Director
Special Review
and Reregistration Division

Enclosures

SUMMARY OF INSTRUCTIONS FOR RESPONDING TO THE REREGISTRATION ELIGIBILITY DECISION (RED)

1. **DATA CALL-IN (DCI) OR "90-DAY RESPONSE"**--If **generic data** are required for reregistration, a DCI letter will be enclosed describing such data. If **product specific data** are required, a DCI letter will be enclosed listing such requirements. If **both generic and product specific data** are required, a combined Generic and Product Specific DCI letter will be enclosed describing such data. However, if you are an end-use product registrant only and have been granted a generic data exemption (GDE) by EPA, you are being sent only the **product specific** response forms (2 forms) with the RED. Registrants responsible for generic data are being sent response forms for both generic and product specific data requirements (4 forms). **You must submit the appropriate response forms (following the instructions provided) within 90 days of the receipt of this RED/DCI letter; otherwise, your product may be suspended.**

2. **TIME EXTENSIONS AND DATA WAIVER REQUESTS**--No time extension requests will be granted for the 90-day response. Time extension requests may be submitted only with respect to actual data submissions. Requests for time extensions for product specific data should be submitted in the 90-day response. Requests for data waivers must be submitted as part of the 90-day response. All data waiver and time extension requests must be accompanied by a full justification. All waivers and time extensions must be granted by EPA in order to go into effect.

3. **APPLICATION FOR REREGISTRATION OR "8-MONTH RESPONSE"--You must submit the following items for each product within eight months of the date of this letter (RED issuance date).**

a. **Application for Reregistration** (EPA Form 8570-1). Use only an original application form. Mark it "Application for Reregistration." Send your Application for Reregistration (along with the other forms listed in b-e below) to the address listed in item 5.

b. **Five copies of draft labeling** which complies with the RED and current regulations and requirements. Only make labeling changes which are required by the RED and current regulations (40 CFR 156.10) and policies. Submit any other amendments (such as formulation changes, or labeling changes not related to reregistration) separately. You may, but are not required to, delete uses which the RED says are ineligible for reregistration. For further labeling guidance, refer to the labeling section of the EPA publication "General Information on Applying for Registration in the U.S., Second Edition, August 1992" (available from the National Technical Information Service, publication #PB92-221811; telephone number 703-487-4650).

c. **Generic or Product Specific Data**. Submit all data in a format which complies with PR Notice 86-5, and/or submit citations of data already submitted and give the EPA identifier (MRID) numbers. Before citing these studies, you must **make sure that they meet the Agency's acceptance criteria** (attached to the DCI).

d. **Two copies of the Confidential Statement of Formula (CSF)** for each basic and each alternate formulation. The labeling and CSF which you submit for each product must comply with P.R. Notice 91-2 by declaring the active ingredient as the **nominal concentration**. You have two options for submitting a CSF: (1) accept the standard certified limits (see 40 CFR §158.175) or (2) provide certified limits that are supported by the analysis of five batches. If you choose the second option, you must submit or cite the data for the five batches along with a certification statement as described in 40 CFR §158.175(e). A copy of the CSF is enclosed; follow the instructions on its back.

e. **Certification With Respect to Data Compensation Requirements**. Complete and sign EPA form 8570-31 for each product.

4. **COMMENTS IN RESPONSE TO FEDERAL REGISTER NOTICE**--Comments pertaining to the content of the RED may be submitted to the address shown in the Federal Register Notice which announces the availability of this RED.

5. **WHERE TO SEND PRODUCT SPECIFIC DCI RESPONSES (90-DAY) AND APPLICATIONS FOR REREGISTRATION (8-MONTH RESPONSES)**

By U.S. Mail:

Document Processing Desk **(RED-SRRD-PRB)**
Office of Pesticide Programs (7504C)
EPA, 401 M St. S.W.
Washington, D.C. 20460-0001

By express:

Document Processing Desk **(RED-SRRD-PRB)**
Office of Pesticide Programs (7504C)
Room 266A, Crystal Mall 2
1921 Jefferson Davis Hwy.
Arlington, VA 22202

6. **EPA'S REVIEWS**--EPA will screen all submissions for completeness; those which are not complete will be returned with a request for corrections. EPA will try to respond to data waiver and time extension requests within 60 days. EPA will also try to respond to all 8-month submissions with a final reregistration determination within 14 months after the RED has been issued.

REREGISTRATION ELIGIBILITY DECISION

Furanone

LIST C

CASE 3138

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
SPECIAL REVIEW AND REREGISTRATION DIVISION

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FURANONE REREGISTRATION ELIGIBILITY DECISION TEAM

Office of Pesticide Programs:

Biological and Economic Analysis Division

Alan Halvorson	Economic Analysis Branch
William Gross	Biological Analysis Branch
Thomas C. Harris	Biological Analysis Branch

Environmental Fate and Effects Division

Mary Powell	Science Analysis and Coordination Staff
Nick Mastrota	Ecological Effects Branch
James Hetrick	Environmental Fate and Groundwater Branch

Health Effects Division

John Redden	Risk Characterization and Analysis Branch
Linnea Hansen	Toxicology Branch I
Winston Dang	Occupational and Residential Exposure Branch

Registration Division

Mark Perry	Registration Support Branch
Sami Malak	Registration Support Branch
Beth Edwards	Insecticide-Rodenticide Branch

Special Review and Reregistration Division

Emily Mitchell	Planning and Reregistration Branch
Barbara Briscoe	Planning and Reregistration Branch

Policy and Special Projects Staff

Jean Frane

Office of Compliance Monitoring:

Phyllis Flaherty	Pesticides Enforcement Policy Branch:

GLOSSARY OF TERMS AND ABBREVIATIONS

ADI	Acceptable Daily Intake. A now defunct term for reference dose (RfD).
AE	Acid Equivalent
a.i.	Active Ingredient
ARC	Anticipated Residue Contribution
CAS	Chemical Abstracts Service
CI	Cation
CNS	Central Nervous System
CSF	Confidential Statement of Formula
DFR	Dislodgeable Foliar Residue
DRES	Dietary Risk Evaluation System
DWEL	Drinking Water Equivalent Level (DWEL) The DWEL represents a medium specific (i.e. drinking water) lifetime exposure at which adverse, non carcinogenic health effects are not anticipated to occur.
EEC	Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.
EP	End-Use Product
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
FOB	Functional Observation Battery
GLC	Gas Liquid Chromatography
GM	Geometric Mean
GRAS	Generally Recognized as Safe as Designated by FDA
HA	Health Advisory (HA). The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.
HDT	Highest Dose Tested
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/l, mg/kg or ppm.
LD_{50}	Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
LD_{lo}	Lethal Dose-low. Lowest Dose at which lethality occurs.
LEL	Lowest Effect Level
LOC	Level of Concern
LOD	Limit of Detection
LOEL	Lowest Observed Effect Level
MATC	Maximum Acceptable Toxicant Concentration
MCLG	Maximum Contaminant Level Goal (MCLG) The MCLG is used by the Agency to regulate contaminants in drinking water under the Safe Drinking Water Act.
µg/g	Micrograms Per Gram
mg/L	Milligrams Per Liter
MOE	Margin of Exposure
MP	Manufacturing-Use Product
MPI	Maximum Permissible Intake
MRID	Master Record Identification (number). EPA's system of recording and tracking studies submitted.
N/A	Not Applicable
NOEC	No effect concentration
NPDES	National Pollutant Discharge Elimination System

GLOSSARY OF TERMS AND ABBREVIATIONS

NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OP	Organophosphate
OPP	Office of Pesticide Programs
PADI	Provisional Acceptable Daily Intake
PAG	Pesticide Assessment Guideline
PAM	Pesticide Analytical Method
PHED	Pesticide Handler's Exposure Data
PHI	Preharvest Interval
ppb	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
PRN	Pesticide Registration Notice
Q^*_1	The Carcinogenic Potential of a Compound, Quantified by the EPA's Cancer Risk Model
RBC	Red Blood Cell
RED	Reregistration Eligibility Decision
REI	Restricted Entry Interval
RfD	Reference Dose
RS	Registration Standard
SLN	Special Local Need (Registrations Under Section 24 (c) of FIFRA)
TC	Toxic Concentration. The concentration at which a substance produces a toxic effect.
TD	Toxic Dose. The dose at which a substance produces a toxic effect.
TEP	Typical End-Use Product
TGAI	Technical Grade Active Ingredient
TLC	Thin Layer Chromatography
TMRC	Theoretical Maximum Residue Contribution
torr	A unit of pressure needed to support a column of mercury 1 mm high under standard conditions.
FAO/WHO	Food and Agriculture Organization/World Health Organization
WP	Wettable Powder
WPS	Worker Protection Standard

EXECUTIVE SUMMARY

This Reregistration Eligibility Decision Document (RED) addresses the reregistration eligibility of two active ingredients: dihydro-5-pentyl-2(3H)-furanone and dihydro-5-heptyl-2(3H)-furanone (also known as gamma-nonalactone and gamma-undecalactone, respectively). Both of these active ingredients are also referred to by the common name furanone. Products containing furanones are registered for use as insecticides, repellents, mammal repellents and mosquito larvicides.

The Agency first registered a product containing furanone in 1983 for use as a cat repellent. Later, the Agency registered six additional products as insect toxicants, including use as a mosquito larvicide, or repellents. At present, there are seven furanone products, which are all registered as mixtures with the active ingredient, limonene (Case 3083). The Agency issued a Reregistered Eligibility Decision (RED) on limonene in September 1994. One of the seven products also contains a third active ingredient, Aliphatic Petroleum Hydrocarbons.

The Agency has determined that all uses of furanone products, labeled and used as specified in the RED, will not pose unreasonable risks to humans or the environment and, therefore, are eligible for reregistration.

Acute toxicity tests to assess both human toxicity and toxicity to non-target organisms were conducted on a formulated product containing : .024% dihydro-5-pentyl-2(3H)-furanone, .049% dihydro-5-heptyl-2(3H)-furanone, and 4.015% limonene. No toxicity data are available on the technical.

Acute oral, dermal, and inhalation toxicity studies indicate low acute toxicity (Category IV). Likewise, eye and dermal irritation studies also classified the formulated product in Category IV. The product also is not a skin sensitizer. Data from the open literature on each of the two compounds considered, the furanones, indicate low toxicity.

Acute toxicity data indicate that the formulated product is practically non-toxic to birds (oral, subacute dietary), mammals, and freshwater fish. The product is slightly toxic to freshwater invertebrates.

The furanones, when used in mosquito larvicides, are applied directly to water (residential and large scale public health use.) The public health larvicidal use of furanones applied at 4.10 lbs/A to 6 inches of water results in exceedances of the level of concern for freshwater invertebrates. The LOC for endangered species is exceeded when the product is applied at 1.64 lbs/A in water 6 inches deep or less, or when it is applied at 4.10 lbs/A in water 1 foot deep or less. This assessment is based on testing done on a formulated product and tells little about the actual toxicity of the furanones as active ingredients. Chronic invertebrate toxicity data (life cycle test with a freshwater invertebrate) and basic environmental fate data

would improve the Agency's understanding and assessment of the potential risk posed by the use of the furanones in mosquito larvicides.

The annual volume of products containing furanones that are used as mosquito larvicides is low and the percentage of furanone in the products is also low. The low volume used and low percent of furanone support the conclusion that widespread adverse impacts are not likely to result from the mosquito larvicide use if continued at the amounts currently produced and used (volume and percent furanone in the product). The Agency, in this RED document, is imposing a production limit on furanone for use in mosquito larvicide of 150 gallons per year. Should the volume produced and used and/or the percent of furanone in the product significantly increase, the Agency may impose additional data requirements to understand and assess potential risks.

It should be recognized that the risk posed to aquatic invertebrates is common to most, if not all, products for this use. It is the nature of mosquito larvicides to be harmful to aquatic invertebrates because the target species is itself an aquatic invertebrate. The mosquito larvicide product containing furanones would cause less harm to aquatic ecosystems than many other products because its risk is limited to aquatic invertebrates, whereas others pose a risk to fish and birds as well.

Before reregistering the products containing furanone, the Agency is requiring that product specific data, revised Confidential Statements of Formula (CSF) and revised labeling be submitted within eight months of the issuance of this document. These data include product chemistry for each registration and acute toxicity testing. After reviewing these data and any revised labels and finding them acceptable in accordance with Section 3(c)(5) of FIFRA, the Agency will reregister a product. Those products which contain other active ingredients will be eligible for reregistration only when the other active ingredients are determined to be eligible for reregistration.

I. INTRODUCTION

In 1988, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. The amended Act provides a schedule for the reregistration process to be completed in nine years. There are five phases to the reregistration process. The first four phases of the process focus on identification of data requirements to support the reregistration of an active ingredient and the generation and submission of data to fulfill the requirements. The fifth phase is a review by the U.S. Environmental Protection Agency (referred to as "the Agency") of all data submitted to support reregistration.

FIFRA Section 4(g)(2)(A) states that in Phase 5 "the Administrator shall determine whether pesticides containing such active ingredient are eligible for reregistration" before calling in data on products and either reregistering products or taking "other appropriate regulatory action." Thus, reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential hazards arising from the currently registered uses of the pesticide; to determine the need for additional data on health and environmental effects; and to determine whether the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA.

This document presents the Agency's decision regarding the reregistration eligibility of the registered uses of furanone. The document consists of six sections. Section I is the introduction. Section II describes furanone, its uses, data requirements and regulatory history. Section III discusses the human health and environmental assessment based on the data available to the Agency. Section IV presents the reregistration decision for furanone . Section V discusses the reregistration requirements for furanone . Finally, Section VI is the Appendices which support this Reregistration Eligibility Decision. Additional details concerning the Agency's review of applicable data are available on request.

II. CASE OVERVIEW

A. Chemical Overview

The following active ingredient(s) are covered by this Reregistration Eligibility Decision:

- **Common Name:** Furanone
- **Chemical Name:** Dihydro-5-pentyl-2(3H)-furanone
- **Alternate Name:** gamma-nonalactone
- **CAS Registry Number:** 104-61-0
- **OPP Chemical Code:** 122301
- **Empirical Formula:** $C_9H_{16}O_2$
- **Trade and Other Names:** Doo-Not
- **Basic Manufacturer:** Rod Products Company

- **Common Name:** Furanone
- **Chemical Name:** Dihydro-5-heptyl-2(3H)-furanone
- **Alternate Name:** gamma-undecalactone
- **CAS Registry Number:** 104-67-6
- **OPP Chemical Code:** 122302
- **Empirical Formula:** $C_{11}H_{20}O_2$
- **Trade and Other Names:** Doo-Not
- **Basic Manufacturer:** Rod Products Company

B. Use Profile

The following is information on the currently registered uses for furanone. A detailed table of eligible uses as well as the methods, application rates, and use restrictions is included in Appendix A.

For: **dihydro-5-pentyl-2(3H) furanone**
For: **dihydro-5-heptyl-2(3H) furanone**

Type of Pesticide: Insecticide/repellent, mammal repellent, mosquito larvicide

Currently registered products, containing these chemicals, have the following uses:

1) dog and cat repellent;
2) fly, cockroach and ant killer;
3) insect repellent;
4) insect repellent strip;
5) insect repellent tablecloth; and
6) mosquito larvicide (contains mineral oil).

Use Sites: Terrestrial Non-Food & Outdoor Residential

Target Pests: cats, dogs, house flies, cockroaches, ants, biting flies, mosquito adults and larvae

Formulation Types Registered:
Granular
Liquid Ready-to-use
Impregnated Material
Emulsifiable Concentrate
Pressurized Liquid

Percentage of the three AIs in all formulations

Active Ingredient	Weight Percentage in formulations
d-Limonene	4.015%
gamma-Undecalactone	0.049%
gamma-Nonalactone	0.024%

There are no formulations that contain the furanones alone.

Method and Rates of Application:

Equipment - Hand, pre-moistened applicator puffs, aerosol sprayers, non-aerosol hand pumped sprayers, hydraulic ground sprayers, aircraft, compressed air sprayers and knapsack.

Method and Rate - Furanone may be applied by the use of impregnated table cloths, premoistened applicator puffs, aersol sprayers, non-aerosol hand pumped sprayer, knapsack sprayers, and the granules may be applied by hand by sprinkling from the bag.

Some product labels do not provide adequate information to calculate rates; however, for those that can be calculated they are as follows:
granules are applied at rates of 0.002 lb ai/1000 square feet, and for the emulsifiable concentrate for mosquito larvae control, the rates range from 0.0393 to 0.0984 lb ai/acre.

Timing - Product labels do not give specific timing of application of this product. The language reads: "when needed."

Use Practice Limitations: Do not apply directly to treated, finished drinking water reservoirs or drinking water receptacles. Avoid application to man-made surfaces sensitive to mineral oils.

C. Estimated Usage of Pesticide

This section summarizes the best estimates available for the pesticide uses of **furanone**. These estimates are derived from a variety of published and proprietary sources available to the Agency. The data, reported on an aggregate and site (crop) basis, reflect annual fluctuations in use patterns as well as the variability in using data from various information sources.

The table below summarizes the pesticides use by site.

Estimated Typical Annual Usage of Furanone (Dihydro-5-pentyl-2(3H)-furanone and 5-Heptyldihydro-2(3H)-furanone)

	Site Available (000)	Site Treated		Lbs a.i. Applied
		(000)	(%)	
Drainage systems	na	na	<1	na
Eating establ incl servg areas	na	na	<1	na
Flooded areas, intermittent	na	na	<1	na
Food marktg/storg/distr facils	na	na	<1	na
Food proc/hndl/stor plts/areas	na	na	<1	na
Garbage dumps/manure	na	na	<1	na
Households, indoor/outdoor	90,000H	<90H	<1	<10
Human body/hair/clothg/footwr	250,000P	<250P	<1	<10
Lakes/ponds/impounded water	na	na	<1	na
Ornam herbaceous plants/lawns	30,000A	<30A	<1	<10
Ornam ponds/fountains/aquaria	na	na	<1	na
Paved areas	na	na	<1	na
Recreation areas	na	na	<1	na
Refuse/solid waste containers	na	na	<1	na
Swamps/marshes/bogs/stdg water	na	na	<1	na
Wide area/gen indr/outdr trtmt	na	na	<1	na
TOTAL	na	na	na	<40

---

na = not available or not applicable
H = households
P = persons
A = acres

Note - Some sites overlap.

Sources --
- Kline & Co., Consumer Markets for Pesticides & Fertilizers, 1990 Update.
- Kline & Co., Professional Markets for Pesticides & Fertilizers, Year 1: 1990 & 1992.
- Research Triangle Institute, National Home & Garden Pesticide Use Survey, 1992.

In the table, "site available" refers to the quantity of a site that potentially could be treated with pesticides: for example, all 90 million households (indoor, outdoor or both), the entire population of about 250 million people (with potential treatments to themselves and/or their clothing or footwear) and 30 million acres of lawns and herbaceous ornamentals. Usage data is sparce, but indicates that typically furanone is used on less than one percent of the total households, less than one percent of the total population and less than one percent of the total lawn and herbaceous ornamental acreage. Although data are lacking for other labeled sites, their treatments with furanone are also likely to be less than one percent per site. For example,

probably considerably less than one percent of available recreation areas, however measured, are treated with furanone.

D. Data Requirements

Data requested in the Phase IV Data Call-In Notice(s) for **furanone** (dihydro-5-pentyl-2(3H)-furanone and dihydro-5-heptyl-2(3H)-furanone) include studies on **product chemistry and environmental fate**. These data were required to support the uses listed in the Phase IV Data Call-In Notice(s), dated July 27, 1992. Appendix B includes all data requirements identified by the Agency for currently registered uses needed to support reregistration.

E. Regulatory History

The Agency first registered a product containing furanone in 1983 for use as a dog and cat repellent. Later, the Agency registered six additional products as insect toxicants, including use as a mosquito larvicide, or repellents. At present, there are seven furanone products, which are all registered as mixtures with the active ingredient, limonene (Case 3083). The Agency issued a Reregistration Eligibility Decision (RED) on limonene in September 1994. One of the seven products also contains a third active ingredient, Aliphatic Petroleum Hydrocarbons.

The compounds dihydro-5-heptyl-2(3H)-furanone (also known as gamma-undecalactone) and dihydro-5-pentyl-2(3H)-furanone (also known as gamma-nonalactone) are compounds that appear on the 'GRAS' ("generally recognized as safe") list of the Expert Panel of the Flavoring Extract Manufacturers' Association of the United States (FEMA). These same compounds are not on the FDA 'GRAS' list, but are approved as food additives by the FDA.

III. SCIENCE ASSESSMENT

A. Physical Chemistry Assessment

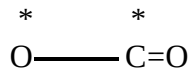
The following physical/chemical property information was extracted from MRID No. 240229307 (Product Chemistry) and is supplied as background material, and from two monographs in Food and Cosmetics Toxicology (FD Cosmet Toxicol Vol. 12, 889 and 921 1975).

The data for the active ingredients were extracted from the Food Chemicals Codex as set forth on pages 456, 457, 844, 845, 553 and 554. The first active, dihydro-5-heptyl-2(3H)-furanone, is referred to as Undecanoic acid, 4-hydroxy, gamma-lactone by the registrant. The active ingredient product name is **gamma-Undecalactone**. The second active dihydro-5-pentyl-2(3H)-furanone (on submitted material this active ingredient is

referred to as: 2(3H)-furanone dihydro-5-pentyl; MW=156.25) is synonymous to **gamma-Nonalactone** (active ingredient product name).

dihydro-5-heptyl-2(3H)-furanone Properties

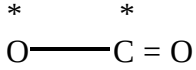
- M.W. - 184.31
- Color - colorless to yellow
- Odor - strong fruity odor suggestive of peach
- Melting point - liquid, not applicable
- Solubility - 60% alcohol in water - 20% soluble
  70% alcohol in water - 33% soluble
  Insoluble in water
- Stability - stable under all normal conditions
- Octanol/water partition coefficient - not available
- Physical state - liquid
- Density - 0.942 - 0.945
- Vapor Pressure - not available
- pH - not available
- Structural Formula:



Extracted from Food and Cosmetics Toxicology Volume 13, 921, 1975.

dihydro-5-pentyl-2(3H) furanone Properties

- M.W. - 156.25
- Color - colorless to yellow
- Odor - strong odor suggestive of coconut
- Melting point - liquid, not applicable
- Solubility - 50% alcohol in water - 20% soluble
  60% alcohol in water - 33% soluble
  Insoluble in water
- Stability - stable under all normal conditions
- Octanol/water partition coefficient - not available
- Physical state - liquid
- Density - 0.958 - 0.966
- Boiling point - 243°C
- Vapor Pressure - not available
- pH - not available
- Structural Formula: $CH_3(CH_2)_4\ CH\ CH_2CH_2$



Extracted from Food and Cosmetics Toxicology Volume 13, 889, 1975.

B. Human Health Assessment

1. Toxicology Assessment

The toxicological data base on furanones is adequate to support reregistration eligibility for dihydro-5-pentyl-2(3H)-furanone (PC CODE: 122301 synonymous to gamma-Nonalactone) and dihydro-5-heptyl-2(3H)-furanone (PC CODE: 122302 synonymous to gamma-Undecalactone).

a. Acute Toxicity

ACUTE TOXICITY DATA FOR FURANONES

ACUTE TOXICITY DATA

TEST	RESULTS	CATEGORY
Oral LD50--rat, Monogram Animal Repellent #100(Limonene) [1]	LD50 > 5 gm/kg	IV
Dermal LD50--rabbit, Monogram Animal Repellent #100(Limonene)[1]	LD50 of dry formulation > 5 gm/kg	IV
Inhalation LC50--rat, Monogram Animal Repellent #100(Limonene)[1]	LC50 > 5 mg/l, using particle size of 297 microns or less.	IV
Eye irritation--rabbit, Monogram Animal Repellent #100(Limonene)[1]	PIS - Washed = 0.0. PIS - Unwashed = 0.0	IV
Dermal irritation--rabbit, Monogram Animal Repellent #100(Limonene)[1]	PIS = 0.0 Primary Irritation Score	IV
Dermal sensitization--Guinea pig, Monogram Animal Repellent #100(Limonene)[1]	No sensitization using draize technique	----

1: At this time there are no products that contai n Furanones as the sole active ingredient. All formulations containing furanones also contain limonene.

It should be noted that only acute studies on formulations were conducted to support the reregistration of gamma-Undecalactone and gamma-Nonalactone. There are no toxicity data available on the technical. Toxicity data on the two compounds which comprise the furanones are available from the open literature and are discussed below:

The following was extracted from two monographs that appeared in Food and Cosmetics Toxicology (FD. Cosmet. Toxicol. Vol. 12, pp 889 and 921, 1975).

gamma Nonalactone:

"The acute oral LD_{50} in rats and guinea pigs was reported as 9.78 and 3.44 g/kg, respectively (Jenner, Hagan, Taylor, Cook & Fitzhugh, 1964). The acute oral LD_{50} in rats was reported as 6.6 g/kg (5.8-7.4 g/kg) (Moreno, 1972a). The acute dermal LD_{50} in rabbits exceeded 5 g/kg (Moreno, 1972b)."

Based on this information the toxicity category established in both rat studies, for nonalactone, would be IV for acute oral toxicity. The toxicity category based on the guinea pig acute oral LD_{50} study would be III for nonalactone.

"Nonalactone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was slightly irritating (Moreno, 1973b). Tested at 10% in petrolatum. It produced no irritation after a 48 hr closed-patch test on human subjects (Kligman, 1972). A maximization test (Kligman, 1966: Kligman & Epstein, 1975) was carried out on 25 volunteers. The material was tested at a concentration of 10% in petrolatum and produced no sensitization reactions (Kligman, 1972)."

gamma Undecalactone:

"The acute oral LD_{50} in rats was reported as 18.5 g/kg (Jenner, Hagan, Taylor, Cook & Fitzhugh. 1964). Fatty infiltration of liver parenchymal cells occurred in rats fed 13-115 mg gamma-undecalactone for 5-9 days (Shillinger, 1950)."

"Gamma-Undecalactone tested at 2% in petrolatum produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1971). A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material was tested at a concentration of 2% in petrolatum and produced no sensitization reactions (Kligman, 1971)."

The acute oral LD_{50} in rats for undecalactone would be classified as toxicity category IV. The studies on undecalactone are classified as **Supplementary** as they are from the open literature.

However, they offer valuable information for the evaluation of the toxicity of the furanones because these data indicate that the two compounds that make up the furanones are of low acute toxicity.

b. Subchronic Toxicity

In an open literature study (Fd Cosmet. Toxicol. Vol. 3, pp. 563-569. Pergamon Press 1965) gamma-Undecalactone and gamma-Nonalactone were two of 23 flavoring compounds that were examined in subchronic feeding studies.

Fifteen female FDRL and fifteen male FDRL rats were fed the following for 90 days: females were fed 14.6 mg/kg body weight/day gamma-undecalactone and 62.8 mg/kg body weight/day gamma-nonalactone; males were fed 16.5 and 72.5 mg/kg body weight/day gamma-undecalactone and gamma-nonalactone, respectively.

Body weight, food consumption, haematological and blood chemical determinations were made on 8 rats of each sex after 6 weeks of dosing, and in all rats at 12 weeks. The study was terminated after 90 days and the rats were necropsied. Liver and kidney weights were recorded for all rats. The following organs from half the animals in each group were taken for histological examinations: liver, kidneys, stomach, small and large intestines, spleen, pancreas, heart, lungs, bone marrow, muscle, brain, spinal cord, bladder, adrenals, thyroid, pituitary, gonads, salivary glands, and lymph nodes.

The study authors conclusions are presented below and the Agency agrees with their assessment:

> "No significant gross pathological change was observed at autopsy in any rats...Several of the animals in the groups receiving the lactones (L,M,N)....show various degrees of mild thyroid hyperplasia but this observation was also noted in the corresponding control groups and is not uncommon finding in these laboratories."
>
> "90-Day feeding studies in groups of rats receiving dietary doses of any of 23 different flavouring matters at levels corresponding to at least 100 times the maximum estimated human dietary levels, revealed no evidence of adverse toxic effects."

Since this is an open literature study it must be core graded **Supplementary**. However, it supplies useful information in evaluating the potential hazard of the furanones in that compounds making up the

furanones did not demonstrate a hazard following subchronic oral administration.

c. Chronic toxicity

The following was extracted from two monographs that appeared in Food and Cosmetics Toxicology (FD. Cosmet. Toxicol. Vol. 12, pp 889 and 921, 1975).

Nonalactone:

> "...In another study 0.1-0.5% fed to groups of 20 male and female rats in the diet for 2 yr produced no effects (Bar & Griepentrog. 1967)."

Undecalactone:

> "...Groups of 20 male and 20 female rats were fed diets containing 0.1 or 0.5% gamma-undecalacone for 2 yr without any specific adverse effects (Bar & Griepentrog. 1967)."

These studies are classified as **Supplementary** as they appear in the open literature. However, they offer valuable information for the determination of the toxicity of the furanones in that the compounds making up the furanones did not demonstrate a hazard following chronic oral exposure.

d. Carcinogenicity

No effects were observed in a 2 year rat study where rats were administered 0.10% to 0.5% furanones in their diet (Bar. F. U. Griepentrog. F. (1967)).

e. Other Toxic Endpoints

The Agency has determined that a 90-day dermal toxicity (safety) study would be required for a product that may result in prolonged human dermal exposure through repeated skin applications. The Agency has decided to waive the requirement for the 90-day dermal toxicity for the furanones based on the following : a combination of low exposure to furanones in the product; comparable concentrations of tanols in this product to those already used in cosmetic products (lotions, detergents,

perfumes); naturally occurring compound and absence of toxicity in the toxicology studies (notwithstanding limited data). Also, although not a FDA GRAS chemical, furanone is approved by FDA as a food additive and exposures to low concentrations are considered safe.

f. Reference Dose

There are no registered food uses for the furanones. The Agency's Peer Review Committee has not considered the furanones. The Joint FAO/WHO Expert Committee on Food Additives established an unconditional ADI of 0-1.25 mg/kg for undecalactone and nonalactone.

2. Exposure Assessment

a. Occupational and Residential

For the following reasons the Agency has no concerns for occupational or residential exposure to the furanones: 1) the acute battery for the furanones indicate toxicity category IV; and 2) the furanones are only in formulations containing limonene and the percentage in formulations is less than 1%.

However, there is a concern that repeated high dose dermal exposure to limonene may cause dermal sensitization based on a two week preliminary study for a rat 90 day dermal toxicity study. The data demonstrated that dermally applied technical limonene at 500, 1000, or 1500 mg/kg/day produced excessive dermal irritation after 1-3 days of dermal application. No overt treatment related systemic toxicity or clinical signs were observed.

Since these effects occur after repeated high dose administration of limonene, for purposes of reregistration, the following from the Limonene RED applies to the tanol derivatives:

> "The primary toxicological concerns for humans are dermal irritation and/or sensitization from dermal exposure at high concentrations. Systemic toxicity is not expected to occur from pesticide uses since dermal irritation, which occurs at high doses, results in self-discontinuation of product use."

Thus, since the most noted dermal effect would be self limiting dermal irritation resulting from the limonene and not the furanones, the Agency does not anticipate occupational or residential risks of concern from exposure to the furanones.

3. Risk Assessment

a. Occupational and Residential

For the reasons discussed above, the Agency does not anticipate occupational or residential risks of concern from exposure to the furanones. However, as the furanones are only in formulations containing limonene, the following from the limonene RED applies to the furanones:

> "Human exposure may occur during application of animal repellent granules or insect spray, or use of impregnated tablecloths. The tablecloth product containing limonene to repel insects was exempted from tolerances. Toxicological concerns for humans from exposure to limonene are dermal irritation and sensitization. Systemic toxicity is not anticipated to occur at doses below the threshold for dermal irritation. Exposure to limonene would be discontinued if dermal irritation occurred and therefore self-limiting. Ocular irritation may also occur if products are accidently placed in the eye and not washed away."
>
> "Additional precautionary statements on label are required to reduce adverse effects."

C. Environmental Assessment

1. Ecological Toxicity Data

The term "furanones" will be used to refer to the two related chemicals covered by this RED: dihydro-5-pentyl-2(3H)-furanone and 5-heptyldihydro-2(3H)-furanone. All of the formulated products containing these furanones also contain a third active ingredient, limonene. All of the toxicity data for furanones are for one formulated product, Doo-Not Dog & Cat Repellent (Reg. No. 45987-1), containing the three active ingredients in the following concentrations:

PC Code	Name	% A.I.
122301	Dihydro-5-pentyl-2(3H)-furanone	0.024%
122302	5-heptyldihydro-2(3H)-furanone	0.049%
079701	Limonene	4.015%

The other registered formulations containing furanones contain the same set of active ingredients as Doo-Not in identical concentrations, although Rodspray Mosquito Larvicide (Reg. No. 45987-6) also contains 22.94% white mineral oil as a fourth active ingredient.

There are adequate data to assess the hazard of the formulated product Doo-Not, as well as other products that contain furanones in identical concentrations as Doo-Not and have low-volume minor uses. Only the six basic studies are required for these use patterns because they are expected to result in low exposure to the environment. The mosquito larvicide use is exceptional in that it may result in relatively large exposures to aquatic habitats. An additional chronic aquatic invertebrate study on the technical grade of the active ingredient is needed to clarify the Agency's understanding and assessment of the potential risk posed by the use of furanone as a mosquito larvicide.

a. Toxicity to Terrestrial Animals

(1) Birds, Acute and Subacute

To establish the toxicity of furanones to birds, the following tests are required using the technical grade material: one avian single-dose oral (LD_{50}) study on one species (preferably mallard or bobwhite quail); two subacute dietary studies (LC_{50}) on one species of waterfowl (preferably the mallard duck) and one species of upland game bird (preferably bobwhite quail). Data that have been submitted for these three tests have been with a formulated product, not the technical grade material. The following tables show the results of these tests.

Avian Acute Oral Toxicity Findings					
Species	**% A.I.** [a]	**LD_{50} mg/kg**	**MRID No. Author/Year**	**Toxicity Category**	**Fulfills Guideline Requirement**
Northern Bobwhite	4.015% 079701 0.024% 122301 0.049% 122302	> 2000	109340 Bottoms, J. (1982)	The formulated product is practically nontoxic	Yes, for the formulated product Doo-Not Dog Repellent

[a] Active ingredients are listed by their Shaughnessey numbers.

Avian Subacute Dietary Toxicity Findings					
Species	**% A.I.**[a]	**LC_{50} ppm**	**MRID No. Author/Year**	**Toxicity Category**	**Fulfills Guideline Requirement**
Northern Bobwhite	4.015% 079701 0.024% 122301 0.049% 122302	>5000	109342 Bottoms, J. (1982)	The formulated product is practically nontoxic	Yes, for the formulated product Doo-Not Dog Repellent
Mallard	4.015% 079701 0.024% 122301 0.049% 122302	>5000	109341 Bottoms, J. (1982)	The formulated product is practically nontoxic	Yes, for the formulated product Doo-Not Dog Repellent

[a] Active ingredients are listed by their Shaughnessey numbers.

These results indicate that the formulated product Doo-Not Dog Repellent is practically nontoxic to avian species on an acute oral and subacute dietary basis (MRID 109340-109341). However, since this product contains only a small percent of active ingredient, it cannot be inferred from these results that furanones are practically nontoxic. If the furanones caused all of the toxicity of the formulated product, an estimated acute oral LD_{50} of the furanones for avian species would be >1.46 mg ai/kg, and an estimated subacute dietary LC_{50} would be >3.65 ppm ai. These results tell little about the actual toxicity of the furanones as active ingredients. Furanones may fall into any of five toxicity classifications.

(2) Mammals

Wild mammal testing is required on a case-by-case basis, depending on the results of the lower tier studies such as acute and subacute testing, intended use pattern, and pertinent environmental fate characteristics.

The oral rat LD_{50} for Doo-Not Dog & Cat Repellent (Reg. No. 45987-1) is >5 g/kg (Accession No. 245190). This result indicates that this formulated product is practically nontoxic, but it tells little about the toxicity of furanones as active ingredients. If all of the toxicity of the formulated product was caused by the furanones, an estimated acute oral LD_{50} of the furanones for avian species would be >3.65 mg ai/kg. Furanones thus may fall into any of five toxicity classifications.

b. Toxicity to Aquatic Animals

(1) Freshwater Fish

To establish the toxicity of a pesticide to freshwater fish, the minimum data required on the technical grade of the active ingredient are two freshwater fish toxicity studies. One study should use a coldwater species (preferably the rainbow trout), and the other should use a warmwater species (preferably the bluegill sunfish). Data that have been submitted for these two tests have been with a formulated product, not the technical grade material. The following table shows the results of these tests.

Freshwater Fish Acute Toxicity Findings

Species	% A.I.[a]	LC_{50} ppm a.i.	MRID No.	Toxicity Category	Fulfills Guideline Requirement
Rainbow trout	4.015% 079701 0.024% 122301 0.049% 122302	569[b]	109343	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent
Fathead minnow	4.015% 079701 0.024% 122301 0.049% 122302	1490[b]	109344	The formulated product is practically nontoxic	Supplemental, for the formulated product Doo-Not Dog Repellent

[a] Active ingredients are listed by their Shaughnessey numbers.
[b] Result is based on the moving average method.

The results of the 96-hour acute toxicity studies indicate that the formulated product is practically nontoxic to fish (MRID 109343-109344). However, since this product contains only a small percent of active ingredient, it cannot be concluded from these results that furanones are practically nontoxic to fish. Assuming that all of the toxicity of the formulated product was caused by the furanones, the estimated acute LC_{50} of the furanones is >0.42 ppm a.i. for the rainbow trout and >1.1 ppm a.i. for the fathead minnow. These results tell little about the toxicity of the furanones as active ingredients. Furanones could fall into any

toxicity classification except very highly toxic. (MRID No. 109343)

Because the tests were conducted with formulated product rather than technical grade active ingredients, they do not fulfill the guideline requirements for furanones. However, these data are considered adequate for assessing the risk of the use of the Doo-Not product. They may also be adequate for other low-use products that contain furanones in identical concentrations.

(2) Freshwater Invertebrates

The minimum testing required to assess the hazard of a pesticide to freshwater invertebrates is a freshwater aquatic invertebrate toxicity test, preferably using first instar *Daphnia magna* or early instar amphipods, stoneflies, mayflies, or midges. Data that have been submitted for a test with *Daphnia magna* have been with a formulated product, not the technical grade material. The table below gives the results of these tests.

Freshwater Invertebrate Toxicity Findings

Species	% A.I.[a]	EC_{50} (ppm)	MRID NO. Author/Year	Toxicity Category	Fulfills Guideline Requirement
Daphnia magna	4.015% 079701 0.024% 122301 0.049% 122302	17[b]	109345 Bottoms, J. (1982)	The formulated product is slightly toxic	Supplemental, for formulated product Doo-Not Dog Repellent

[a] Active ingredients are listed by their Shaughnessey numbers.

[b] Result is based on the moving average method.

There is sufficient information to characterize the formulated product containing furanones as slightly toxic to aquatic invertebrates (MRID 109345). However, since this product contains only a small percent of active ingredient, it cannot be inferred from these results that furanones are slightly toxic to freshwater invertebrates. Assuming that all of the toxicity of the formulated product was caused by the furanones, the estimated acute LC_{50} of the furanones is >0.012 ppm a.i. for *Daphnia magna*. These results probably overestimate the toxicity of furanone because much of the toxicity of the formulated product could be the result of the other active ingredient, limonene, or of inert ingredients. These results tell little about the actual toxicity of the furanones as active ingredients. Furanones may fall into any of five toxicity classifications (MRID 109345).

Because the tests were conducted with formulated product rather than technical grade active ingredients, they do not fulfill the guideline requirements for furanones. However, these data are considered adequate for assessing the risk of the use of the Doo-Not product. They may also be adequate for other low-use products that contain furanones in identical concentrations.

A life-cycle test with a freshwater invertebrate (guideline 72-4b) is needed for the mosquito larvicide use of furanones because it involves direct application to water and because some of the acute risk quotients for freshwater invertebrates are greater than 0.01. This guideline requirement is not fulfilled.

2. Environmental Fate Assessment

Environmental fate data are not required to support low-volume outdoor residential uses for the furanones. Environmental fate data generally are required to support aquatic nonfood uses. To complete an ecological risk assessment for the mosquito larvicide use, the Agency needs the following data to assess the dissipation of the furanones in the environment:

161-1, Hydrolysis
161-2, Photodegradation in Water
162-3, Anaerobic Aquatic Metabolism
162-4, Aerobic Aquatic Metabolism
163-1, Leaching/Adsorption-Desorption
164-2, Aquatic Field Dissipation

A quantitative environmental fate assessment for the furanones cannot be made at this time because no environmental fate data have been submitted for review. However, the furanones are classified as lactones, and some open literature data are available on physiochemical properties of lactones and their possible effect on the environment.

Lactones are cyclic esters that can be synthesized by acidification of alpha-hydroxy acids. Detoxification mechanisms of lactones can be accomplished through base-catalyzed hydrolysis or microbial-mediated degradation of reactive groups. De-esterification may be catalyzed by metal ions, enzymes, and nucleophilic attack. The reported data indicate lactones may not be stable in alkaline environments.

3. Exposure and Risk Characterization

a. Ecological Exposure and Risk Characterization

Explanation of the Risk Quotient (RQ) and the Level of Concern (LOC): The Levels of Concern are criteria used to indicate potential risk to nontarget organisms. The criteria indicate that a chemical, when used as directed, has the potential to cause undesirable effects on nontarget organisms. There are two general categories of LOC (acute and chronic) for each of the four nontarget faunal groups and one category (acute) for each of two nontarget floral groups. In order to determine if an LOC has been exceeded, a risk quotient must be derived and compared to the LOCs. A risk quotient is calculated by dividing an appropriate exposure estimate, e.g. the estimated environmental concentration (EEC), by an appropriate toxicity test effect level, e.g. the LC_{50}. The acute effect levels typically are:

- EC_{25} (terrestrial plants),
- EC_{50} (aquatic plants and invertebrates),
- LC_{50} (fish and birds), and
- LD_{50} (birds and mammals)

The chronic test results are the:

-NOEL (or NOEC) for avian and mammal reproduction studies, and either the NOEL for chronic aquatic studies, or the Maximum Allowable Toxicant Concentration (MATC), which is the geometric mean of the NOEL and the LOEL (or LOEC) for chronic aquatic studies.

When the risk quotient exceeds the LOC for a particular category, risk to that particular category is presumed to exist. Risk presumptions are presented along with the corresponding LOCs.

Levels of Concern (LOC) and associated Risk Presumption

Mammals, Birds		
IF THE	LOC	PRESUMPTION
acute RQ>	0.5	High acute risk
acute RQ>	0.2	Risk that may be mitigated through restricted use
acute RQ>	0.1	Endangered species may be affected acutely
chronic RQ>	1	Chronic risk, endangered species may be affected chronically,
Fish, Aquatic invertebrates		
IF THE	LOC	PRESUMPTION
acute RQ>	0.5	High acute risk
acute RQ>	0.1	Risk that may be mitigated through restricted use
acute RQ>	0.05	Endangered species may be affected acutely

Mammals, Birds		
chronic RQ>	1	Chronic risk, endangered species may be affected chronically
Plants		
IF THE	LOC	PRESUMPTION
RQ>	1	High risk
RQ>	1	Endangered plants may be affected
Currently, no separate criteria for restricted use or chronic effects for plants exist.		

4. Exposure and Risk to Nontarget Terrestrial Animals

a. Birds

Birds may ingest granules of the product Doo-Not. They may also be exposed by other routes, such as dermal exposure when walking on top of granules. The number of lethal doses (LD_{50}s) that are available on one square foot immediately after application is normally used to assess the risk from such exposure. However, the available toxicity data are inadequate for this approach because definitive LD_{50} values are not available.

The only acceptable study on acute effects to birds used the formulated product Doo-Not. This study determined only that the LD_{50} is something greater than 2000 mg/kg. This information is inadequate to assess quantitatively the risk of Doo-Not to birds.

Although the risk of furanones to avian species cannot be quantified, several factors indicate that the risk is small. First, there was no mortality or signs of toxicity to birds that received the relatively high dose of 2000 mg/kg. Birds would need to consume more than 2000 mg/kg of this product before toxic effects may be expected. This is a relatively large quantity of granules for a bird to consume. Second, Doo-Not is labeled for use on lawns and ornamental turf around homes. It is likely to be applied only to small isolated areas. Any risk to birds that may result would be small in terms of area exposed and number of birds affected. Third, the formulation repels mammals and insects, and thus is probably somewhat repellent to birds as well. In conclusion, it is reasonable to assume that normal use of this product would not cause sufficient exposure to birds to result in any significant ecological damage.

Birds also could be exposed to furanones if they drink from bodies of water treated with Rodspray Mosquito Larvicide (Reg. No. 45987-6). The EECs in water immediately following application of the mosquito larvicide are not greater than 3.01 ppm (see section 3.a.2). In avian dietary toxicity tests with Doo-Not, a product that contains the same concentrations of furanones as the mosquito larvicide, the dietary LC_{50} for

both the bobwhite and mallard were greater than 5000 ppm. While there is uncertainty associated with comparing the toxicity of different formulated products and using a dietary toxicity test to assess the risk from drinking contaminated water, the huge difference (>1000 fold) between the EEC and the concentrations that may cause effects is large enough to safely presume minimal risk from this type of exposure.

Other products that contain furanones are expected to result in negligible exposure to birds. The Rodspray Fly, Cockroach, & Ant Killer (Reg. No. 45987-2) is sold in a pump-spray bottle and is designed for application to very small areas indoors and outdoor premises of domestic dwellings. Homeowners would not be likely to use this product on wildlife food. The other products are used as insect repellents and would not result in exposure to birds.

b. Mammals

Mammals may be exposed to furanones by consuming the granular product Doo-Not Dog & Cat Repellent (Reg. No. 45987-1) and by exposure to residues of the liquid product Rodspray Fly, Cockroach, & Ant Killer (Reg. No. 45987-2). An acute oral toxicity study with Doo-Not showed that no mortality occurred at doses as great as 5000 mg/kg. The fly, cockroach, & ant killer product has the same concentration of furanones as Doo-Not. Because of this apparent lack of toxicity of the formulated products containing furanone, and because these formulations repel mammals, it is anticipated that mammals will not be exposed to large enough quantities to cause any significant ecological damage. The EECs of the formulated product Rodspray mosquito larvicide are not expected to exceed 3.01 ppm. This concentration is too small to cause any appreciable risk to mammals that may drink treated water. The use pattern of other products should not lead to any appreciable exposure to mammals. Thus, the currently registered products containing furanones pose minimal risk to wild mammals.

c. Insects

None of the products containing furanones are used in a manner that is expected to cause any appreciable exposure to honey bees.

5. Exposure and Risk to Nontarget Aquatic Animals

With the exception of Rodspray Mosquito Larvicide (Reg. No. 45987-6), all registered products containing furanones should pose very little exposure to

aquatic organisms when used as directed. These products, which are for domestic use only, are used to repel dogs and cats, repel insects from human skin, and kill household insect pests. The dog and cat repellent use and insecticide use are for spot treatment of very small areas around homes, such as compost piles and garbage pails. Considering that the toxicities of these formulations are at most only slightly toxic to aquatic organisms, the risk they pose to aquatic organisms is minute. The potential of exposure to aquatic organisms is much greater with the mosquito larvacide use because it is applied directly to bodies of water. Furthermore, it is registered for public use as well as household use, and thus may be applied to much larger areas than the other products. The risk that the mosquito larvacide use poses to aquatic organisms is discussed in detail below.

Expected Aquatic Concentrations: The toxicity of furanones to aquatic organisms is not known. However, data on the formulated product Doo-Not (Reg. No. 45987-1) indicate that it is practically nontoxic to freshwater fish and slightly toxic to freshwater invertebrates. This formulated product contains the same concentration of furanones as the mosquito larvicide (0.024% dihydro-5-pentyl-2(3H)-furanone and 0.049% 5-heptyldihydro-2(3H)-furanone). Therefore, aquatic risk will be assessed by comparing the expected environmental exposure to the larvicide formulated product to the toxicity of the Doo-Not formulated product. This risk assessment approach is less than ideal because the larvicide contains an additional active ingredient, mineral oil, as well as different inert ingredients than Doo-Not.

The following table summarizes the EECs for the mosquito larvicide.

ESTIMATED ENVIRONMENTAL CONCENTRATIONS (EECs)					
Use Type	Application Method	Application Rate	Initial EEC (ppm)		
			6 inches	1 foot	6 feet
Mosquito Larvicide, Domestic Use	Pour-on	1.64 lb/A (2 gal/A)	1.200	0.603	0.100
Mosquito Larvicide, Public Health Use	Spray	4.10 lb/A (5 gal/A)	3.010	1.510	0.251

6. Freshwater Fish

The following table summarizes the risk quotients (EEC/LC_{50}) for freshwater fish.

Risk Quotients (RQ) for Freshwater Fish				
Crop/application rate	Species	Acute Risk Quotient		
		6 inches	1 foot	6 feet
Mosquito Larvicide, Domestic Use, 1.64 lb/A	Rainbow trout Fathead minnow	0.0021 0.00081	0.0011 0.00040	0.00018 0.000067
Mosquito Larvicide, Public Health Use, 4.10 lb/A	Rainbow trout Fathead minnow	0.0053 0.0020	0.0027 0.0010	0.00044 0.00017

None of the acute RQs listed above exceed any of the LOCs. Therefore, use of the mosquito larvicide containing furanones is presumed to pose low acute risk to freshwater fish and to cause no appreciable acute effects to endangered species.

As stated previously, use of the other formulated products containing furanones are expected to result in very little exposure to aquatic habitats, much less than the mosquito larvacide use described above. Since the mosquito larvacide is presumed to pose low acute risk to freshwater fish, the other uses are also presumed to pose low acute risk.

a. Freshwater Invertebrates

The following table summarizes the risk quotients (EEC/LC_{50}) for freshwater invertebrates.

Risk Quotients (RQ) for Freshwater Invertebrates				
Crop/application rate	Species	Acute Risk Quotient		
		6 inches	1 foot	6 feet
Mosquito Larvicide, Domestic Use, 1.64 lb/A	*Daphnia magna*	0.071	0.035	0.0059
Mosquito Larvicide, Public Health Use, 4.10 lb/A	*Daphnia magna*	0.18	0.089	0.015

None of the acute RQ's listed above exceed the LOC for high acute risk. The acute level of concern for which restricted use may be appropriate (0.1) are not exceeded for either use of the larvicide when the depth of the water is 1 foot or greater. However, public health use of the larvicide at the rate of 4.10 lbs/A (5 gal/A) exceeds this LOC when applied to 6 inches of water.

The level of concern for endangered species is also exceeded when the product is applied at 1.64 lb/A (2 gal/A) in water 6 inches deep or less, or when it is applied at 4.10 lb/A (5 gal/A) in water 1 foot deep or less. These risk quotients are plausible, since mosquito larvicides are often applied to shallow, stagnant water.

The risk quotients also suggest that some risk could be posed to the aquatic stages of amphibians (e.g. tadpoles), although the extrapolation of risk from aquatic invertebrates to amphibian larvae is highly uncertain.

It should be recognized that the risk posed to aquatic invertebrates would be shared with most, if not all, products for this use. It is the nature of mosquito larvicides to be harmful to aquatic invertebrates because the target species is itself an aquatic invertebrate. The mosquito larvicide containing furanone would cause less harm to aquatic ecosystems than many other products because its risk is limited to aquatic invertebrates, whereas others pose a risk to fish and birds as well.

Persistence of furanones in water or repeated applications of the product could cause chronic exposure to furanones. A chronic freshwater invertebrate toxicity study is needed to complete the aquatic risk assessment.

As stated previously, use of the other formulated products containing furanones is expected to result in very little exposure to aquatic habitats, much less than the mosquito larvicide use described above. A high-risk scenario would be if 1% of the formulated product applied to a 1-acre lot runs off into a 1-acre pond. This scenario would result in aquatic exposures approximately 100 times smaller than those for the mosquito larvicide use. Since the mosquito larvicide generally is presumed to pose low acute risk (except for the extreme case of application to 6 inches of water), the other uses are also presumed to pose low acute risk.

b. Endangered Species

When the Endangered Species Protection Program becomes final, limitations in the use of furanones may be required to protect endangered and threatened species. These limitations have not been defined and may be formulation specific. EPA anticipates that a consultation with the Fish and Wildlife Service may be conducted in accordance with the species-based priority approach described in the Program. After completion of consultation, registrants would be informed if any required label modifications are necessary. Such modifications would most likely consist of the generic label statement referring pesticide users to use limitations contained in county Bulletins.

Use of the mosquito larvicide containing furanones (Reg. No. 45987-6) may have harmful effects on endangered species of aquatic invertebrates. The level of concern for endangered species are exceeded when the product is applied at 1.64 lb/A (2 gal/A) in water 6 inches deep or less, or when it is applied at 4.10 lb/A (5 gal/A) in water 1 foot deep or less. In general, mosquito larvicide of any kind should not be applied to stagnant or slow-flowing bodies of water that provide habitat for endangered aquatic invertebrates. The two primary types of habitats that are of concern are vernal pools in California and water that drains into caves. The California state-initiative plan will protect species in vernal pools. No federal protection is therefore required for these species as long as the state-initiative plan continues to be implemented. Use of this mosquito larvicide should be restricted around caves that harbor endemic populations of endangered crayfish, shrimp, or other aquatic invertebrates.

Other than the possible effect of the mosquito larvicide to endangered aquatic invertebrates, the use of products containing furanones should not cause effects to endangered species.

IV. RISK MANAGEMENT AND REREGISTRATION DECISION

A. Determination of Eligibility

Section 4(g)(2)(A) of FIFRA calls for the Agency to determine, after submission of relevant data concerning an active ingredient, whether products containing the active ingredients are eligible for reregistration. The Agency has previously identified and required the submission of the data required to support reregistration of products containing furanone as active ingredients. The Agency has completed its review of these generic data, and has determined that the data are sufficient to support reregistration of all products containing furanone. Appendix B identifies the data requirements that the Agency reviewed as part of its determination of reregistration eligibility of furanone and lists the submitted studies that the Agency found acceptable.

The data identified in Appendix B were sufficient to allow the Agency to assess the registered uses of furanone and to determine that furanone can be used without resulting in unreasonable adverse effects to humans and the environment provided the the production cap is not exceeded. The Agency therefore finds that all products containing furanone as the active ingredients are eligible for reregistration. The reregistration of particular products is addressed in Section V of this document.

The Agency made its reregistration eligibility determination based upon the target data base required for reregistration, the current guidelines for conducting acceptable studies to generate such data, published scientific literature, etc. and the data identified in Appendix B. Although the Agency has found that all uses of furanone are eligible for reregistration, it should be understood that the Agency may take appropriate regulatory action, and/or require the submission of additional data to support the registration of products containing furanone, if new information comes to the Agency's attention or if the data requirements for registration (or the guidelines for generating such data) change.

B. Determination of Eligibility Decision

1. Eligibility Decision

Based on the reviews of the generic data for the furanone active ingredients, the Agency has sufficient information on the health effects of furanone and on its potential for causing adverse effects in fish and wildlife and the environment. The Agency has determined that furanone products, labeled and used as specified in this Reregistration Eligibility Decision, will not pose unreasonable risks or adverse effects to humans or the environment. Therefore,

the Agency concludes that all uses of products containing furanone are eligible for reregistration.

2. Eligible and Ineligible Uses

The Agency has determined that all uses of furanone are eligible for reregistration.

C. Regulatory Position

The following is a summary of the regulatory positions and rationales for furanone. Where labeling revisions are imposed, specific language is set forth in Section V of this document.

1. Mosquito Larvicide Use-Production Limit

The furanones, when used as mosquito larvicides, are applied directly to water (residential and large scale public health use.) The public health larvicidal use of furanones applied at 4.10 lbs/A to 6 inches of water results in exceedances of the level of concern for freshwater invertebrates. The LOC for endangered species is exceeded when the product is applied at 1.64 lb/A in water 6 inches deep or less, or when it is applied at 4.10 lb/A in water 1 foot deep or less. This assessment is based on testing done on a formulated product and tells little about the actual toxicity of the furanones as active ingredients. Chronic invertebrate toxicity data (life cycle test with a freshwater invertebrate) and basic environmental fate data would improve the Agency's understanding and assessment of the potential risk posed by the use the of furanones in mosquito larvicides.

The volume of furanone products used annually as mosquito larvicides is low and the percentage of furanones in the product is also low. The low volume and low percent furanones support the conclusion that widespread adverse impacts are not likely to result from the mosquito larvicide use if continued at the amounts currently produced and used (volume and percent furanone in product). The Agency, in this RED document, is imposing a production limit on furanone for use in a mosquito larvicide of 150 gallons furanone per year. Should the volume produced and used and/or the percent of furanone in the product significantly increase, the Agency may impose additional data requirements to understand and assess potential risks.

It should be recognized that the risk posed to aquatic invertebrates is common to most, if not all, products for this use. It is the nature of mosquito larvicides to be harmful to aquatic invertebrates because the target species is itself an aquatic invertebrate. The mosquito larvicide product containing furanones would cause less harm to aquatic ecosystems than many other products because its

risk is limited to aquatic invertebrates, whereas others pose a risk to fish and birds as well.

2. Endangered Species Statement

Currently, the Agency is developing a program ("The Endangered Species Protection Program") to identify all pesticides whose use may cause adverse impacts on endangered and threatened species and to implement mitigation measures that will eliminate the adverse impacts. The program would require use restrictions to protect endangered and threatened species at the county level. Consultations with the Fish and Wildlife Service may be necessary to assess risks to newly listed species or from proposed new uses.

In the future, the Agency plans to publish a description of the Endangered Species Program in the Federal Register. Because the Agency is taking this approach for protecting endangered and threatened species, it is not imposing label modifications at this time through the RED. Rather, any requirements for product use modifications will occur in the future under the Endangered Species Protection Program.

V. ACTIONS REQUIRED OF REGISTRANTS

This section specifies the data requirements and responses necessary for the reregistration of both manufacturing-use and end-use products.

A. Manufacturing-Use Products

1. Additional Generic Data Requirements

The generic data base supporting the reregistration of furanone for the above eligible uses has been reviewed and determined to be substantially complete.

Environmental fate and chronic invertebrate toxicity data are not available and are needed to complete the assessment of risks posed by the use of the furanones as mosquito larvicides. However, the Agency is not requiring these data as long as the product of furanone for this use does not exceed 150 gallons per year. Should the volume produced and used and/or the percent of active ingredient in the product significantly increase, the Agency may impose additional data requirements to understand and assess potential risks.

2. Labeling Requirements for Manufacturing-Use Products

To remain in compliance with FIFRA, manufacturing use product (MP) labeling must be revised to comply with all current EPA regulations, PR Notices and applicable policies. The MP labeling must bear the following statement under Directions for Use:

> "Only for formulation into an [fill blank with Insecticide, Herbicide or the applicable term which describes the type of pesticide use(s)] for the following use(s) [fill blank only with those uses that are being supported by MP registrant."

An MP registrant may, at his/her discretion, add one of the following statements to an MP label under

> "Directions for Use" to permit the reformulation of the product for a specific use or all additional uses supported by a formulator or user group:

(a) "This product may be used to formulate products for specific use(s) not listed on the MP label if the formulator, user group, or grower has complied with U.S. EPA submission requirements regarding support of such use(s)."

(b) "This product may be used to formulate products for any additional use(s) not listed on the MP label if the formulator, user group, or grower has complied with U.S. EPA submission requirements regarding support of such use(s)."

Effluent Discharge Labeling Statements

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA."

B. End-Use Products

1. Additional Product-Specific Data Requirements

Section 4(g)(2)(B) of FIFRA calls for the Agency to obtain any needed product-specific data regarding the pesticide after a determination of eligibility has been made. The product specific data requirements are listed in Appendix G, the Product Specific Data Call-In Notice.

Registrants must review previous data submissions to ensure that they meet current EPA acceptance criteria (Appendix F; Attachment E) and if not, commit to conduct new studies. If a registrant believes that previously submitted data meet current testing standards, then study MRID numbers should be cited according to the instructions in the Requirement Status and Registrants Response Form provided for each product.

2. Labeling Requirements for End-Use Products

Worker Protection Standard

Any product whose labeling reasonably permits use in the production of an agricultural plant on any farm, forest, nursery, or greenhouse must comply with the labeling requirements of PR Notice 93-7, "Labeling Revisions Required by the Worker Protection Standard (WPS), and PR Notice 93-11, "Supplemental Guidance for PR Notice 93-7, which reflect the requirements of EPA' s labeling regulations for worker protection statements (4 0 CFR part 156, subpart K). These labeling revisions are necessary to implement the Worker Protection Standard for Agricultural Pesticides (40 CFR part 170) and must be completed in accordance with, and within the deadlines specified in, PR Notices 93-7 and 93-11. Unless otherwise specifically directed in this RED, all statements required by PR Notices 93-7 and 93-11 are to be on the product label exactly as instructed in those notices.

After April 21, 1994, except as otherwise provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR Notice complying labeling when they are distributed or sold by the primary registrant or any supplementally registered distributor.

After October 23, 1995, except as otherwise provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR Notice complying labeling when they are distributed or sold by any person.

The labels and labeling of all products must comply with EPA's current regulations and requirements as specified in 40 CFR §156.10 and other applicable notices.

Effluent Discharge Labeling Statements

Refer to subsection A. above for labeling requirements for effluent discharge.

C. Existing Stocks

Registrants may generally distribute and sell products bearing old labels/labeling for 26 months from the date of the issuance of this Reregistration Eligibility Decision (RED). Persons other than the registrant may generally distribute or sell such products for 50 months from the date of the issuance of this RED. However, existing stocks time frames will be established case-by-case, depending on the number of products involved, the number of label changes, and other factors. Refer to "Existing Stocks of Pesticide Products; Statement of Policy"; Federal Register, Volume 56, No. 123, June 26, 1991.

The Agency has determined that registrants may distribute and sell [add chemical names here] products bearing old labels/labeling for 26 months from the date of issuance of this RED. Persons other than the registrant may distribute or sell such products for 50 months from the date of the issuance of this RED. Registrants and persons other than registrants remain obligated to meet pre-existing Agency imposed label changes and existing stocks requirements applicable to products they sell or distribute.

VI. APPENDICES

Case 3138[Tanol derivs.] Chemical 122301[Dihydro-5-pentyl-2(3H)-furanone]

SITE Application Type, Application Timing, Application Equipment) Surface Type (Antimicrobial only) & Efficacy Influencing Factor (Antimicrobial only)	Form(s)	Min. Appl. Rate (AI unless noted otherwise)	Max. Appl. Rate (AI unless noted otherwise)	Soil Tex. Dose	Max. # Apps @ Max. Rate Max. /crop cycle	/year	Max. Dose [(AI unless noted otherwise)/A] /crop cycle	/year	Min. Interv (days)	Re-Entry Intv.	Geographic Limitations Allowed	Disallowed	Use Limitations Codes
USES ELIGIBLE FOR REREGISTRATION													
FOOD/FEED USES													
COMPOST/COMPOST PILES				Use Group: TERRESTRIAL FOOD+FEED CROP									
Compost treatment, When needed, Pump spray bottle	RTU	NA		UC	*	NS	NS	NS	NS	NS	NS		
MANURE				Use Group: TERRESTRIAL FOOD+FEED CROP									
Spray, When needed, Pump spray bottle	RTU	NA		UC	*	NS	NS	NS	NS	NS	NS		
NON-FOOD/NON-FEED													
DRAINAGE SYSTEMS				Use Group: AQUATIC NON-FOOD INDUSTRIAL									
Water treatment (surface), When needed, By hand	EC*RTU	NA	3.936E-04 lb A		*	NS	NS	NS	NS	NS	NS		
HOUSEHOLD/DOMESTIC DWELLINGS INDOOR PREMISES				Use Group: INDOOR RESIDENTIAL									
Indoor general surface treatment, When needed, Pump spray bottle	RTU	NA		UC	*	NS	NS	NS	NS	NS	NS		
Insecticidal strip, When needed, Not applicable	IMPR	NA			*	NS	NS	NS	NS	3	NS		
HOUSEHOLD/DOMESTIC DWELLINGS OUTDOOR PREMISES				Use Group: OUTDOOR RESIDENTIAL									
Impregnated covering, When needed, By hand	IMPR	NA		UC	*	NS	NS	NS	NS	NS	NS		
Insecticidal strip, When needed, Not applicable	IMPR	NA		UC	*	NS	NS	NS	NS	3	NS		
Outdoor general surface spray, When needed, Pump spray bottle	RTU	NA		UC	*	NS	NS	NS	NS	NS	NS		
Sprinkle, When needed, Not on label	G	NA	.0024 lb 1K sq.ft		*	NS	NS	NS	NS	AN	NS		

APPENDIX A REPORT

Case 3138[Tanol derivs.] Chemical 122301[Dihydro-5-pentyl-2(3H)-furanone]

SITE Application Type, Application Timing, Application Equipment) Surface Type (Antimicrobial only) & Efficacy Influencing Factor (Antimicrobial only)	Form(s)	Min. Appl. Rate (AI unless noted otherwise)	Max. Appl. Rate (AI unless noted otherwise)	Soil Tex. @ Max. Dose	Max. # Apps /crop cycle	Max. # Apps /year	Max. Dose [(AI unless noted otherwise)/A] /crop cycle	Max. Dose [(AI unless noted otherwise)/A] /year	Min. Interv (days)	Re-Entry Intv.	Geographic Limitations Allowed	Geographic Limitations Disallowed	Use Limitations Codes
USES ELIGIBLE FOR REREGISTRATION													
NON-FOOD/NON-FEED (con't)													
HUMAN BODY/CLOTHING WHILE BEING WORN (INSECT CONTROL)				Use Group: INDOOR RESIDENTIAL									
Clothing treatment, When needed, Sprayer	RTU	NA		UC	*	NS	NS	NS	NS	0.12	NS		
Skin contact treatment, When needed, By hand	RTU	NA		UC	*	NS	NS	NS	NS	0.12	NS		
Spray, When needed, Hand held sprayer	RTU	NA		UC	*	NS	NS	NS	NS	0.12	NS		
ORNAMENTAL HERBACEOUS PLANTS				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0024 lb 1K sq.ft		*	NS	NS	NS	NS	AN	NS		
ORNAMENTAL LAWNS AND TURF				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0024 lb 1K sq.ft		*	NS	NS	NS	NS	AN	NS		
ORNAMENTAL PONDS/AQUARIA				Use Group: AQUATIC NON-FOOD RESIDENTIAL									
Water treatment (surface), When needed, By hand	EC*RTU	NA	3.936E-04 lb A		*	NS	NS	NS	NS	NS	NS		
PAVED AREAS (PRIVATE ROADS/SIDEWALKS)				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0024 lb 1K sq.ft		*	NS	NS	NS	NS	AN	NS		
SWAMPS/MARSHES/WETLANDS/STAGNANT WATER				Use Group: AQUATIC NON-FOOD OUTDOOR									
Water treatment (surface), When needed, By hand	EC*RTU	NA	3.936E-04 lb A		*	NS	NS	NS	NS	NS	NS		
WIDE AREA/GENERAL OUTDOOR TREATMENT (PUBLIC HEALTH USE)				Use Group: TERRESTRIAL NON-FOOD CROP									
Spray, When needed, Sprayer	EC	NA	9.840E-04 lb A		*	NS	NS	NS	NS	14	NS		

APPENDIX A REPORT

Case 3138[Tanol derivs.] Chemical 122301[Dihydro-5-pentyl-2(3H)-furanone]
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LEGEND
444444

Sort: Uses Eligible or Ineligible for Re-registration, Food/Feed or Non-Food/Non-Feed Uses, Alpha Site Name, Use Group Name, Alpha Application Type/Timing/Equipment Description, Formulation, Maximum Application Rate Unit/Area Quantity, Minimum Application Rate

HEADER ABBREVIATIONS

Min. Appl. Rate (AI unless noted otherwise)	: Minimum dose for a single application to a single site. System calculated. Microbial claims only.
Max. Appl. Rate (AI unless noted otherwise)	: Maximum dose for a single application to a single site. System calculated.
Soil Tex. Max. Dose	: Maximum dose for a single application to a single site as related to soil texture (Herbicide claims only).
Max. # Apps @ Max. Rate	: Maximum number of Applications at Maximum Dosage Rate. Example: "4 applications per year" is expressed as "4/1 yr"; "4 applications per 3 years" is expressed as "4/3 yr"
Max. Dose [(AI unless noted otherwise)/A]	: Maximum dose applied to a site over a single crop cycle or year. System calculated.
Min. Interv (days)	: Minimum Interval between Applications (days)
Re-Entry Intv.	: Reentry Intervals
PRD Report Date	: LUIS contains all products that were active or suspended (and that were available from OPP Document Center) as of this date. Some products registered after this date may have data included in this report, but LUIS does not guarantee that all products registered after this date have data that has been captured.

SOIL TEXTURE FOR MAX APP. RATE

*	: Non-specific
C	: Coarse
M	: Medium
F	: Fine
O	: Others

FORMULATION CODES

EC	: EMULSIFIABLE CONCENTRATE
G	: GRANULAR
IMPR	: IMPREGNATED MATERIAL
RTU	: LIQUID-READY TO USE
xx*RTU	: Form xx with LIQUID-READY TO USE

ABBREVIATIONS

AN	: As Needed
NA	: Not Applicable
NS	: Not Specified (on label)
UC	: Unconverted due to lack of data (on label), or with one of following units: bag, bait, bait block, bait pack, bait station, bait station(s), block, briquet, briquets, bursts, cake, can, canister, capsule, cartridges, coil, collar, container, dispenser, drop, eartag, grains, lure, pack, packet, packets, pad, part, parts, pellets, piece, pieces, pill, pumps, sec, sec burst, sheet, spike, stake, stick, strip, tab, tablet, tablets, tag, tape, towelette, tray, unit, --

APPLICATION RATE

DCNC	: Dosage Can Not be Calculated
No Calc	: No Calculation can be made
W	: PPM calculated by weight
V	: PPM Calculated by volume
U	: Unknown whether PPM is given by weight or by volume
cwt	: Hundred Weight
nnE-xx	: nn times (10 power -xx); for instance, "1.234E-04" is equivalent to ".0001234"

APPENDIX A REPORT

Case 3138[Tanol derivs.] Chemical 122302[5-Heptyldihydro-2(3H)-furanone]

SITE Application Type, Application Timing, Application Equipment) Surface Type (Antimicrobial only) & Efficacy Influencing Factor (Antimicrobial only)	Form(s)	Min. Appl. Rate (AI unless noted otherwise)	Max. Appl. Rate (AI unless noted otherwise)	Soil Tex. Dose	Max. # Apps @ Max. Rate /crop /year cycle	Max. Dose [(AI unless noted otherwise)/A] /crop /year cycle	Min. Interv (days)	Re-Entry Intv.	Geographic Limitations Allowed	Disallowed	Use Limitations Codes
USES ELIGIBLE FOR REREGISTRATION											
FOOD/FEED USES											
COMPOST/COMPOST PILES				Use Group: TERRESTRIAL FOOD+FEED CROP							
Compost treatment, When needed, Pump spray bottle	RTU	NA		UC	* NS NS NS NS NS NS						
MANURE				Use Group: TERRESTRIAL FOOD+FEED CROP							
Spray, When needed, Pump spray bottle	RTU	NA		UC	* NS NS NS NS NS NS						
NON-FOOD/NON-FEED											
DRAINAGE SYSTEMS				Use Group: AQUATIC NON-FOOD INDUSTRIAL							
Water treatment (surface), When needed, By hand	EC*RTU	NA	8.036E-04 lb A		* NS NS NS NS NS NS						
HOUSEHOLD/DOMESTIC DWELLINGS INDOOR PREMISES				Use Group: INDOOR RESIDENTIAL							
Indoor general surface treatment, When needed, Pump spray bottle	RTU	NA		UC	* NS NS NS NS NS NS						
Insecticidal strip, When needed, Not applicable	IMPR	NA			* NS NS NS NS 3 NS						
HOUSEHOLD/DOMESTIC DWELLINGS OUTDOOR PREMISES				Use Group: OUTDOOR RESIDENTIAL							
Impregnated covering, When needed, By hand	IMPR	NA		UC	* NS NS NS NS NS NS						
Insecticidal strip, When needed, Not applicable	IMPR	NA		UC	* NS NS NS NS 3 NS						
Outdoor general surface spray, When needed, Pump spray bottle	RTU	NA		UC	* NS NS NS NS NS NS						
Sprinkle, When needed, Not on label	G	NA	.0049 lb 1K sq.ft		* NS NS NS NS AN NS						

APPENDIX A REPORT

Case 3138[Tanol derivs.] Chemical 122302[5-Heptyldihydro-2(3H)-furanone]

SITE Application Type, Application Timing, Application Equipment) Surface Type (Antimicrobial only) & Efficacy Influencing Factor (Antimicrobial only)	Form(s)	Min. Appl. Rate (AI unless noted otherwise)	Max. Appl. Rate (AI unless noted otherwise)	Soil Tex. @ Max. Dose	Max. # Apps /crop cycle	/year	Max. Dose [(AI unless noted otherwise)/A] /crop	/year	Min. Interv (days)	Re-Entry Intv.	Geographic Limitations Allowed	Disallowed	Use Limitations Codes
USES ELIGIBLE FOR REREGISTRATION													
NON-FOOD/NON-FEED (con't)													
HUMAN BODY/CLOTHING WHILE BEING WORN (INSECT CONTROL)				Use Group: INDOOR RESIDENTIAL									
Clothing treatment, When needed, Sprayer	RTU	NA		UC	*	NS	NS		NS	NS	0.12	NS	
Skin contact treatment, When needed, By hand	RTU	NA		UC	*	NS	NS		NS	NS	0.12	NS	
Spray, When needed, Hand held sprayer	RTU	NA		UC	*	NS	NS		NS	NS	0.12	NS	
ORNAMENTAL HERBACEOUS PLANTS				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0049 lb 1K sq.ft		*	NS	NS		NS	NS	AN	NS	
ORNAMENTAL LAWNS AND TURF				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0049 lb 1K sq.ft		*	NS	NS		NS	NS	AN	NS	
ORNAMENTAL PONDS/AQUARIA				Use Group: AQUATIC NON-FOOD RESIDENTIAL									
Water treatment (surface), When needed, By hand	EC*RTU	NA	8.036E-04 lb A		*	NS	NS		NS	NS	NS	NS	
PAVED AREAS (PRIVATE ROADS/SIDEWALKS)				Use Group: TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL									
Sprinkle, When needed, Not on label	G	NA	.0049 lb 1K sq.ft		*	NS	NS		NS	NS	AN	NS	
SWAMPS/MARSHES/WETLANDS/STAGNANT WATER				Use Group: AQUATIC NON-FOOD OUTDOOR									
Water treatment (surface), When needed, By hand	EC*RTU	NA	8.036E-04 lb A		*	NS	NS		NS	NS	NS	NS	
WIDE AREA/GENERAL OUTDOOR TREATMENT (PUBLIC HEALTH USE)				Use Group: TERRESTRIAL NON-FOOD CROP									
Spray, When needed, Sprayer	EC	NA	.002009 lb A		*	NS	NS		NS	NS	14	NS	

APPENDIX A REPORT

Case 3138[Tanol derivs.] Chemical 122302[5-Heptyldihydro-2(3H)-furanone]
44444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444

LEGEND
444444

Sort: Uses Eligible or Ineligible for Re-registration, Food/Feed or Non-Food/Non-Feed Uses, Alpha Site Name, Use Group Name, Alpha Application Type/Timing/Equipment Description, Formulation, Maximum Application Rate Unit/Area Quantity, Minimum Application Rate

HEADER ABBREVIATIONS

Min. Appl. Rate (AI unless noted otherwise) : Minimum dose for a single application to a single site. System calculated. Microbial claims only.
Max. Appl. Rate (AI unless noted otherwise) : Maximum dose for a single application to a single site. System calculated.
Soil Tex. Max. Dose : Maximum dose for a single application to a single site as related to soil texture (Herbicide claims only).
Max. # Apps @ Max. Rate : Maximum number of Applications at Maximum Dosage Rate. Example: "4 applications per year" is expressed as "4/1 yr"; "4 applications per 3 years" is expressed as "4/3 yr"
Max. Dose [(AI unless noted otherwise)/A] : Maximum dose applied to a site over a single crop cycle or year. System calculated.
Min. Interv (days) : Minimum Interval between Applications (days)
Re-Entry Intv. : Reentry Intervals
PRD Report Date : LUIS contains all products that were active or suspended (and that were available from OPP Document Center) as of this date. Some products registered after this date may have data included in this report, but LUIS does not guarantee that all products registered after this date have data that has been captured.

SOIL TEXTURE FOR MAX APP. RATE
* : Non-specific
C : Coarse
M : Medium
F : Fine
O : Others

FORMULATION CODES
EC : EMULSIFIABLE CONCENTRATE
G : GRANULAR
IMPR : IMPREGNATED MATERIAL
RTU : LIQUID-READY TO USE
xx*RTU : Form xx with LIQUID-READY TO USE

ABBREVIATIONS
AN : As Needed
NA : Not Applicable
NS : Not Specified (on label)
UC : Unconverted due to lack of data (on label), or with one of following units: bag, bait, bait block, bait pack, bait station, bait station(s), block, briquet, briquets, bursts, cake, can, canister, capsule, cartridges, coil, collar, container, dispenser, drop, eartag, grains, lure, pack, packet, packets, pad, part, parts, pellets, piece, pieces, pill, pumps, sec, sec burst, sheet, spike, stake, stick, strip, tab, tablet, tablets, tag, tape, towelette, tray, unit, --

APPLICATION RATE
DCNC : Dosage Can Not be Calculated
No Calc : No Calculation can be made
W : PPM calculated by weight
V : PPM Calculated by volume
U : Unknown whether PPM is given by weight or by volume
cwt : Hundred Weight
nnE-xx : nn times (10 power -xx); for instance, "1.234E-04" is equivalent to ".0001234"

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for active ingredients within the case Furanone covered by this Reregistration Eligibility Decision Document. It contains generic data requirements that apply to Furanone in all products, including data requirements for which a "typical formulation" is the test substance.

The data table is organized in the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR Part 158. the reference numbers accompanying each test refer to the test protocols set in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 487-4650.

2. Use Pattern (Column 2). This column indicates the use patterns for which the data requirements apply. The following letter designations are used for the given use patterns:

A	Terrestrial food
B	Terrestrial feed
C	Terrestrial non-food
D	Aquatic food
E	Aquatic non-food outdoor
F	Aquatic non-food industrial
G	Aquatic non-food residential
H	Greenhouse food
I	Greenhouse non-food
J	Forestry
K	Residential
L	Indoor food
M	Indoor non-food
N	Indoor medical
O	Indoor residential

3. Bibliographic citation (Column 3). If the Agency has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a "GS" number if no MRID number has been assigned. Refer to the Bibliography appendix for a complete citation of the study.

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Furanone 122301

REQUIREMENT		USE PATTERN	CITATION(S)
PRODUCT CHEMISTRY			
61-1	Chemical Identity	ALL	00077001
61-2A	Start. Mat. & Mnfg. Process	ALL	00077001
61-2B	Formation of Impurities	ALL	00077001
62-1	Preliminary Analysis		
62-2	Certification of limits	ALL	00077001
62-3	Analytical Method	ALL	00077001
63-2	Color	ALL	00077001
63-3	Physical State	ALL	00077001
63-4	Odor	ALL	00077001
63-5	Melting Point	ALL	00077001
63-6	Boiling Point	ALL	00077001
63-7	Density	ALL	00077001
63-8	Solubility	ALL	00077001
63-9	Vapor Pressure	ALL	00077001
63-12	pH	ALL	00077001
63-13	Stability	ALL	00077001
63-14	Oxidizing/Reducing Action	ALL	00077001
63-15	Flammability	ALL	00077001
63-16	Explodability	ALL	00077001

Data Supporting Guideline Requirements for the Reregistration of Furanone 122301

REQUIREMENT		USE PATTERN	CITATION(S)
63-17	Storage stability	ALL	00077001
63-18	Viscosity	ALL	00077001
63-19	Miscibility	ALL	00077001
63-20	Corrosion characteristics	ALL	00077001
63-21	Dielectric breakdown volt	ALL	00077001
ECOLOGICAL EFFECTS			
71-1A	Acute Avian Oral - Quail/Duck	ALL	00109340
71-2A	Avian Dietary - Quail	ALL	00109342
72-1B	Fish Toxicity Bluegill - TEP	ALL	00109344
72-1C	Fish Toxicity Rainbow Trout	ALL	00077001, 00109343 & 00109344
72-1D	Fish Toxicity Rainbow Trout- TEP	ALL	00077001 & 00109343
72-2A	Invertebrate Toxicity	ALL	00077001 & 00109345
72-2B	Invertebrate Toxicity - TEP	ALL	00109345
72-4B	Life Cycle Invertebrate	EG	DATA GAP
81-1	Acute Oral Toxicity - Rat	ALL	00109339
81-2	Acute Dermal Toxicity - Rabbit/Rat	ALL	00109339
81-3	Acute Inhalation Toxicity - Rat	ALL	00109339
81-4	Primary Eye Irritation - Rabbit	ALL	00109339
81-5	Primary Dermal Irritation - Rabbit	ALL	00109339

Data Supporting Guideline Requirements for the Reregistration of Furanone 122301

REQUIREMENT		USE PATTERN	CITATION(S)
81-6	Dermal Sensitization - Guinea Pig	ALL	00109339
ENVIRONMENTAL FATE			
160-5	Chemical Identity	ALL	00077001
161-1	Hydrolysis	EG	DATA GAP
161-2	Photodegradation - Water	EG	DATA GAP
161-4	Photodegradation - Air	EG	Reserved
162-3	Anaerobic Aquatic Metabolism	EG	DATA GAP
162-4	Aerobic Aquatic Metabolism	EG	DATA GAP
163-1	Leaching/Adsorption/Desorption	EG	DATA GAP
164-1	Terrestrial Field Dissipation	EG	Reserved
164-2	Aquatic Field Dissipation	EG	DATA GAP

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Furanone 122302

REQUIREMENT		USE PATTERN	CITATION(S)
PRODUCT CHEMISTRY			
61-2A	Start. Mat. & Mnfg. Process	ALL	00077001
62-1	Preliminary Analysis	ALL	00077001
62-3	Analytical Method	ALL	00077001
63-2	Color	ALL	00077001
63-3	Physical State	ALL	00077001
63-6	Boiling Point	ALL	00077001
63-7	Density	ALL	00077001
63-8	Solubility	ALL	00077001
63-9	Vapor Pressure	ALL	00077001
63-10	Dissociation Constant	ALL	00077001
63-11	Octanol/Water Partition	ALL	00077001
63-12	pH	ALL	00077001
63-15	Flammability	ALL	00077001
63-17	Storage stability	ALL	00077001
63-18	Viscosity	ALL	00077001
63-19	Miscibility	ALL	00077001
63-20	Corrosion characteristics	ALL	00077001

Data Supporting Guideline Requirements for the Reregistration of Furanone 122302

REQUIREMENT		USE PATTERN	CITATION(S)
63-21	Dielectric breakdown volt	ALL	00077001
ECOLOGICAL EFFECTS			
71-1A	Acute Avian Oral - Quail/Duck	ALL	00109340
71-2A	Avian Dietary - Quail	ALL	00077001
71-2B	Avian Dietary - Duck	ALL	00077001 & 00109341
72-1A	Fish Toxicity Bluegill	ALL	00077001 & 00109344
72-1B	Fish Toxicity Bluegill - TEP	ALL	00109344
72-1C	Fish Toxicity Rainbow Trout	ALL	00077001 & 00109343
72-1D	Fish Toxicity Rainbow Trout- TEP	ALL	00109343
72-2A	Invertebrate Toxicity	ALL	00109345
72-2B	Invertebrate Toxicity - TEP	ALL	00109345
72-4B	Life Cycle Invertebrate	EG	DATA GAP
81-1	Acute Oral Toxicity - Rat	ALL	00109339
81-2	Acute Dermal Toxicity - Rabbit/Rat	ALL	00077001 & 00109339
81-3	Acute Inhalation Toxicity - Rat	ALL	00109339
81-4	Primary Eye Irritation - Rabbit	ALL	00109339
81-5	Primary Dermal Irritation - Rabbit	ALL	00109339
81-6	Dermal Sensitization - Guinea Pig	ALL	00077001 & 00109339
160-5	Chemical Identity	ALL	00077001
161-1	Hydrolysis	EG	DATA GAP

Data Supporting Guideline Requirements for the Reregistration of Furanone 122302

REQUIREMENT		USE PATTERN	CITATION(S)
161-2	**Photodegradation - Water**	**EG**	**DATA GAP**
161-4	**Photodegradation - Air**	**EG**	**Reserved**
162-3	**Anaerobic Aquatic Metabolism**	**EG**	**DATA GAP**
162-4	**Aerobic Aquatic Metabolism**	**EG**	**DATA GAP**
163-1	**Leaching/Adsorption/Desorption**	**EG**	**DATA GAP**
164-1	**Terrestrial Field Dissipation**	**EG**	**Reserved**
164-2	**Aquatic Field Dissipation**	**EG**	**DATA GAP**

GUIDE TO APPENDIX C

1. CONTENTS OF BIBLIOGRAPHY. This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Document. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including the published literature, in those instances where they have been considered, are included.

2. UNITS OF ENTRY. The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review and can be described with a conventional bibliographic citation. The Agency has also attempted to unite basic documents and commentaries upon them, treating them as a single study.

3. IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted numerically by Master Record Identifier, or "MRID number". This number is unique to the citation, and should be used whenever a specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies (see paragraph 4(d)(4) below for further explanation). In a few cases, entries added to the bibliography late in the review may be preceded by a nine character temporary identifier. These entries are listed after all MRID entries. This temporary identifying number is also to be used whenever specific reference is needed.

4. FORM OF ENTRY. In addition to the Master Record Identifier (MRID), each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standard of the American National Standards Institute (ANSI), expanded to provide for certain special needs.

   a Author. Whenever the author could confidently be identified, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as the author. When no author or laboratory could be identified, the Agency has shown the first submitter as the author.

   b. Document date. The date of the study is taken directly from the document. When the date is followed by a question mark, the bibliographer has deduced the date from the evidence contained in the document. When the date appears

as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. In some cases, it has been necessary for the Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission date. The date of the earliest known submission appears immediately following the word "received."

(2) Administrative number. The next element immediately following the word "under" is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter. When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," which stands for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume.

BIBLIOGRAPHY

MRID	CITATION
00077001	Monogram Industries, Incorporated (19??) Product Chemistry. (Unpublished study received May 5, 1981 under 45987-1; CDL: 245189-A)
40543600	Rod Products Company (1988) Submission of Efficacy Data on Doo-Not (EPA Reg. No. 45987-1). Transmittal of 1 study.
40543601	Rod, R. (1987) Doo-Not Test Procedure TP-109B: Efficacy Tests with Cats. Unpublished study prepared by Connie's Kitty Castle. 13 p.
41864500	Rod Products Co. (1991) Submission of Products Chemistry Data to Support the Application for Amended Registration of RodSpray Fly , Cockroach, and Ant Killer Spray. Transmittal of 1 Study.
41864501	Rod, R. (1991) Subpart C-Product Chemistry Data. Unpublished study prepared by Rod Products Co. 58 p.
42380700	Rod Products Comp. (1992) Submission of Product Chemistry Data in Support of Registration for Fly, Cockroach, and Ant Killer. Transmittal of 1 study.
42380701	Rod, R. (1991) Subpart C--Product Chemistry Data Requirements: Unpublished study prepared by Rod Products Co. 58 p.
42678100	Rod Products Co. (1993) Submission of product chemistry, toxicity, and efficacy data to support Rodspray registration. Transmittal of 4 studies.
42678101	Rod, R. (1993) Subpart C: Product Chemistry: Rodspray Mosquito Larvicide. Unpublished study prepared by Rod Products Co. 15 p.
42678102	Rod, R. (1993) Subpart D: Data Requirements Tables-Rodspray Mosquito Larvicide. Unpublished study prepared by Rod Products Co. 10 p.
42678103	Rod, R. (1993) Efficacy Study: Rodspray Mosquito Larvicide. Unpublished study prepared by Rod Products Co. 23 p.
42678104	Kuhn, J. (1993) Rodspray: Acute Dermal Toxicity Study in Rabbits: Lab Project Number: 9684-92. Unpublished study prepared by Stillmeadow, Inc. 11 p.

BIBLIOGRAPHY

MRID	CITATION
42864100	Rod Products Co. (1993) Submission of efficacy data in support of registration for Bugchaser Insect Repellent Strip. Transmittal of 1 study.
42864101	Rod, R. (1993) Efficacy Studies: Various Rodspray Products. Unpublished study prepared by Rod Products Co. 14 p.
43013700	Rod Products Co. (1993) Submission of Efficacy Data in Support of Application for Registration of BUGCHASER Insect Repellent Strip. Transmittal of 1 Study.
43013701	Vargo, A. (1993) Efficacy Study: Bugchaser Wrist Band Insect Repellent Strip. Unpublished study prepared by American Samoa Community College. 5 p.
43355000	Rod Products Co. (1994) Submission of Product Chemistry Data in Support of Application for Registration of Rodspray IndoorOutdoor Crawling & Flying Insect Killer. Transmittal of 1 Study.
43355001	Rod, R. (1994) Product Chemistry Data Requirements (Rodspray Indoor-Outdoor Crawling & Flying Insect Killer). Unpublished study prepared by Rod Products Co. 68 p.
93207000	Rod Products Company (1990) Reregistration Phase 3 Response: Furanone, dihydro-5-pentyl(8CI,9CI).
93207001	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00077001 and Related MRIDs 00077002, 00077003. (Product Identity: Doo-Not). 8 p.
93207002	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109340. Acute Avian Oral: Quail: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratory, Inc. 11 p.
93207003	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109342. Acute Avian Diet.: Quail: No-Go Dog Repellent: ABSL No. 18866. Prepared by Applied Biological Sciences Laboratory, Inc. 12 p.
93207004	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109341. Acute Avian Diet. Duck No-Go Dog Repellent: ABSL No. 18866. Prepared by Applied Biological Sciences Laboratory, Inc. 12 p.

BIBLIOGRAPHY

MRID	CITATION
93207005	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109344. Fish Toxicity Fathead Minnows: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratory, Inc. 11 p.
93207006	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109343. Fish Toxicity Rainbow Trout: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratories, Inc. 11 p.
93207007	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109345. Invertebrate Toxicity: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratories, Inc. 11 p.
93207008	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Oral Toxicity Rat: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-2. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93207009	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Dermal Toxicity Rabbit…Monogram Animal Repellant No. 100 Test Report No. 1-2-27836-1. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93207010	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Inhalation Toxicity Rat: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-4. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93207011	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Primary Eye Irritation Rabbit: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-3. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93207012	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Primary Dermal Irritation: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-5. Prepared by Bio-Technics Laboratories, Inc. 10 p.
93207013	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Dermal Sensitization: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-6. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93207999	Rod Products Company (1990) Reregistration Phase 3 Response: Furanone, dihydro-5-pentyl(8CI,9CI). Correspondence and Supporting Material.

BIBLIOGRAPHY

MRID	CITATION
93208000	Rod Products Company (1990) Reregistration Phase 3 Response: Furanone,5-heptyldihydro-.
93208001	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00077001 and Related MRIDs 00077002, 00077003. (Product Identity: Doo-Not). 8 p.
93208002	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109340. Acute Avian Oral Toxicity: No-Go Dog Repellent: Quail: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratory, Inc. 11 p.
93208003	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109342. Acute Avian Diet. Toxicity: No-Go Dog Repellent: Quail: ABSL No. 18866. Prepared by Applied Biological Sciences Laboratory, Inc. 12 p.
93208004	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109341. Acute Avian Diet. Toxicity: No-Go Dog Repellent: Duck: ABSL No. 18866. Prepared by Applied Biological Sciences Laboratory, Inc. 12 p.
93208005	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109344. Fish Toxicity Fathead Minnows: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratory, Inc. 11 p.
93208006	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109343. Fish Toxicity Rainbow Trout: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratories, Inc. 11 p.
93208007	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109345. Invertebrate Toxicity: No-Go Dog Repellent: ABSL No. 18766. Prepared by Applied Biological Sciences Laboratories, Inc. 11 p.
93208008	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Oral Toxicity Rat: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-2. Prepared by Bio-Technics Laboratories, Inc. 9 p.
93208009	Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Dermal Toxicity: Rabbit…(Monogram Animal Repellant No. 100): Test Report No. 1-2-27836-1. Prepared by Bio-Technics Laboratories, Inc. 9 p.

BIBLIOGRAPHY

MRID **CITATION**

93208010 Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Acute Inhalation Toxicity Rat: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-4. Prepared by Bio-Technics Laboratories, Inc. 9 p.

93208011 Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Primary Eye Irritation Rabbit: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-3. Prepared by Bio-Technics Laboratories, Inc. 9 p.

93208012 Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Primary Dermal Irritation: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-5. Prepared by Bio-Technics Laboratories, Inc. 10 p.

93208013 Rod, R. (1990) Rod Products Company Phase 3 Summary of MRID 00109339. Dermal Sensitization: Monogram Animal Repellant No. 100: Test Report No. 1-2-27836-6. Prepared by Bio-Technics Laboratories, Inc. 9 p.

93208999 Rod Products Company (1990) Reregistration Phase 3 Response: Furanone,5-heptyldihydro-. Correspondence and Supporting Material.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

GENERIC DATA CALL-IN NOTICE

CERTIFIED MAIL

Dear Sir or Madam:

This Notice requires you and other registrants of pesticide products containing the active ingredient(s) identified in Attachment 1 of this Notice, the Data Call-In Chemical Status Sheet, to submit certain data as noted herein to the U.S. Environmental Protection Agency (EPA, the Agency). These data are necessary to maintain the continued registration of your product(s) containing this active ingredient(s). Within 90 days after you receive this Notice you must respond as set forth in Section III below. Your response must state:

1. how you will comply with the requirements set forth in this Notice and its Attachments 1 through 4; or,

2. why you believe you are exempt from the requirements listed in this Notice and in Attachment 3, Requirements Status and Registrant's Response Form, (see section III-B); or,

3. why you believe EPA should not require your submission of data in the manner specified by this Notice (see section III-D).

If you do not respond to this Notice, or if you do not satisfy EPA that you will comply with its requirements or should be exempt or excused from doing so, then the registration of your product(s) subject to this Notice will be subject to suspension. We have provided a list of all of your products subject to this Notice in Attachment 2, Data Call-In Response Form, as well as a list of all registrants who were sent this Notice (Attachment 4).

The authority for this Notice is section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act as amended (FIFRA), 7 U.S.C. section 136a(c)(2)(B). Collection of this

information is authorized under the Paperwork Reduction Act by OMB Approval No. 2070-0107 and 2070-0057 (expiration date 3-31-96).

This Notice is divided into six sections and five Attachments. The Notice itself contains information and instructions applicable to all Data Call-In Notices. The Attachments contain specific chemical information and instructions. The six sections of the Notice are:

Section I	-	Why You Are Receiving This Notice
Section II	-	Data Required By This Notice
Section III	-	Compliance With Requirements Of This Notice
Section IV	-	Consequences Of Failure To Comply With This Notice
Section V	-	Registrants' Obligation To Report Possible Unreasonable Adverse Effects
Section VI	-	Inquiries And Responses To This Notice

The Attachments to this Notice are:

Attachment 1	-	Data Call-In Chemical Status Sheet
Attachment 2	-	Data Call-In Response Form
Attachment 3	-	Requirements Status And Registrant's Response Form
Attachment 4	-	List Of All Registrants Sent This Data Call-In Notice

SECTION I. WHY YOU ARE RECEIVING THIS NOTICE

The Agency has reviewed existing data for this active ingredient(s) and reevaluated the data needed to support continued registration of the subject active ingredient(s). This reevaluation identified additional data necessary to assess the health and safety of the continued use of products containing this active ingredient(s). You have been sent this Notice because you have product(s) containing the subject active ingredient(s).

SECTION II. DATA REQUIRED BY THIS NOTICE

A. DATA REQUIRED

The data required by this Notice are specified in Attachment 3, Requirements Status and Registrant's Response Form. Depending on the results of the studies required in this Notice, additional testing may be required.

B. SCHEDULE FOR SUBMISSION OF DATA

You are required to submit the data or otherwise satisfy the data requirements specified in Attachment 3, Requirements Status and Registrant's Response Form, within the time frames provided.

C. TESTING PROTOCOL

All studies required under this Notice must be conducted in accordance with test standards outlined in the Pesticide Assessment Guidelines for those studies for which guidelines have been established.

These EPA Guidelines are available from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, Va 22161 (tel: 703-487-4650).

Protocols approved by the Organization for Economic Cooperation and Development (OECD) are also acceptable if the OECD-recommended test standards conform to those specified in the Pesticide Data Requirements regulation (40 CFR § 158.70). When using the OECD protocols, they should be modified as appropriate so that the data generated by the study will satisfy the requirements of 40 CFR § 158. Normally, the Agency will not extend deadlines for complying with data requirements when the studies were not conducted in accordance with acceptable standards. The OECD protocols are available from 2001 L Street, N.W., Washington, D.C. 20036 (Telephone number 202-785-6323; Fax telephone number 202-785-0350).

All new studies and proposed protocols submitted in response to this Data Call-In Notice must be in accordance with Good Laboratory Practices [40 CFR Part 160.3(a)(6)].

D. REGISTRANTS RECEIVING PREVIOUS SECTION 3(c)(2)(B) NOTICES ISSUED BY THE AGENCY

Unless otherwise noted herein, this Data Call-In does not in any way supersede or change the requirements of any previous Data Call-In(s), or any other agreements entered into with the Agency pertaining to such prior Notice. Registrants must comply with the requirements of all Notices to avoid issuance of a Notice of Intent to Suspend their affected products.

SECTION III. COMPLIANCE WITH REQUIREMENTS OF THIS NOTICE

A. SCHEDULE FOR RESPONDING TO THE AGENCY

The appropriate responses initially required by this Notice must be submitted to the Agency within 90 days after your receipt of this Notice. Failure to adequately respond

to this Notice within 90 days of your receipt will be a basis for issuing a Notice of Intent to Suspend (NOIS) affecting your products. This and other bases for issuance of NOIS due to failure to comply with this Notice are presented in Section IV-A and IV-B.

B. OPTIONS FOR RESPONDING TO THE AGENCY

The options for responding to this Notice are: 1) voluntary cancellation, 2) delete use(s), (3) claim generic data exemption, (4) agree to satisfy the data requirements imposed by this Notice or (5) request a data waiver(s).

A discussion of how to respond if you chose the Voluntary Cancellation option, the Delete Use(s) option or the Generic Data Exemption option is presented below. A discussion of the various options available for satisfying the data requirements of this Notice is contained in Section III-C. A discussion of options relating to requests for data waivers is contained in Section III-D.

There are two forms that accompany this Notice of which, depending upon your response, one or both must be used in your response to the Agency. These forms are the Data-Call-In Response Form (Attachment 2) and the Requirements Status and Registrant's Response Form (Attachment 3). The Data Call-In Response Form must be submitted as part of every response to this Notice. Please note that the company's authorized representative is required to sign the first page of the Data Call-In Response Form and Requirements Status and Registrant's Response Form (if this form is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person identified in Attachment 1.

1. Voluntary Cancellation - You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient(s) that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit a completed Data Call-In Response Form, indicating your election of this option. Voluntary cancellation is item number 5 on the Data Call-In Response Form. If you choose this option, this is the only form that you are required to complete.

   If you choose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice which are contained in Section IV-C.

2. Use Deletion - You may avoid the requirements of this Notice by eliminating the uses of your product to which the requirements apply. If you wish to amend your registration to delete uses, you must submit the Requirements Status and Registrant's Response Form, a completed application for amendment, a copy

of your proposed amended labeling, and all other information required for processing the application. Use deletion is option number 7 on the Requirements Status and Registrant's Response Form. You must also complete a Data Call-In Response Form by signing the certification, item number 8. Application forms for amending registrations may be obtained from the Registration Support and Emergency Response Branch, Registration Division, (703) 308-8358.

If you choose to delete the use(s) subject to this Notice or uses subject to specific data requirements, further sale, distribution, or use of your product after one year from the due date of your 90 day response, must bear an amended label.

3. Generic Data Exemption - Under section 3(c)(2)(D) of FIFRA, an applicant for registration of a product is exempt from the requirement to submit or cite generic data concerning an active ingredient(s) if the active ingredient(s) in the product is derived exclusively from purchased, registered pesticide products containing the active ingredient(s). EPA has concluded, as an exercise of its discretion, that it normally will not suspend the registration of a product which would qualify and continue to qualify for the generic data exemption in section 3(c)(2)(D) of FIFRA. To qualify, all of the following requirements must be met:

a. The active ingredient(s) in your registered product must be present solely because of incorporation of another registered product which contains the subject active ingredient(s) and is purchased from a source not connected with you; and,

b. every registrant who is the ultimate source of the active ingredient(s) in your product subject to this DCI must be in compliance with the requirements of this Notice and must remain in compliance; and

c. you must have provided to EPA an accurate and current "Confidential Statement of Formula" for each of your products to which this Notice applies.

To apply for the Generic Data Exemption you must submit a completed Data Call-In Response Form, Attachment 2 and all supporting documentation. The Generic Data Exemption is item number 6a on the Data Call-In Response Form. If you claim a generic data exemption you are not required to complete the Requirements Status and Registrant's Response Form. Generic Data Exemption cannot be selected as an option for product specific data.

If you are granted a Generic Data Exemption, you rely on the efforts of other persons to provide the Agency with the required data. If the registrant(s) who have committed to generate and submit the required data fail to take appropriate steps to meet the requirements or are no longer in compliance with this

Data Call-In Notice, the Agency will consider that both they and you are not in compliance and will normally initiate proceedings to suspend the registrations of both your and their product(s), unless you commit to submit and do submit the required data within the specified time. In such cases the Agency generally will not grant a time extension for submitting the data.

4. Satisfying the Data Requirements of this Notice - There are various options available to satisfy the data requirements of this Notice. These options are discussed in Section III-C of this Notice and comprise options 1 through 6 on the Requirements Status and Registrant's Response Form and option 6b and 7 on the Data Call-In Response Form. If you choose option 6b or 7, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

5. Request for Data Waivers. Data waivers are discussed in Section III-D of this Notice and are covered by options 8 and 9 on the Requirements Status and Registrant's Response Form. If you choose one of these options, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

C. SATISFYING THE DATA REQUIREMENTS OF THIS NOTICE

If you acknowledge on the Data Call-In Response Form that you agree to satisfy the data requirements (i.e. you select option 6b and/or 7), then you must select one of the six options on the Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide registrants to additional instructions provided in this Section. The options are:

1. I will generate and submit data within the specified time frame (Developing Data),

2. I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing),

3. I have made offers to cost-share (Offers to Cost Share),

4. I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study),

5. I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study),

6. I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study).

Option 1, Developing Data --

If you choose to develop the required data it must be in conformance with Agency deadlines and with other Agency requirements as referenced herein and in the attachments. All data generated and submitted must comply with the Good Laboratory Practice (GLP) rule (40 CFR Part 160), be conducted according to the Pesticide Assessment Guidelines (PAG), and be in conformance with the requirements of PR Notice 86-5. In addition, certain studies require Agency approval of test protocols in advance of study initiation. Those studies for which a protocol must be submitted have been identified in the Requirements Status and Registrant's Response Form and/or footnotes to the form. If you wish to use a protocol which differs from the options discussed in Section II-C of this Notice, you must submit a detailed description of the proposed protocol and your reason for wishing to use it. The Agency may choose to reject a protocol not specified in Section II-C. If the Agency rejects your protocol you will be notified in writing, however, you should be aware that rejection of a proposed protocol will not be a basis for extending the deadline for submission of data.

A progress report must be submitted for each study within 90 days from the date you are required to commit to generate or undertake some other means to address that study requirement, such as making an offer to cost-share or agreeing to share in the cost of developing that study. A 90-day progress report must be submitted for all studies. This 90-day progress report must include the date the study was or will be initiated and, for studies to be started within 12 months of commitment, the name and address of the laboratory(ies) or individuals who are or will be conducting the study.

In addition, if the time frame for submission of a final report is more than 1 year, interim reports must be submitted at 12 month intervals from the date you are required to commit to generate or otherwise address the requirement for the study. In addition to the other information specified in the preceding paragraph, at a minimum, a brief description of current activity on and the status of the study must be included as well as a full description of any problems encountered since the last progress report.

The time frames in the Requirements Status and Registrant's Response Form are the time frames that the Agency is allowing for the submission of

completed study reports or protocols. The noted deadlines run from the date of the receipt of this Notice by the registrant. If the data are not submitted by the deadline, each registrant is subject to receipt of a Notice of Intent to Suspend the affected registration(s).

If you cannot submit the data/reports to the Agency in the time required by this Notice and intend to seek additional time to meet the requirement(s), you must submit a request to the Agency which includes: (1) a detailed description of the expected difficulty and (2) a proposed schedule including alternative dates for meeting such requirements on a step-by-step basis. You must explain any technical or laboratory difficulties and provide documentation from the laboratory performing the testing. While EPA is considering your request, the original deadline remains. The Agency will respond to your request in writing. If EPA does not grant your request, the original deadline remains. Normally, extensions can be requested only in cases of extraordinary testing problems beyond the expectation or control of the registrant. Extensions will not be given in submitting the 90-day responses. Extensions will not be considered if the request for extension is not made in a timely fashion; in no event shall an extension request be considered if it is submitted at or after the lapse of the subject deadline.

Option 2, Agreement to Share in Cost to Develop Data --

If you choose to enter into an agreement to share in the cost of producing the required data but will not be submitting the data yourself, you must provide the name of the registrant who will be submitting the data. You must also provide EPA with documentary evidence that an agreement has been formed. Such evidence may be your letter offering to join in an agreement and the other registrant's acceptance of your offer, or a written statement by the parties that an agreement exists. The agreement to produce the data need not specify all of the terms of the final arrangement between the parties or the mechanism to resolve the terms. Section 3(c)(2)(B) provides that if the parties cannot resolve the terms of the agreement they may resolve their differences through binding arbitration.

Option 3, Offer to Share in the Cost of Data Development --

If you have made an offer to pay in an attempt to enter into an agreement or amend an existing agreement to meet the requirements of this Notice and have been unsuccessful, you may request EPA (by selecting this option) to exercise its discretion not to suspend your registration(s), although you do not comply with the data submission requirements of this Notice. EPA has determined that as a general policy, absent other relevant considerations, it will not suspend the registration of a product of a registrant who has in good faith sought and continues to seek to enter into a joint data development/cost sharing program, but the other registrant(s) developing the data has refused to accept your offer. To qualify for this option,

you must submit documentation to the Agency proving that you have made an offer to another registrant (who has an obligation to submit data) to share in the burden of developing that data. You must also submit to the Agency a completed EPA Form 8570-32, Certification of Offer to Cost Share in the Development of Data. In addition, you must demonstrate that the other registrant to whom the offer was made has not accepted your offer to enter into a cost sharing agreement by including a copy of your offer and proof of the other registrant's receipt of that offer (such as a certified mail receipt). Your offer must, in addition to anything else, offer to share in the burden of producing the data upon terms to be agreed or failing agreement to be bound by binding arbitration as provided by FIFRA section 3(c)(2)(B)(iii) and must not qualify this offer. The other registrant must also inform EPA of its election of an option to develop and submit the data required by this Notice by submitting a Data Call-In Response Form and a Requirements Status and Registrant's Response Form committing to develop and submit the data required by this Notice.

In order for you to avoid suspension under this option, you may not withdraw your offer to share in the burdens of developing the data. In addition, the other registrant must fulfill its commitment to develop and submit the data as required by this Notice. If the other registrant fails to develop the data or for some other reason is subject to suspension, your registration as well as that of the other registrant will normally be subject to initiation of suspension proceedings, unless you commit to submit, and do submit the required data in the specified time frame. In such cases, the Agency generally will not grant a time extension for submitting the data.

Option 4, Submitting an Existing Study --

If you choose to submit an existing study in response to this Notice, you must determine that the study satisfies the requirements imposed by this Notice. You may only submit a study that has not been previously submitted to the Agency or previously cited by anyone. Existing studies are studies which predate issuance of this Notice. Do not use this option if you are submitting data to upgrade a study. (See Option 5).

You should be aware that if the Agency determines that the study is not acceptable, the Agency will require you to comply with this Notice, normally without an extension of the required date of submission. The Agency may determine at any time that a study is not valid and needs to be repeated.

To meet the requirements of the DCI Notice for submitting an existing study, all of the following three criteria must be clearly met:

a. You must certify at the time that the existing study is submitted that the raw data and specimens from the study are available for audit and review and you must identify where they are available. This must be done in accordance with the requirements of the Good Laboratory Practice (GLP) regulation, 40 CFR Part 160. As stated in 40 CFR 160.3(7) " *raw data* means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. *Raw data* may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments." The term "specimens", according to 40 CFR 160.3(7), means "any material derived from a test system for examination or analysis."

b. Health and safety studies completed after May 1984 must also contain all GLP-required quality assurance and quality control information, pursuant to the requirements of 40 CFR Part 160. Registrants must also certify at the time of submitting the existing study that such GLP information is available for post-May 1984 studies by including an appropriate statement on or attached to the study signed by an authorized official or representative of the registrant.

c. You must certify that each study fulfills the acceptance criteria for the Guideline relevant to the study provided in the FIFRA Accelerated Reregistration Phase 3 Technical Guidance and that the study has been conducted according to the Pesticide Assessment Guidelines (PAG) or meets the purpose of the PAG (both available from NTIS). A study not conducted according to the PAG may be submitted to the Agency for consideration if the registrant believes that the study clearly meets the purpose of the PAG. The registrant is referred to 40 CFR 158.70 which states the Agency's policy regarding acceptable protocols. If you wish to submit the study, you must, in addition to certifying that the purposes of the PAG are met by the study, clearly articulate the rationale why you believe the study meets the purpose of the PAG, including copies of any supporting information or data. It has been the Agency's experience that studies completed prior to January 1970 rarely satisfied the purpose of the PAG and that necessary raw data are usually not available for such studies.

If you submit an existing study, you must certify that the study meets all requirements of the criteria outlined above.

If EPA has previously reviewed a protocol for a study you are submitting, you must identify any action taken by the Agency on the protocol and must indicate, as part of your certification, the manner in which all Agency comments, concerns, or issues were addressed in the final protocol and study.

If you know of a study pertaining to any requirement in this Notice which does not meet the criteria outlined above but does contain factual information regarding unreasonable adverse effects, you must notify the Agency of such a study. If such a study is in the Agency's files, you need only cite it along with the notification. If not in the Agency's files, you must submit a summary and copies as required by PR Notice 86-5.

Option 5, Upgrading a Study --

If a study has been classified as partially acceptable and upgradeable, you may submit data to upgrade that study. The Agency will review the data submitted and determine if the requirement is satisfied. If the Agency decides the requirement is not satisfied, you may still be required to submit new data normally without any time extension. Deficient, but upgradeable studies will normally be classified as supplemental. However, it is important to note that not all studies classified as supplemental are upgradeable. If you have questions regarding the classification of a study or whether a study may be upgraded, call or write the contact person listed in Attachment 1. If you submit data to upgrade an existing study you must satisfy or supply information to correct all deficiencies in the study identified by EPA. You must provide a clearly articulated rationale of how the deficiencies have been remedied or corrected and why the study should be rated as acceptable to EPA. Your submission must also specify the MRID number(s) of the study which you are attempting to upgrade and must be in conformance with PR Notice 86-5.

Do not submit additional data for the purpose of upgrading a study classified as unacceptable and determined by the Agency as not capable of being upgraded.

This option should also be used to cite data that has been previously submitted to upgrade a study, but has not yet been reviewed by the Agency. You must provide the MRID number of the data submission as well as the MRID number of the study being upgraded.

The criteria for submitting an existing study, as specified in Option 4 above, apply to all data submissions intended to upgrade studies. Additionally your submission of data intended to upgrade studies must be accompanied by a certification that you comply with each of those criteria as well as a certification regarding protocol compliance with Agency requirements.

Option 6, Citing Existing Studies --

If you choose to cite a study that has been previously submitted to EPA, that study must have been previously classified by EPA as acceptable or it must be a study which has not yet been reviewed by the Agency. Acceptable toxicology studies generally will have been classified as "core-guideline" or "core minimum." For ecological effects studies, the classification generally would be a rating of "core." For all other disciplines the classification would be "acceptable." With respect to any studies for which you wish to select this option you must provide the MRID number of the study you are citing and, if the study has been reviewed by the Agency, you must provide the Agency's classification of the study.

If you are citing a study of which you are not the original data submitter, you must submit a completed copy of EPA Form 8570-31, Certification with Respect to Data Compensation Requirements.

D. REQUESTS FOR DATA WAIVERS

There are two types of data waiver responses to this Notice. The first is a request for a low volume/minor use waiver and the second is a waiver request based on your belief that the data requirement(s) are inapplicable and do not apply to your product.

1. Low Volume/Minor Use Waiver -- Option 8 on the Requirements Status and Registrant's Response Form. Section 3(c)(2)(A) of FIFRA requires EPA to consider the appropriateness of requiring data for low volume, minor use pesticides. In implementing this provision EPA considers as low volume pesticides only those active ingredient(s) whose total production volume for all pesticide registrants is small. In determining whether to grant a low volume, minor use waiver the Agency will consider the extent, pattern and volume of use, the economic incentive to conduct the testing, the importance of the pesticide, and the exposure and risk from use of the pesticide. If an active ingredient(s) is used for both high volume and low volume uses, a low volume exemption will not be approved. If all uses of an active ingredient(s) are low volume and the combined volumes for all uses are also low, then an exemption may be granted, depending on review of other information outlined below. An exemption will not be granted if any registrant of the active ingredient(s) elects to conduct the testing. Any registrant receiving a low volume minor use waiver must remain within the sales figures in their forecast supporting the waiver request in order to remain qualified

for such waiver. If granted a waiver, a registrant will be required, as a condition of the waiver, to submit annual sales reports. The Agency will respond to requests for waivers in writing.

To apply for a low volume, minor use waiver, you must submit the following information, as applicable to your product(s), as part of your 90-day response to this Notice:

a. Total company sales (pounds and dollars) of all registered product(s) containing the active ingredient(s). If applicable to the active ingredient(s), include foreign sales for those products that are not registered in this country but are applied to sugar (cane or beet), coffee, bananas, cocoa, and other such crops. Present the above information by year for each of the past five years.

b. Provide an estimate of the sales (pounds and dollars) of the active ingredient(s) for each major use site. Present the above information by year for each of the past five years.

c. Total direct production cost of product(s) containing the active ingredient(s) by year for the past five years. Include information on raw material cost, direct labor cost, advertising, sales and marketing, and any other significant costs listed separately.

d. Total indirect production cost (e.g. plant overhead, amortized plant and equipment) charged to product(s) containing the active ingredient(s) by year for the past five years. Exclude all non-recurring costs that were directly related to the active ingredient(s), such as costs of initial registration and any data development.

e. A list of each data requirement for which you seek a waiver. Indicate the type of waiver sought and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

f. A list of each data requirement for which you are not seeking any waiver and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

g. For each of the next ten years, a year-by-year forecast of company sales (pounds and dollars) of the active ingredient(s), direct production costs of product(s) containing the active ingredient(s) (following the parameters in item c above), indirect production costs of product(s)

containing the active ingredient(s) (following the parameters in item d above), and costs of data development pertaining to the active ingredient(s).

h. A description of the importance and unique benefits of the active ingredient(s) to users. Discuss the use patterns and the effectiveness of the active ingredient(s) relative to registered alternative chemicals and non-chemical control strategies. Focus on benefits unique to the active ingredient(s), providing information that is as quantitative as possible. If you do not have quantitative data upon which to base your estimates, then present the reasoning used to derive your estimates. To assist the Agency in determining the degree of importance of the active ingredient(s) in terms of its benefits, you should provide information on any of the following factors, as applicable to your product(s):

(1) documentation of the usefulness of the active ingredient(s) in Integrated Pest Management, (b) description of the beneficial impacts on the environment of use of the active ingredient(s), as opposed to its registered alternatives, (c) information on the breakdown of the active ingredient(s) after use and on its persistence in the environment, and (d) description of its usefulness against a pest(s) of public health significance.

Failure to submit sufficient information for the Agency to make a determination regarding a request for a low volume minor use waiver will result in denial of the request for a waiver.

2. Request for Waiver of Data --Option 9 on the Requirements Status and Registrant's Response Form. This option may be used if you believe that a particular data requirement should not apply because the corresponding use is no longer registered or the requirement is inappropriate. You must submit a rationale explaining why you believe the data requirements should not apply. You must also submit the current label(s) of your product(s) and, if a current copy of your Confidential Statement of Formula is not already on file you must submit a current copy.

You will be informed of the Agency's decision in writing. If the Agency determines that the data requirements of this Notice do not apply to your product(s), you will not be required to supply the data pursuant to section 3(c)(2)(B). If EPA determines that the data are required for your product(s), you must choose a method of meeting the requirements of this Notice within the time frame provided by this Notice. Within 30 days of your receipt of the Agency's written decision, you must submit a revised Requirements Status and Registrant's Response Form indicating the option chosen.

IV. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS NOTICE

A. NOTICE OF INTENT TO SUSPEND

The Agency may issue a Notice of Intent to Suspend products subject to this Notice due to failure by a registrant to comply with the requirements of this Data Call-In Notice, pursuant to FIFRA section 3(c)(2)(B). Events which may be the basis for issuance of a Notice of Intent to Suspend include, but are not limited to, the following:

1. Failure to respond as required by this Notice within 90 days of your receipt of this Notice.

2. Failure to submit on the required schedule an acceptable proposed or final protocol when such is required to be submitted to the Agency for review.

3. Failure to submit on the required schedule an adequate progress report on a study as required by this Notice.

4. Failure to submit on the required schedule acceptable data as required by this Notice.

5. Failure to take a required action or submit adequate information pertaining to any option chosen to address the data requirements (e.g., any required action or information pertaining to submission or citation of existing studies or offers, arrangements, or arbitration on the sharing of costs or the formation of Task Forces, failure to comply with the terms of an agreement or arbitration concerning joint data development or failure to comply with any terms of a data waiver).

6. Failure to submit supportable certifications as to the conditions of submitted studies, as required by Section III-C of this Notice.

7. Withdrawal of an offer to share in the cost of developing required data.

8. Failure of the registrant to whom you have tendered an offer to share in the cost of developing data and provided proof of the registrant's receipt of such offer, or failure of a registrant on whom you rely for a generic data exemption either to:

   a. inform EPA of intent to develop and submit the data required by this Notice on a Data Call-In Response Form and a Requirements Status and Registrant's Response Form; or,

b. fulfill the commitment to develop and submit the data as required by this Notice; or,

c. otherwise take appropriate steps to meet the requirements stated in this Notice, unless you commit to submit and do submit the required data in the specified time frame.

9. Failure to take any required or appropriate steps, not mentioned above, at any time following the issuance of this Notice.

B. BASIS FOR DETERMINATION THAT SUBMITTED STUDY IS UNACCEPTABLE

The Agency may determine that a study (even if submitted within the required time) is unacceptable and constitutes a basis for issuance of a Notice of Intent to Suspend. The grounds for suspension include, but are not limited to, failure to meet any of the following:

1. EPA requirements specified in the Data Call-In Notice or other documents incorporated by reference (including, as applicable, EPA Pesticide Assessment Guidelines, Data Reporting Guidelines, and GeneTox Health Effects Test Guidelines) regarding the design, conduct, and reporting of required studies. Such requirements include, but are not limited to, those relating to test material, test procedures, selection of species, number of animals, sex and distribution of animals, dose and effect levels to be tested or attained, duration of test, and, as applicable, Good Laboratory Practices.

2. EPA requirements regarding the submission of protocols, including the incorporation of any changes required by the Agency following review.

3. EPA requirements regarding the reporting of data, including the manner of reporting, the completeness of results, and the adequacy of any required supporting (or raw) data, including, but not limited to, requirements referenced or included in this Notice or contained in PR 86-5. All studies must be submitted in the form of a final report; a preliminary report will not be considered to fulfill the submission requirement.

C. EXISTING STOCKS OF SUSPENDED OR CANCELLED PRODUCTS

EPA has statutory authority to permit continued sale, distribution and use of existing stocks of a pesticide product which has been suspended or cancelled if doing so

would be consistent with the purposes of the Federal Insecticide, Fungicide, and Rodenticide Act.

The Agency has determined that such disposition by registrants of existing stocks for a suspended registration when a section 3(c)(2)(B) data request is outstanding would generally not be consistent with the Act's purposes. Accordingly, the Agency anticipates granting registrants permission to sell, distribute, or use existing stocks of suspended product(s) only in exceptional circumstances. If you believe such disposition of existing stocks of your product(s) which may be suspended for failure to comply with this Notice should be permitted, you have the burden of clearly demonstrating to EPA that granting such permission would be consistent with the Act. You must also explain why an "existing stocks" provision is necessary, including a statement of the quantity of existing stocks and your estimate of the time required for their sale, distribution, and use. Unless you meet this burden the Agency will not consider any request pertaining to the continued sale, distribution, or use of your existing stocks after suspension.

If you request a voluntary cancellation of your product(s) as a response to this Notice and your product is in full compliance with all Agency requirements, you will have, under most circumstances, one year from the date your 90 day response to this Notice is due, to sell, distribute, or use existing stocks. Normally, the Agency will allow persons other than the registrant such as independent distributors, retailers and end users to sell, distribute or use such existing stocks until the stocks are exhausted. Any sale, distribution or use of stocks of voluntarily cancelled products containing an active ingredient(s) for which the Agency has particular risk concerns will be determined on case-by-case basis.

Requests for voluntary cancellation received after the 90 day response period required by this Notice will not result in the Agency granting any additional time to sell, distribute, or use existing stocks beyond a year from the date the 90 day response was due unless you demonstrate to the Agency that you are in full compliance with all Agency requirements, including the requirements of this Notice. For example, if you decide to voluntarily cancel your registration six months before a 3 year study is scheduled to be submitted, all progress reports and other information necessary to establish that you have been conducting the study in an acceptable and good faith manner must have been submitted to the Agency, before EPA will consider granting an existing stocks provision.

SECTION V. REGISTRANTS' OBLIGATION TO REPORT POSSIBLE UNREASONABLE ADVERSE EFFECTS

Registrants are reminded that FIFRA section 6(a)(2) states that if at any time after a pesticide is registered a registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit the information to the Agency. Registrants must notify the Agency of any factual information they have, from

whatever source, including but not limited to interim or preliminary results of studies, regarding unreasonable adverse effects on man or the environment. This requirement continues as long as the products are registered by the Agency.

SECTION VI. INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the requirements and procedures established by this Notice, call the contact person listed in Attachment 1, the Data Call-In Chemical Status Sheet.

All responses to this Notice (other than voluntary cancellation requests and generic data exemption claims) must include a completed Data Call-In Response Form (Attachment 2) and a completed Requirements Status and Registrant's Response Form (Attachment 3) and any other documents required by this Notice, and should be submitted to the contact person identified in Attachment 1. If the voluntary cancellation or generic data exemption option is chosen, only the Data Call-In Response Form need be submitted.

The Office of Compliance (OC) of the Office of Enforcement and Compliance Assurance (OECA), EPA, will be monitoring the data being generated in response to this Notice.

Sincerely yours,

Lois Rossi, Division Director
Special Review
and Reregistration Division

Attachment 1. Chemical Status Sheets

FURANONE DATA CALL-IN CHEMICAL STATUS SHEET

INTRODUCTION

You have been sent this Generic Data Call-In Notice because you have product(s) containing Furanone.

This Generic Data Call-In Chemical Status Sheet, contains an overview of data required by this notice, and point of contact for inquiries pertaining to the reregistration of Furanone. This attachment is to be used in conjunction with (1) the Generic Data Call-In Notice, (2) the Generic Data Call-In Response Form (Attachment 2), (3) the Requirements Status and Registrant's Form (Attachment 2), (4) a list of registrants receiving this DCI (Attachment 4), (5) the EPA Acceptance Criteria (Attachment 5), and (6) the Cost Share and Data Compensation Forms in replying to this Furanone Generic Data Call In (Attachment F). Instructions and guidance accompany each form.

DATA REQUIRED BY THIS NOTICE

The additional data requirements needed to complete the generic database for Furanone are contained in the Requirements Status and Registrant's Response, Attachment C. The Agency has concluded that additional product chemistry data on Furanone are needed. These data are needed to fully complete the reregistration of all eligible Furanone products.

INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the generic data requirements and procedures established by this Notice, please contact Emily Mitchell at (703) 308-8583.

All responses to this Notice for the generic data requirements should be submitted to:

Emily Mitchell, Chemical Review Manager
Planning and Reregistration
Special Review and Registration Division (H7508W)
Office of Pesticiafde Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460
RE: **Furanone**

SPECIFIC INSTRUCTIONS FOR THE GENERIC DATA CALL-IN RESPONSE FORM

This Form is designed to be used to respond to call-ins for generic and product specific data for the purpose of reregistering pesticides under the Federal Insecticide Fungicide and Rodenticide Act. Fill out this form each time you are responding to a data call-in for which EPA has sent you the form entitled "Requirements Status and Registrant's Response."

Items 1-4 will have been preprinted on the form Items 5 through 7 must be completed by the registrant as appropriate Items 8 through 11 must be completed by the registrant before submitting a response to the Agency.

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggesting for reducing this burden, to Chief, Information Policy Branch, PM-223, U S Environmental Protection Agency, 401 M St , S W , Washington, D C 20460; and to the Office of Management and Budget, Paperwork Reduction Project 2070-0107, Washington, D C 20503.

INSTRUCTIONS

Item 1. This item identifies your company name, number and address.

Item 2. This item identifies the ease number, ease name, EPA chemical number and chemical name.

Item 3. This item identifies the date and type of data call-in.

Item 4. This item identifies the EPA product registrations relevant to the data call-in. Please note that you are also responsible for informing the Agency of your response regarding any product that you believe may be covered by this data call-in but that is not listed by the Agency in Item 4. You must bring any such apparent omission to the Agency's attention within the period required for submission of this response form.

Item 5. Cheek this item for each product registration you wish to cancel voluntarily. If a registration number is listed for a product for which you previously requested voluntary cancellation, indicate in Item 5 the date of that request. You do not need to complete any item on the Requirements Status and Registrant's Response Form for any product that is voluntarily cancelled.

Item 6a. Check this item if this data call-in is for generic data as indicated in Item 3 and if you are eligible for a Generic Data Exemption for the chemical listed in Item 2 and used in the subject product. By electing this exemption, you agree to the terms and conditions of a Generic Data Exemption as explained in the Data Call-In Notice.

If you are eligible for or claim a Generic Data Exemption, enter the EPA registration Number of each registered source of that active ingredient that you use in your product.

Typically, if you purchase an EPA-registered product from one or more other producers (who, with respect to the incorporated product, are in compliance with this and-any other outstanding Data Call-In Notice), and incorporate that product into all your products, you may complete this item for all products listed on this form If, however, you produce the active ingredient yourself, or use any unregistered product (regardless of the fact that some of your sources are registered), you may not claim a Generic Data Exemption and you may not select this item.

Item 6b. Check this Item if the data call-in is a generic data call-in as indicated in Item 3 and if you are agreeing to satisfy the generic data requirements of this data call-in. Attach the Requirements Status and Registrant's Response Form that indicates how you will satisfy those requirements.

Item 7a. Check this item if this call-in if a data call-in as indicated in Item 3 for a manufacturing use product (MUP), and if your product is a manufacturing use product for which you agree to supply product-specific data. Attach the Requirements Status and Registrants' Response Form that indicates how you will satisfy those requirements.

Item 7b. Check this item if this call-in is a data call-in for an end use product (EUP) as indicated in Item 3 and if your product is an end use product for which you agree to supply product-specific data. Attach the Requirements Status and Registrant's Response Form that indicates how you will satisfy those requirements.

Item 8. This certification statement must be signed by an authorized representative of your company and the person signing must include his/her title. Additional pages used in your response must be initialled and dated in the space provided for the certification.

Item 9. Enter the date of signature.

Item 10. Enter the name of the person EPA should contact with questions regarding your response.

Item 11. Enter the phone number of your company contact.

SPECIFIC INSTRUCTIONS FOR COMPLETING THE REQUIREMENTS STATUS AND REGISTRANTS RESPONSE FORM

Generic Data

This form is designed to be used for registrants to respond to call-in- for generic and product-specific data as part of EPA's reregistration program under the Federal Insecticide Fungicide and Rodenticide Act. Although the form is the same for both product specific and generic data, instructions for completing the forms differ slightly. Specifically, options for satisfying product specific data requirements do not include (1) deletion of uses or (2) request for a low volume/minor use waiver. These instructions are for completion of generic data requirements.

EPA has developed this form individually for each data call-in addressed to each registrant, and has preprinted this form with a number of items. DO NOT use this form for any other active ingredient.

Items 1 through 8 (inclusive) will have been preprinted on the form. You must complete all other items on this form by typing or printing legibly.

Public reporting burden for this collection of information is estimated to average 30 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggesting for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Project 2070-0107, Washington, D.C. 20503.

INSTRUCTIONS

Item 1. This item identifies your company name, number, and address.

Item 2. This item identifies the case number, case name, EPA chemical number and chemical name.

Item 3. This item identifies the date and type of data call-in.

Item 4. This item identifies the guideline reference numbers of studies required to support the product(s) being reregistered. These guidelines, in addition to requirements specified in the Data Call-In Notice, govern the conduct of the required studies.

Item 5. This item identifies the study title associated with the guideline reference number and whether protocols and 1, 2, or 3-year progress reports are required to be

submitted in connection with the study. As noted in Section III of the Data Call-In Notice, 90-day progress reports are required for all studies.

If an asterisk appears in Item 5, EPA has attached information relevant to this guideline reference number to the Requirements Status and Registrant's Response Form.

Item 6. This item identifies the code associated with the use pattern of the pesticide. A brief description of each code follows:

A.	Terrestrial food
B.	Terrestrial feed
C.	Terrestrial non-food
D.	Aquatic food
E.	Aquatic non-food outdoor
F.	Aquatic non-food industrial
G.	Aquatic non-food residential
H.	Greenhouse food
I.	Greenhouse non-food crop
J.	Forestry
K.	Residential
L.	Indoor food
M.	Indoor non-food
N.	Indoor medical
O.	Indoor residential

Item 7. This item identifies the code assigned to the substance that must be used for testing. A brief description of each code follows.

EP	End-Use Product
MP	Manufacturing-Use Product
MP/TGAI	Manufacturing-Use Product and Technical Grade Active Ingredient
PAI	Pure Active Ingredient
PAI/M	Pure Active Ingredient and Metabolites
PAI/PAIRA	Pure Active Ingredient or Pure Active Ingredient Radiolabelled
PAIRA	Pure Active Ingredient Radiolabelled
PAIRA/M	Pure Active Ingredient Radiolabelled and Metabolites
PAIRA/PM	Pure Active Ingredient Radiolabelled and Plant Metabolites
TEP	Typical End-Use Product
TEP _ *	Typical End-Use Product, Percent Active Ingredient Specified

TEP/MET	Typical End-Use Product and Metabolites
TEP/PAI/M	Typical End-Use Product or Pure Active Ingredient and Metabolites
TGAI/PAIRA	Technical Grade Active Ingredient or Pure Active Ingredient Radiolabelled
TGAI	Technical Grade Active Ingredient
TGAI/TEP	Technical Grade Active Ingredient or Typical End-Use Product
TGAI/PAI	Technical Grade Active Ingredient or Pure Active Ingredient
MET	Metabolites
IMP	Impurities
DEGR	Degradates

*See: guideline comment

Item 8. This item identifies the time frame allowed for submission of the study or protocol identified in item 2. The time frame runs from the date **of your** receipt of the Data Call-In Notice.

Item 9. Enter the appropriate Response Code or Codes to show how you intend to comply with each data requirement. Brief descriptions of each code follow. The Data Call-In Notice contains a fuller description of each of these options.

1. (Developing Data) I will conduct a new study and submit it within the time frames specified in item 8 above. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to the conditions for submittal of this study as outlined in the Data Call-In Notice and that I will provide the protocol and progress reports required in item 5 above.

2. (Agreement to Cost Share) I have entered into an agreement with one or more registrants to develop data jointly. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to sharing in the cost of developing data as outlined in the Data Call-ln Notice.

3. (Offer to Cost Share) I have made an offer to enter into an agreement with one or more registrants to develop data jointly. I am submitting a copy of the form "Certification of Offer to Cost Share in the Development of Data" that describes this offer/agreement. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to making an offer to share in the cost of developing data as outlined in the Data Call-In Notice.

4. (Submitting Existing Data) I am submitting an existing study that has never before been submitted to EPA. By indicating that I have chosen this option, I certify that this study meets all the requirements pertaining to the conditions for submittal of existing data outlined in the Data Call-In Notice and I have attached the needed supporting information along with this response.

5. (Upgrading a Study) I am submitting or citing data to upgrade a study that EPA has classified as partially acceptable and potentially upgradeable. By indicating that I have chosen this option, I certify that I have met all the requirements pertaining to the conditions for submitting or citing existing data to upgrade a study described in the Data Call-In Notice. I am indicating on attached correspondence the Master Record Identification Number (MRID) that EPA has assigned to the data that I am citing as well as the MRID of the study I am attempting to upgrade.

6. (Citing a Study) I am citing an existing study that has been previously classified by EPA as acceptable, core, core minimum, or a study that has not yet been reviewed by the Agency. I am providing the Agency's classification of the study.

7. (Deleting Uses) I am attaching an application for amendment to my registration deleting the uses for which the data are required.

8. (Low Volume/Minor Use Waiver Request) I have read the statements concerning low volume-minor use data waivers in the Data Call-In Notice and I request a low-volume minor use waiver of the data requirement. I am attaching a detailed justification to support this waiver request including, among other things, all information required to support the request. I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

9. (Request for Waiver of Data) I have read the statements concerning data waivers other than low volume minor-use data waivers in the Data Call-In Notice and I request a waiver of the data requirement. I am attaching an identification of the basis for this waiver and a detailed justification to support this waiver request. The justification includes, among other things, all information required to support the request. I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

Item 10. This item must be signed by an authorized representative of your company. The person signing must include his/her title, and must initial and date all other pages of this form.

Item 11. Enter the date of signature.

Item 12. Enter the name of the person EPA should contact with questions regarding your response.

Item 13. Enter the phone number of your company contact.

Attachment 4. List of Registrant(s) sent this DCI (Insert)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

DATA CALL-IN NOTICE

CERTIFIED MAIL

Dear Sir or Madam:

This Notice requires you and other registrants of pesticide products containing the active ingredient identified in Attachment 1 of this Notice, the Data Call-In Chemical Status Sheet, to submit certain product specific data as noted herein to the U.S. Environmental Protection Agency (EPA, the Agency). These data are necessary to maintain the continued registration of your product(s) containing this active ingredient. Within 90 days after you receive this Notice you must respond as set forth in Section III below. Your response must state:

1. How you will comply with the requirements set forth in this Notice and its Attachments 1 through 6; or

2. Why you believe you are exempt from the requirements listed in this Notice and in Attachment 3, Requirements Status and Registrant's Response Form, (see section III-B); or

3. Why you believe EPA should not require your submission of product specific data in the manner specified by this Notice (see section III-D).

If you do not respond to this Notice, or if you do not satisfy EPA that you will comply with its requirements or should be exempt or excused from doing so, then the registration of your product(s) subject to this Notice will be subject to suspension. We have provided a list of

all of your products subject to this Notice in Attachment 2, Data Call-In Response Form, as well as a list of all registrants who were sent this Notice (Attachment 6).

The authority for this Notice is section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act as amended (FIFRA), 7 U.S.C. section 136a(c)(2)(B). Collection of this information is authorized under the Paperwork Reduction Act by OMB Approval No. 2070-0107 and 2070-0057 (expiration date 03-31-96).

This Notice is divided into six sections and six Attachments. The Notice itself contains information and instructions applicable to all Data Call-In Notices. The Attachments contain specific chemical information and instructions. The six sections of the Notice are:

Section I - Why You Are Receiving This Notice
Section II - Data Required By This Notice
Section III - Compliance With Requirements Of This Notice
Section IV - Consequences Of Failure To Comply With This Notice
Section V - Registrants' Obligation To Report Possible Unreasonable Adverse Effects
Section VI - Inquiries And Responses To This Notice

The Attachments to this Notice are:

1 - Data Call-In Chemical Status Sheet
2 - Product-Specific Data Call-In Response Form
3 - Requirements Status and Registrant's Response Form
4 - EPA Batching of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
5 - List of Registrants Receiving This Notice
6 - Cost Share and Data Compensation Forms

SECTION I. WHY YOU ARE RECEIVING THIS NOTICE

The Agency has reviewed existing data for this active ingredient and reevaluated the data needed to support continued registration of the subject active ingredient. The Agency has concluded that the only additional data necessary are product specific data. No additional generic data requirements are being imposed. You have been sent this Notice because you have product(s) containing the subject active ingredient.

SECTION II. DATA REQUIRED BY THIS NOTICE

II-A. DATA REQUIRED

The product specific data required by this Notice are specified in Attachment 3, Requirements Status and Registrant's Response Form. Depending on the results of the studies required in this Notice, additional testing may be required.

II-B. SCHEDULE FOR SUBMISSION OF DATA

You are required to submit the data or otherwise satisfy the data requirements specified in Attachment 3, Requirements Status and Registrant's Response Form, within the time frames provided.

II-C. TESTING PROTOCOL

All studies required under this Notice must be conducted in accordance with test standards outlined in the Pesticide Assessment Guidelines for those studies for which guidelines have been established.

These EPA Guidelines are available from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, Va 22161 (tel: 703-487-4650).

Protocols approved by the Organization for Economic Cooperation and Development (OECD) are also acceptable if the OECD-recommended test standards conform to those specified in the Pesticide Data Requirements regulation (40 CFR § 158.70). When using the OECD protocols, they should be modified as appropriate so that the data generated by the study will satisfy the requirements of 40 CFR § 158. Normally, the Agency will not extend deadlines for complying with data requirements when the studies were not conducted in accordance with acceptable standards. The OECD protocols are available from OECD, 2001 L Street, N.W., Washington, D.C. 20036 (Telephone number 202-785-6323; Fax telephone number 202-785-0350).

All new studies and proposed protocols submitted in response to this Data Call-In Notice must be in accordance with Good Laboratory Practices [40 CFR Part 160.3(a)(6)].

II-D. REGISTRANTS RECEIVING PREVIOUS SECTION 3(c)(2)(B) NOTICES ISSUED BY THE AGENCY

Unless otherwise noted herein, this Data Call-In does not in any way supersede or change the requirements of any previous Data Call-In(s), or any other agreements entered into with the Agency pertaining to such prior Notice. Registrants must comply with the requirements of all Notices to avoid issuance of a Notice of Intent to Suspend their affected products.

SECTION III. COMPLIANCE WITH REQUIREMENTS OF THIS NOTICE

III-A. SCHEDULE FOR RESPONDING TO THE AGENCY

The appropriate responses initially required by this Notice for product specific data must be submitted to the Agency within 90 days after your receipt of this Notice. Failure to adequately respond to this Notice within 90 days of your receipt will be a basis for issuing a Notice of Intent to Suspend (NOIS) affecting your products. This and other bases for issuance of NOIS due to failure to comply with this Notice are presented in Section IV-A and IV-B.

III-B. OPTIONS FOR RESPONDING TO THE AGENCY

The options for responding to this Notice for product specific data are: (a) voluntary cancellation, (b) agree to satisfy the product specific data requirements imposed by this notice or (c) request a data waiver(s).

A discussion of how to respond if you chose the Voluntary Cancellation option is presented below. A discussion of the various options available for satisfying the product specific data requirements of this Notice is contained in Section III-C. A discussion of options relating to requests for data waivers is contained in Section III-D.

There are two forms that accompany this Notice of which, depending upon your response, one or both must be used in your response to the Agency. These forms are the Data-Call-In Response Form, and the Requirements Status and Registrant's Response Form, Attachment 2 and Attachment 3. The Data Call-In Response Form must be submitted as part of every response to this Notice. In addition, one copy of the Requirements Status and Registrant's Response Form must be submitted for each product listed on the Data Call-In Response Form unless the voluntary cancellation option is selected or unless the product is identical to another (refer to the instructions for completing the Data Call-In Response Form in Attachment 2). Please note that the company's authorized representative is required to sign the first page of the Data Call-In Response Form and Requirements Status and Registrant's Response Form (if this form is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person(s) identified in Attachment 1.

1. Voluntary Cancellation - You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit a completed Data Call-In Response Form, indicating your election of this option. Voluntary cancellation is item number 5 on the Data Call-In Response Form. If you choose this option, this is the only form that you are required to complete.

If you chose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice which are contained in Section IV-C.

2. Satisfying the Product Specific Data Requirements of this Notice There are various options available to satisfy the product specific data requirements of this Notice. These options are discussed in Section III-C of this Notice and comprise options 1 through 6 on the Requirements Status and Registrant's Response Form and item numbers 7a and 7b on the Data Call-In Response Form. Deletion of a use(s) and the low volume/minor use option are not valid options for fulfilling product specific data requirements.

3. Request for Product Specific Data Waivers. Waivers for product specific data are discussed in Section III-D of this Notice and are covered by option 7 on the Requirements Status and Registrant's Response Form. If you choose one of these options, you must submit

both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

III-C SATISFYING THE DATA REQUIREMENTS OF THIS NOTICE

If you acknowledge on the Data Call-In Response Form that you agree to satisfy the product specific data requirements (i.e. you select item number 7a or 7b), then you must select one of the six options on the Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide registrants to additional instructions provided in this Section. The options are:

(1) I will generate and submit data within the specified time frame (Developing Data)
(2) I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing)
(3) I have made offers to cost-share (Offers to Cost Share)
(4) I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study)
(5) I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study)
(6) I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study)

Option 1, Developing Data -- If you choose to develop the required data it must be in conformance with Agency deadlines and with other Agency requirements as referenced herein and in the attachments. All data generated and submitted must comply with the Good Laboratory Practice (GLP) rule (40 CFR Part 160), be conducted according to the Pesticide Assessment Guidelines (PAG), and be in conformance with the requirements of PR Notice 86-5.

The time frames in the Requirements Status and Registrant's Response Form are the time frames that the Agency is allowing for the submission of completed study reports. The noted deadlines run from the date of the receipt of this Notice by the registrant. If the data are not submitted by the deadline, each registrant is subject to receipt of a Notice of Intent to Suspend the affected registration(s).

If you cannot submit the data/reports to the Agency in the time required by this Notice and intend to seek additional time to meet the requirements(s), you must submit a request to the Agency which includes: (1) a detailed description of the expected difficulty and (2) a proposed schedule including alternative dates for meeting such requirements on a step-by-step basis. You must explain any technical or laboratory difficulties and provide documentation from the laboratory performing the testing. While EPA is considering your request, the original

deadline remains. The Agency will respond to your request in writing. If EPA does not grant your request, the original deadline remains. Normally, extensions can be requested only in cases of extraordinary testing problems beyond the expectation or control of the registrant. Extensions will not be given in submitting the 90-day responses. Extensions will not be considered if the request for extension is not made in a timely fashion; in no event shall an extension request be considered if it is submitted at or after the lapse of the subject deadline.

Option 2, Agreement to Share in Cost to Develop Data -- Registrants may only choose this option for acute toxicity data and certain efficacy data and only if EPA has indicated in the attached data tables that your product and at least one other product are similar for purposes of depending on the same data. If this is the case, data may be generated for just one of the products in the group. The registration number of the product for which data will be submitted must be noted in the agreement to cost share by the registrant selecting this option. If you choose to enter into an agreement to share in the cost of producing the required data but will not be submitting the data yourself, you must provide the name of the registrant who will be submitting the data. You must also provide EPA with documentary evidence that an agreement has been formed. Such evidence may be your letter offering to join in an agreement and the other registrant's acceptance of your offer, or a written statement by the parties that an agreement exists. The agreement to produce the data need not specify all of the terms of the final arrangement between the parties or the mechanism to resolve the terms. Section 3(c)(2)(B) provides that if the parties cannot resolve the terms of the agreement they may resolve their differences through binding arbitration.

Option 3, Offer to Share in the Cost of Data Development -- This option only applies to acute toxicity and certain efficacy data as described in option 2 above. If you have made an offer to pay in an attempt to enter into an agreement or amend an existing agreement to meet the requirements of this Notice and have been unsuccessful, you may request EPA (by selecting this option) to exercise its discretion not to suspend your registration(s), although you do not comply with the data submission requirements of this Notice. EPA has determined that as a general policy, absent other relevant considerations, it will not suspend the registration of a product of a registrant who has in good faith sought and continues to seek to enter into a joint data development/cost sharing program, but the other registrant(s) developing the data has refused to accept your offer. To qualify for this option, you must submit documentation to the Agency proving that you have made an offer to another registrant (who has an obligation to submit data) to share in the burden of developing that data. You must also submit to the Agency a completed EPA Form 8570-32, Certification of Offer to Cost Share in the Development of Data, Attachment 7. In addition, you must demonstrate that the other registrant to whom the offer was made has not accepted your offer to enter into a cost sharing agreement by including a copy of your offer and proof of the other registrant's receipt of that offer (such as a certified mail receipt). Your offer must, in addition to anything else, offer to share in the burden of producing the data upon terms to be agreed or failing agreement to be bound by binding arbitration as provided by FIFRA section 3(c)(2)(B)(iii) and must not qualify this offer. The other registrant must also inform EPA of its election of an option to develop and submit the data required by this Notice by submitting a Data Call-In Response Form and a Requirements Status and Registrant's Response Form committing to develop and submit the data required by this Notice.

In order for you to avoid suspension under this option, you may not withdraw your offer to share in the burdens of developing the data. In addition, the other registrant must fulfill its commitment to develop and submit the data as required by this Notice. If the other registrant fails to develop the data or for some other reason is subject to suspension, your registration as well as that of the other registrant will normally be subject to initiation of suspension proceedings, unless you commit to submit, and do submit the required data in the specified time frame. In such cases, the Agency generally will not grant a time extension for submitting the data.

Option 4, Submitting an Existing Study -- If you choose to submit an existing study in response to this Notice, you must determine that the study satisfies the requirements imposed by this Notice. You may only submit a study that has not been previously submitted to the Agency or previously cited by anyone. Existing studies are studies which predate issuance of this Notice. Do not use this option if you are submitting data to upgrade a study. (See Option 5).

You should be aware that if the Agency determines that the study is not acceptable, the Agency will require you to comply with this Notice, normally without an extension of the required date of submission. The Agency may determine at any time that a study is not valid and needs to be repeated.

To meet the requirements of the DCI Notice for submitting an existing study, all of the following three criteria must be clearly met:

a. You must certify at the time that the existing study is submitted that the raw data and specimens from the study are available for audit and review and you must identify where they are available. This must be done in accordance with the requirements of the Good Laboratory Practice (GLP) regulation, 40 CFR Part 160. As stated in 40 CFR 160.3(j) " 'raw data' means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. 'Raw data' may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments." The term "specimens", according to 40 CFR 160.3(k), means "any material derived from a test system for examination or analysis."

b. Health and safety studies completed after May 1984 must also contain all GLP-required quality assurance and quality control information, pursuant to the requirements of 40 CFR Part 160. Registrants must also certify at the time of submitting the existing study that such GLP information is available for post-May 1984 studies by including an appropriate statement on or attached to the study signed by an authorized official or representative of the registrant.

c. You must certify that each study fulfills the acceptance criteria for the Guideline relevant to the study provided in the FIFRA Accelerated Reregistration Phase 3 Technical Guidance and that the study has been conducted according to the Pesticide Assessment Guidelines (PAG) or meets the purpose of the PAG (both available from NTIS). A study not conducted according to the PAG may be submitted to the Agency for consideration if the registrant believes that the study clearly meets the purpose of the PAG. The registrant is referred to 40 CFR 158.70 which states the Agency's policy regarding acceptable protocols. If you wish to submit the study, you must, in addition to certifying that the purposes of the PAG are met by the study, clearly articulate the rationale why you believe the study meets the purpose of the PAG, including copies of any supporting information or data. It has been the Agency's experience that studies completed prior to January 1970 rarely satisfied the purpose of the PAG and that necessary raw data are usually not available for such studies.

If you submit an existing study, you must certify that the study meets all requirements of the criteria outlined above.

If you know of a study pertaining to any requirement in this Notice which does not meet the criteria outlined above but does contain factual information regarding unreasonable adverse effects, you must notify the Agency of such a study. If such study is in the Agency's files, you need only cite it along with the notification. If not in the Agency's files, you must submit a summary and copies as required by PR Notice 86-5.

Option 5, Upgrading a Study -- If a study has been classified as partially acceptable and upgradeable, you may submit data to upgrade that study. The Agency will review the data submitted and determine if the requirement is satisfied. If the Agency decides the requirement is not satisfied, you may still be required to submit new data normally without any time extension. Deficient, but upgradeable studies will normally be classified as supplemental. However, it is important to note that not all studies classified as supplemental are upgradeable. If you have questions regarding the classification of a study or whether a study may be upgraded, call or write the contact person listed in Attachment 1. If you submit data to upgrade an existing study you must satisfy or supply information to correct all deficiencies in the study identified by EPA. You must provide a clearly articulated rationale of how the deficiencies have been remedied or corrected and why the study should be rated as acceptable to EPA. Your submission must also specify the MRID number(s) of the study which you are attempting to upgrade and must be in conformance with PR Notice 86-5.

Do not submit additional data for the purpose of upgrading a study classified as unacceptable and determined by the Agency as not capable of being upgraded.

This option should also be used to cite data that has been previously submitted to upgrade a study, but has not yet been reviewed by the Agency. You must provide the MRID number of the data submission as well as the MRID number of the study being upgraded.

The criteria for submitting an existing study, as specified in Option 4 above, apply to all data submissions intended to upgrade studies. Additionally your submission of data intended to upgrade studies must be accompanied by a certification that you comply with each of those criteria as well as a certification regarding protocol compliance with Agency requirements.

Option 6, Citing Existing Studies -- If you choose to cite a study that has been previously submitted to EPA, that study must have been previously classified by EPA as acceptable or it must be a study which has not yet been reviewed by the Agency. Acceptable toxicology studies generally will have been classified as "core-guideline" or "core minimum." For all other disciplines the classification would be "acceptable." With respect to any studies for which you wish to select this option you must provide the MRID number of the study you are citing and, if the study has been reviewed by the Agency, you must provide the Agency's classification of the study.

If you are citing a study of which you are not the original data submitter, you must submit a completed copy of EPA Form 8570-31, Certification with Respect to Data Compensation Requirements.

Registrants who select one of the above 6 options must meet all of the requirements described in the instructions for completing the Data Call-In Response Form and the Requirements Status and Registrant's Response Form, as appropriate.

III-D REQUESTS FOR DATA WAIVERS

If you request a waiver for product specific data because you believe it is inappropriate, you must attach a complete justification for the request, including technical reasons, data and references to relevant EPA regulations, guidelines or policies. (Note: any supplemental data must be submitted in the format required by PR Notice 86-5). This will be the only opportunity to state the reasons or provide information in support of your request. If the Agency approves your waiver request, you will not be required to supply the data pursuant to section 3(c)(2)(B) of FIFRA. If the Agency denies your waiver request, you must choose an option for meeting the data requirements of this Notice within 30 days of the receipt of the Agency's decision. You must indicate and submit the option chosen on the Requirements Status and Registrant's Response Form. Product specific data requirements for product chemistry, acute toxicity and efficacy (where appropriate) are required for all products and the Agency would grant a waiver only under extraordinary circumstances. You should also be aware that submitting a waiver request will not automatically extend the due date for the study in question. Waiver requests submitted without adequate supporting rationale will be denied and the original due date will remain in force.

IV. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS NOTICE

IV-A NOTICE OF INTENT TO SUSPEND

The Agency may issue a Notice of Intent to Suspend products subject to this Notice due to failure by a registrant to comply with the requirements of this Data Call-In Notice, pursuant

to FIFRA section 3(c)(2)(B). Events which may be the basis for issuance of a Notice of Intent to Suspend include, but are not limited to, the following:

1. Failure to respond as required by this Notice within 90 days of your receipt of this Notice.

2. Failure to submit on the required schedule an acceptable proposed or final protocol when such is required to be submitted to the Agency for review.

3. Failure to submit on the required schedule an adequate progress report on a study as required by this Notice.

4. Failure to submit on the required schedule acceptable data as required by this Notice.

5. Failure to take a required action or submit adequate information pertaining to any option chosen to address the data requirements (e.g., any required action or information pertaining to submission or citation of existing studies or offers, arrangements, or arbitration on the sharing of costs or the formation of Task Forces, failure to comply with the terms of an agreement or arbitration concerning joint data development or failure to comply with any terms of a data waiver).

6. Failure to submit supportable certifications as to the conditions of submitted studies, as required by Section III-C of this Notice.

7. Withdrawal of an offer to share in the cost of developing required data.

8. Failure of the registrant to whom you have tendered an offer to share in the cost of developing data and provided proof of the registrant's receipt of such offer or failure of a registrant on whom you rely for a generic data exemption either to:

   a. inform EPA of intent to develop and submit the data required by this Notice on a Data Call-In Response Form and a Requirements Status and Registrant's Response Form;

   b. fulfill the commitment to develop and submit the data as required by this Notice; or

   c. otherwise take appropriate steps to meet the requirements stated in this Notice, unless you commit to submit and do submit the required data in the specified time frame.

9. Failure to take any required or appropriate steps, not mentioned above, at any time following the issuance of this Notice.

IV-B. BASIS FOR DETERMINATION THAT SUBMITTED STUDY IS UNACCEPTABLE

The Agency may determine that a study (even if submitted within the required time) is unacceptable and constitutes a basis for issuance of a Notice of Intent to Suspend. The grounds for suspension include, but are not limited to, failure to meet any of the following:

1. EPA requirements specified in the Data Call-In Notice or other documents incorporated by reference (including, as applicable, EPA Pesticide Assessment Guidelines, Data Reporting Guidelines, and GeneTox Health Effects Test Guidelines) regarding the design, conduct, and reporting of required studies. Such requirements include, but are not limited to, those relating to test material, test procedures, selection of species, number of animals, sex and distribution of animals, dose and effect levels to be tested or attained, duration of test, and, as applicable, Good Laboratory Practices.

2. EPA requirements regarding the submission of protocols, including the incorporation of any changes required by the Agency following review.

3. EPA requirements regarding the reporting of data, including the manner of reporting, the completeness of results, and the adequacy of any required supporting (or raw) data, including, but not limited to, requirements referenced or included in this Notice or contained in PR 86-5. All studies must be submitted in the form of a final report; a preliminary report will not be considered to fulfill the submission requirement.

IV-C EXISTING STOCKS OF SUSPENDED OR CANCELLED PRODUCTS

EPA has statutory authority to permit continued sale, distribution and use of existing stocks of a pesticide product which has been suspended or cancelled if doing so would be consistent with the purposes of the Act.

The Agency has determined that such disposition by registrants of existing stocks for a suspended registration when a section 3(c)(2)(B) data request is outstanding would generally not be consistent with the Act's purposes. Accordingly, the Agency anticipates granting registrants permission to sell, distribute, or use existing stocks of suspended product(s) only in exceptional circumstances. If you believe such disposition of existing stocks of your product(s) which may be suspended for failure to comply with this Notice should be permitted, you have the burden of clearly demonstrating to EPA that granting such permission would be consistent with the Act. You must also explain why an "existing stocks" provision is necessary, including a statement of the quantity of existing stocks and your estimate of the time required for their sale, distribution, and use. Unless you meet this burden the Agency will not consider any request pertaining to the continued sale, distribution, or use of your existing stocks after suspension.

If you request a voluntary cancellation of your product(s) as a response to this Notice and your product is in full compliance with all Agency requirements, you will have, under most circumstances, one year from the date your 90 day response to this Notice is due, to sell, distribute, or use existing stocks. Normally, the Agency will allow persons other than the

registrant such as independent distributors, retailers and end users to sell, distribute or use such existing stocks until the stocks are exhausted. Any sale, distribution or use of stocks of voluntarily cancelled products containing an active ingredient for which the Agency has particular risk concerns will be determined on case-by-case basis.

Requests for voluntary cancellation received after the 90 day response period required by this Notice will not result in the Agency granting any additional time to sell, distribute, or use existing stocks beyond a year from the date the 90 day response was due unless you demonstrate to the Agency that you are in full compliance with all Agency requirements, including the requirements of this Notice. For example, if you decide to voluntarily cancel your registration six months before a 3 year study is scheduled to be submitted, all progress reports and other information necessary to establish that you have been conducting the study in an acceptable and good faith manner must have been submitted to the Agency, before EPA will consider granting an existing stocks provision.

SECTION V. REGISTRANTS' OBLIGATION TO REPORT POSSIBLE UNREASONABLE ADVERSE EFFECTS

Registrants are reminded that FIFRA section 6(a)(2) states that if at any time after a pesticide is registered a registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit the information to the Agency. Registrants must notify the Agency of any factual information they have, from whatever source, including but not limited to interim or preliminary results of studies, regarding unreasonable adverse effects on man or the environment. This requirement continues as long as the products are registered by the Agency.

SECTION VI. INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the requirements and procedures established by this Notice, call the contact person(s) listed in Attachment 1, the Data Call-In Chemical Status Sheet.

All responses to this Notice (other than voluntary cancellation requests and generic data exemption claims) must include a completed Data Call-In Response Form and a completed Requirements Status and Registrant's Response Form (Attachment 2 and Attachment 3 for product specific data) and any other documents required by this Notice, and should be submitted to the contact person(s) identified in Attachment 1. If the voluntary cancellation or generic data exemption option is chosen, only the Data Call-In Response Form need be submitted.

The Office of Compliance Monitoring (OCM) of the Office of Pesticides and Toxic Substances (OPTS), EPA, will be monitoring the data being generated in response to this Notice.

Sincerely yours,

Lois Rossi, Division Director
Special Review and
Reregistration Division

Attachments

1 - Data Call-In Chemical Status Sheet
2 - Product-Specific Data Call-In Response Form
3 - Requirements Status and Registrant's Response Form
4 - EPA Batching of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
5 - List of Registrants Receiving This Notice
6 - Cost Share and Data Compensation Forms and the Confidential Statement of Formula Form

FURANONE DATA CALL-IN CHEMICAL STATUS SHEET

INTRODUCTION

You have been sent this Product Specific Data Call-In Notice because you have product(s) containing Furanone.

This Product Specific Data Call-In Chemical Status Sheet, contains an overview of data required by this notice, and point of contact for inquiries pertaining to the reregistration of Furanone. This attachment is to be used in conjunction with (1) the Product Specific Data Call-In Notice, (2) the Product Specific Data Call-In Response Form (Attachment 2), (3) the Requirements Status and Registrant's Form (Attachment 3), (4) EPA's Grouping of End-Use Products for Meeting Acute Toxicology Data Requirement (Attachment 4), (5) the EPA Acceptance Criteria (Attachment 5), (6) a list of registrants receiving this DCI (Attachment 6) and (7) the Cost Share and Data Compensation Forms in replying to this Furanone Product Specific Data Call-In (Attachment 7). Instructions and guidance accompany each form.

DATA REQUIRED BY THIS NOTICE

The additional data requirements needed to complete the database for Furanone are contained in the Requirements Status and Registrant's Response, Attachment 3. The Agency has concluded that additional data on Furanone are needed for specific products. These data are required to be submitted to the Agency within the time frame listed. These data are needed to fully complete the reregistration of all eligible Furanone products.

INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding this product specific data requirements and procedures established by this Notice, please contact Emily Mitchell at (703) 308-8583.

All responses to this Notice for the Product Specific data requirements should be submitted to:

Emily Mitchell
Chemical Review Manager Team 81
Product Reregistration Branch
Special Review and Reregistration Branch 7508W
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460

RE: **Furanone**

INSTRUCTIONS FOR COMPLETING THE **DATA CALL-IN RESPONSE FORM** FOR **PRODUCT SPECIFIC DATA**

Item 1-4. Already completed by EPA.

Item 5. If you wish to **voluntarily cancel** your product, answer "**yes**." If you choose this option, you will not have to provide the data required by the Data Call-In Notice and you will not have to complete any other forms. Further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provision of the Data Call-In Notice (Section IV-C).

Item 6. Not applicable since this form calls in product specific data only. However, if your product is **identical** to another product and you qualify for a **data exemption**, you must respond with "**yes**" to Item 7a (MUP) or 7B (EUP) on this form, provide the **EPA registration numbers of your source(s)**; you would **not** complete the "Requirements Status and Registrant's Response" form. Examples of such products include **repackaged** products and **Special Local Needs (Section 24c)** products which are identical to federally registered products.

Item 7a. For each **manufacturing use product** (MUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "**yes**."

Item 7b. For each **end use product** (EUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "**yes**." If you are requesting a **data waiver**, answer "**yes**" here; in addition, on the "Requirements Status and Registrant's Response" form under Item 9, you must respond with **Option 7** (Waiver Request) for each study for which you are requesting a waiver. See Item 6 with regard to identical products and data exemptions.

Items 8-11. Self-explanatory.

NOTE: You may provide **additional information** that does not fit on this form in a signed letter that accompanies this form. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily canceled this product. For these cases, please supply all relevant details so that EPA can ensure that its records are correct.

This page is replaced with the Part A section from the PSDCI module.

INSTRUCTIONS FOR COMPLETING THE **REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORM** FOR **PRODUCT SPECIFIC DATA**

Item 1-3 Completed by EPA. Note the **unique identifier number** assigned by EPA in Item 3. This number **must be used in the transmittal document for any data submissions** in response to this Data Call-In Notice.

Item 4. The guideline reference numbers of studies required to support the product's continued registration are identified. These guidelines, in addition to the requirements specified in the Notice, govern the conduct of the required studies. Note that series 61 and 62 in product chemistry are now listed under 40 CFR 158.155 through 158.180, Subpart C.

Item 5. The study title associated with the guideline reference number is identified.

Item 6. The use pattern(s) of the pesticide associated with the product specific requirements is (are) identified. For most product specific data requirements, all use patterns are covered by the data requirements. In the case of efficacy data, the required studies only pertain to products which have the use sites and/or pests indicated.

Item 7. The substance to be tested is identified by EPA. For product specific data, the product as formulated for sale and distribution is the test substance, except in rare cases.

Item 8. The due date for submission of each study is identified. It is normally based on **8 months after issuance of the Reregistration Eligibility Document** unless EPA determines that a longer time period is necessary.

Item 9. **Enter only one of the following response codes for each data requirement to show how you intend to comply with the data requirements listed in this table.** Fuller descriptions of each option are contained in the Data Call-In Notice.

1. I will generate and submit data by the specified due date (**Developing Data**). By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to the conditions for submittal of this study as outlined in the Data Call-In Notice. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements**" **form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

2. I have entered into an agreement with one or more registrants to develop data jointly (**Cost Sharing**). I am submitting a **copy of this agreement**. I understand that this option is available **only** for acute toxicity or certain efficacy data and **only** if EPA indicates in an attachment to this Notice that my product is similar enough to another product to qualify for this option. I certify that another party in the

agreement is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

3. I have made offers to share in the cost to develop data (**Offers to Cost Share**). I understand that this option is available **only** for acute toxicity or certain efficacy data and **only** if EPA indicates in an attachment to this Data Call-In Notice that my product is similar enough to another product to qualify for this option. I am submitting **evidence that I have made an offer** to another registrant (who has an obligation to submit data) to share in the cost of that data. I am also submitting a completed "**Certification of Offer to Cost Share in the Development Data" form**. I am including a copy of my offer and proof of the other registrant's receipt of that offer. I am identifying the party which is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension. I understand that other terms under Option 3 in the Data Call-In Notice (Section III-C.1.) apply as well. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

4. By the specified due date, I will submit an existing study that has not been submitted previously to the Agency by anyone (**Submitting an Existing Study**). I certify that this study will meet all the requirements for submittal of existing data outlined in Option 4 in the Data Call-In Notice (Section III-C.1.) and will meet the attached acceptance criteria (for acute toxicity and product chemistry data). I will attach the needed supporting information along with this response. I also certify that I have determined that this study will fill the data requirement for which I have indicated this choice. By the specified due date, I will also submit a completed "**Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29)** to show what data compensation option I have chosen. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

5. By the specified due date, I will submit or cite data to upgrade a study classified by the Agency as partially acceptable and upgradable (**Upgrading a Study**). I will submit **evidence of the Agency's review** indicating that the study may be upgraded and what information is required to do so. I will provide the MRID or Accession number of the study at the due date. I understand that the conditions for this option outlined Option 5 in the Data Call-In Notice (Section III-C.1.) apply. By the specified due date, I will also submit: (1) a completed "**Certification With**

Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

6. By the specified due date, I will cite an existing study that the Agency has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (**Citing an Existing Study**). If I am citing another registrant's study, I understand that this option is available **only** for acute toxicity or certain efficacy data and **only** if the cited study was conducted on my product, an identical product or a product which EPA has "grouped" with one or more other products for purposes of depending on the same data. I may also choose this option if I am citing my own data. In either case, I will provide the **MRID or Accession number(s)** for the cited data on a "Product Specific Data Report" form or in a similar format. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements**" **form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

7. I request a waiver for this study because it is inappropriate for my product (**Waiver Request**). I am attaching a complete justification for this request, including technical reasons, data and references to relevant EPA regulations, guidelines or policies. [Note: any supplemental data must be submitted in the format required by P.R. Notice 86-5]. I understand that this is my **only** opportunity to state the reasons or provide information in support of my request. If the Agency approves my waiver request, I will **not** be required to supply the data pursuant to Section 3(c)(2)(B) of FIFRA. If the Agency denies my waiver request, I **must choose** a method of meeting the data requirements of this Notice by the due date stated by this Notice. In this case, I must, within **30 days** of my receipt of the Agency's written decision, submit a revised "Requirements Status and Registrant's Response" Form indicating the option chosen. I also understand that the deadline for submission of data as specified by the original data call-in notice will not change. By the specified due date, I will also submit: (1) a completed "**Certification With Respect To Data Compensation Requirements**" **form (EPA Form 8570-29)** and (2) two completed and signed copies of the **Confidential Statement of Formula (EPA Form 8570-4)**.

Items 10-13. Self-explanatory.

NOTE: You may provide **additional information** that does not fit on this form in a signed letter that accompanies this form. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily canceled this product. For these cases, please supply all relevant details so that EPA can ensure that its records are correct.

This page is replaced with part B page 1 from the PSDCI module.

This page is replaced with part B page 2 from the PSDCI module.

This page is replaced with part B page 3 from the PSDCI module.

This page is replaced with part B page 4 from the PSDCI module.

No tox batching for this case

Attachment 5. List of Registrant(s) sent this DCI (Insert)

Instructions for Completing the Confidential Statement of Formula

The Confidential Statement of Formula (CSF) Form 8570-4 must be used. Two legible, signed copies of the form are required. Following are basic instructions:

a. All the blocks on the form must be filled in and answered completely.

b. If any block is not applicable, mark it N/A.

c. The CSF must be signed, dated and the telephone number of the responsible party must be provided.

d. All applicable information which is on the product specific data submission must also be reported on the CSF.

e. All weights reported under item 7 must be in pounds per gallon for liquids and pounds per cubic feet for solids.

f. Flashpoint must be in degrees Fahrenheit and flame extension in inches.

g. For all active ingredients, the EPA Registration Numbers for the currently registered source products must be reported under column 12.

h. The Chemical Abstracts Service (CAS) Numbers for all actives and inerts and all common names for the trade names must be reported.

i. For the active ingredients, the percent purity of the source products must be reported under column 10 and must be exactly the same as on the source product's label.

j. All the weights in columns 13.a. and 13.b. must be in pounds, kilograms, or grams. In no case will volumes be accepted. Do not mix English and metric system units (i.e., pounds and kilograms).

k. All the items under column 13.b. must total 100 percent.

1. All items under columns 14.a. and 14.b. for the active ingredients must represent pure active form.

m. The upper and lower certified limits for ail active and inert ingredients must follow the 40 CFR 158.175 instructions. An explanation must be provided if the proposed limits are different than standard certified limits.

n. When new CSFs are submitted and approved, all previously submitted CSFs become obsolete for that specific formulation.

Confidential Business Information: Does Not Contain National Security Information (E.O. 12065) Form Approved. OMB No. 2070-0060. Approval Expires 2/28/94

EPA

United States Environmental Protection Agency
Office of Pesticide Programs (TS-767)
Washington, DC 20460

Confidential Statement of Formula

A. ☐ Basic Formulation ☐ Alternate Formulation

B. Page of

See Instructions on Back

1. Name and Address of Applicant/Registrant *(Include ZIP Code)*

2. Name and Address of Producer *(Include ZIP Code)*

3. Product Name

4. Registration No./File Symbol

5. EPA Product Mgr/Team No.

6. Country Where Formulated

7. Pounds/Gal or Bulk Density

8. pH

9. Flash Point/Flame Extension

EPA USE ONLY	10. Components in Formulation *(List as actually introduced into the formulation. Give commonly accepted chemical name, trade name, and CAS number.)*	11. Supplier Name & Address	12. EPA Reg. No.	13. Each Component in Formulation a. Amount	13. b. % by Weight	14. Certified Limits % by Weight a. Upper Limit	14. b. Lower Limit	15. Purpose in Formulation
	16. Typed Name of Approving Official			17. Total Weight	100%			

18. Signature of Approving Official	19. Title	20. Phone No. *(Include Area Code)*	21. Date

EPA Form 8570-4 (Rev. 12-90) **Previous editions are obsolete.** If you can photocopy this, please submit an additional copy. White - EPA File Copy (original) Yellow - Applicant copy

EPA	United States Environmental Protection Agency Washington, DC 20460 **CERTIFICATION OF OFFER TO COST SHARE IN THE DEVELOPMENT OF DATA**	Form Approved OMB No. 2070-0106 2070-0057 Approval Expires 3-31-96

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0106), Washington, DC 20503.

Please fill in blanks below.

Company Name	Company Number
Product Name	EPA Reg. No.

I Certify that:

My company is willing to develop and submit the data required by EPA under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), if necessary. However, my company would prefer to enter into an agreement with one or more registrants to develop jointly or share in the cost of developing data.

My firm has offered in writing to enter into such an agreement. That offer was irrevocable and included an offer to be bound by arbitration decision under section 3(c)(2)(B)(iii) of FIFRA if final agreement on all terms could not be reached otherwise. This offer was made to the following firm(s) on the following date(s):

Name of Firm(s)	Date of Offer

Certification:

I certify that I am duly authorized to represent the company named above, and that the statements that I have made on this form and all attachments therein are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature of Company's Authorized Representative	Date
Name and Title (Please Type or Print)	

EPA Form 8570-32 (5/91) Replaces EPA Form 8580, which is obsolete

United States Environmental Protection Agency
Washington, DC 20460

Form Approved
OMB No. 2070-0107, 2070-0057
Approval Expires 3-31-96

CERTIFICATION WITH RESPECT TO DATA COMPENSATION REQUIREMENTS

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to, Chief Information Policy Branch, PM-233, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0106), Washington, DC 20503.

Please fill in blanks below.

Company Name	Company Number

Product Name	EPA Reg. No.

I Certify that:

1. For each study cited in support of registration or reregistratiion under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) that is an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter to cite that study.

2. That for each study cited in support of registration or reregistration under FIFRA that is NOT an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter, or I have notified in writing the company(ies) that submitted data I have cited and have offered to: (a) Pay compensation for those data in accordance with sections 3(c)(1)(F) and 3(c)(2)(D) of FIFRA; and (b) Commence negotiation to determine which data are subject to the compensation requirement of FIFRA and the amount of compensation due, if any. The companies I have notified are. (check one)

[ ] The companies who have submitted the studies listed on the back of this form or attached sheets, or indicated on the attached "Requirements Status and Registrants' Response Form,"

3. That I have previously complied with section 3(c)(1)(F) of FIFRA for the studies I have cited in support of registration or reregistration under FIFRA.

Signature	Date

Name and Title (Please Type or Print)

GENERAL OFFER TO PAY: I hereby offer and agree to pay compensation to other persons, with regard to the registration or reregistration of my products, to the extent required by FIFRA section 3(c)(1)(F) and 3(c)(2)(D).

Signature	Date

Name and Title (Please Type or Print)

EPA Form 8570-31 (4-96)

The following is a list of available documents for Furanone that my further assist you in responding to this Reregistration Eligibility Decision document. These documents may be obtained by the following methods:

Electronic
File format: Portable Document Format (.PDF) Requires Adobe® Acrobat or compatible reader. Electronic copies can be downloaded from the Pesticide Special Review and Reregistration Information System at 703-308-7224. They also are available on the Internet on EPA's gopher server, GOPHER.EPA.GOV, or using ftp on FTP.EPA.GOV, or using WWW (World Wide Web) on WWW.EPA.GOV., or contact Emily Mitchell at (703)-308-8583.

1. PR Notice 86-5.

2. PR Notice 91-2 (pertains to the Label Ingredient Statement).

3. A full copy of this RED document.

4. A copy of the fact sheet for Furanone.

The following documents are part of the Administrative Record for Furanone and may included in the EPA's Office of Pesticide Programs Public Docket. Copies of these documents are not available electronically, but may be obtained by contacting the person listed on the Chemical Status Sheet.

1.Health and Environmental Effects Science Chapters.

2.Detailed Label Usage Information System (LUIS) Report.

The following Agency reference documents are not available electronically, but may be obtained by contacting the person listed on the Chemical Status Sheet of this RED document.

1. The Label Review Manual.

2. EPA Acceptance Criteria

US EPA ARCHIVE DOCUMENT

United States Environmental Protection Agency | Prevention, Pesticides And Toxic Substances (7508W) | EPA-738-F-96-009 May 1996

R.E.D. FACTS

Furanone

Pesticide Reregistration

All pesticides sold or distributed in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered before November 1, 1984, be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency develops any mitigation measures or regulatory controls needed to effectively reduce each pesticide's risks. EPA then reregisters pesticides that can be used without posing unreasonable risks to human health or the environment.

When a pesticide is eligible for reregistration, EPA explains the basis for its decision in a Reregistration Eligibility Decision (RED) document. This fact sheet summarizes the information in the RED document for reregistration case 3138, furanone.

Use Profile

Dihydro-5-pentyl-2(3H)-furanone and dihydro-5-heptyl-2(3H)-furanone (also known as gamma-nonalactone and gamma-undeccalactone, respectively) are also referred to by the common name furanone. Products containing furanones are registered for use as insecticides, insect and mammal repellents, and mosquito larvicides.

Pesticide products containing furanones may be used used as a dog and cat repellent; fly, cockroach and ant killer; insect repellent, insect repellent strip, and insect repellent tablecloth; and mosquito larvicide. Formulations include liquid ready-to-use solutions, emulsifiable concentrates, granulars and impregnated material.

Furanone is applied by the use of impregnated table cloths, premoistened applicator puffs, aersol sprayers, non-aerosol hand pump sprayer, knapsack sprayers, and granules which may be applied by hand.

Use practice limitations include a label statement prohibiting application directly to treated, finished drinking water reservoirs or drinking water receptacles. Users also must avoid application to man-made surfaces sensitive to mineral oils.

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

Regulatory History

Furanone was first registered as a pesticide in the U.S. in 1983 for use as a cat repellent. Currently, there are seven furanone products, which are registered as mixtures with the active ingredient, limonene (Case 3083). The Agency issued a Reregistered Eligibility Decision (RED) on limonene in September 1994. One of the seven products also contains a third active ingredient, Aliphatic Petroleum Hydrocarbons.

Human Health Assessment

Toxicity

Acute oral, dermal and inhalation toxicity studies indicate low acute toxicity (Category IV). Likewise, eye and dermal irritation studies also classified the formulated product in Category IV. The product is not a skin sensitizer. Data from the open literature on each of the two compounds considered the furanones to be of low toxicity.

An open literature study provided useful information in evaluating the potential hazard of the furanones, indicating that compounds making up the furanones did not demonstrate a hazard following subchronic and chronic oral administration.

The Agency usually would require a 90-day dermal toxicity study for a product that may result in prolonged human dermal exposure through repeated skin applications. The Agency has decided to waive this requirement for the furanones based on a combination of the following: low exposure to furanones in the product; comparable concentrations of tanols in this product to those already used in cosmetic products (lotions, detergents, perfumes); a naturally occurring compound; and absence of toxicity in the toxicology studies (notwithstanding limited data).

Dietary Exposure

Currently registered pesticide products containing furanones have no food uses so dietary exposure is not anticipated. Although it is not a chemical that is Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA), furanone is approved by FDA as a food additive, and exposures to low concentrations are considered safe.

Occupational and Residential Exposure

For the following reasons, the Agency has no concerns regarding occupational or residential exposure to the furanones: (1) all acute studies for the furanones indicate that they have low acute toxicity (category IV); and (2) the furanones are only in pesticide products that also contain limonene, and the percentage of furanones in these formulations is very low--less than 1%.

Human Risk Assessment

The Agency does not anticipate occupational or residential risks of concern from exposure to the furanones. However, as the furanones are only in pesticide products that also contain limonene, the following concerns from the limonene RED apply to the furanones.

US EPA ARCHIVE DOCUMENT

Human exposure to furanones/limonene may occur during application of animal repellent granules or insect spray, or use of impregnated tablecloths. Toxicological concerns for humans from exposure to limonene include dermal irritation and sensitization. Systemic toxicity is not anticipated to occur at doses below the threshold for dermal irritation. Ocular irritation also may occur if products are accidently placed in the eye and not washed away.

The tablecloth insect repellent product containing limonene was exempted from tolerance requirements and is not believed to cause exposure through food. Exposure to limonene would be discontinued by most users if dermal irritation occurred, and exposure therefore is believed to be self-limiting. Product labeling required by the limonene RED is intended to prevent risks of potential skin and eye irritation to users.

Environmental Assessment

Environmental Fate

Environmental fate data are not required to support the low-volume, outdoor, residential uses of the furanones. However, environmental fate data generally are required to support aquatic nonfood uses such as the mosquito larvicide use. A quantitative environmental fate assessment cannot be made for the furanones at this time because no environmental fate data have been submitted for review. The furanones are classified as lactones, and some open literature data are available on physiochemical properties of lactones and their possible effect on the environment. The reported data indicate lactones may not be stable in alkaline environments.

Ecological Effects

Acute toxicity data indicate that the formulated product is practically non-toxic on an acute oral and subacute dietary basis to birds, mammals, and freshwater fish. The product is slightly toxic to freshwater invertebrates.

Ecological Effects Risk Assessment

The furanones, when used as mosquito larvicides, are applied directly to water. The public health larvicidal use of furanones applied at 4.10 lbs/acre (A) to 6 inches of water results in exceedances of the level of concern (LOC) for freshwater invertebrates. The LOC for endangered species is exceeded when the product is applied at 1.64 lbs/A in water 6 inches deep or less, or when it is applied at 4.10 lbs/A in water 1 foot deep or less.

This assessment is based on testing done on a formulated product and tells little about the actual toxicity of the furanones as active ingredients. Chronic invertebrate toxicity data and basic environmental fata data would improve the Agency's understanding and assessment of the potential risk posed by the use of the furanones in mosquito larvicides.

US EPA ARCHIVE DOCUMENT

The volume of products containing furanones that are used annually as mosquito larvicides is low, and the percentage of furanone in the products is also low. The low volume and low percent of furanone support the conclusion that widespread adverse impacts are not likely to result from the mosquito larvicide use if continued at the amounts currently produced and used.

It should be recognized that the risk posed to aquatic invertebrates is common to most, if not all, products registered for mosquito larvicide use. It is the nature of these products to be harmful to aquatic invertebrates because the target species is itself an aquatic invertebrate. The mosquito larvicide product containing furanones would cause less harm to aquatic ecosystems than many other products because its risk is limited to aquatic invertebrates, whereas others pose a risk to fish and birds, as well.

Risk Mitigation

To lessen the risks to aquatic invertebrates posed by furanones used as mosquito larvicides, EPA is imposing a production limit on furanone for use in mosquito larvicides of 150 gallons per year. Should the volume produced and used and/or the percent of furanone in the product significantly increase, the Agency would impose the following additional data requirements to understand and assess potential risks:

161-1, Hydrolysis
161-2, Photodegradation in Water
162-3, Anaerobic Aquatic Metabolism
162-4, Aerobic Aquatic Metabolism
163-1, Leaching/Adsorption-Desorption
164-2, Aquatic Field Dissipation

Additional Data Required

EPA is requiring the following additional generic study for furanone to confirm its regulatory assessments and conclusions:

72-4(b), Life Cycle Invertebrate

The Agency also is requiring product-specific data including product chemistry and acute toxicity studies, revised Confidential Statements of Formula (CSFs), and revised labeling for reregistration.

Product Labeling Changes Required

All furanone end-use products must comply with EPA's current pesticide product labeling requirements and with the following. For a comprehensive list of labeling requirements, please see the furanone RED document.

US EPA ARCHIVE DOCUMENT

The manufacturing use product (MP) labeling must be revised to comply will all current EPA regulations, PR Notices and applicable policies. The MP labeling must bear the following statement under Direction For Use:

> "Only for formulation into an [fill blank with Insecticide, Herbicide or the applicable term which describes the type of pesticide use(s)] for the following use(s)[fill blank only with those uses that are being supported by the MP registrant."

An MP registrant may, at his/her discretion, add one of the following statements to an MP label under

> "Directions for Use" to permit the reformulation of the product for a specific use or all additional uses supported by a formulation or user group:

(a) "This product may be used to formulate products for specific use(s) not listed on the MP label if the formulator, user group, or grower has complied with U.S. EPA submission requirements regarding support of such use(s)."

(b) "This product may be used to formulate products for any additional use(s) not listed on the MP label if the formulator, user group, or grower has complied with U.S. EPA submission requirements regarding support of such use(s)."

End-use products without the mosquito larvicide use must bear the following statement:

> "Do not apply directly to water, or to areas where surface water is present or to interidal areas below the mean high-water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

Regulatory Conclusion

The use of currently registered products containing furanone in accordance with approved labeling will not pose unreasonable risks or adverse effects to humans or the environment. Therefore, all furanone products for all uses are eligible for reregistration.

These products will be reregistered once the required product-specific data, revised Confidential Statements of Formula, and revised labeling are received and accepted by EPA. Products that contain active ingredients in

addition to furanone and limonene will be reregistered when their other active ingredients also are eligible for reregistration.

Environmental fate and chronic invertebrate toxicity data are not available and are needed to complete the assessment of risks posed by the use of the furanones as mosquito larvicides. However, EPA is not requiring additional data as long as the production of furanone for this use does not exceed 150 gallons per year. Should the volume produced and used and/or the percent of active ingredient in the product significantly increase, the Agency may impose additional data requirements in order to develop a more complete data base regarding these uses of furanone.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Decision (RED) document for furanone during a 60-day time period, as announced in a Notice of Availability published in the Federal Register. To obtain a copy of the RED document or to submit written comments, please contact the Pesticide Docket, Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

Electronic copies of the RED and this fact sheet can be downloaded from the Pesticide Special Review and Reregistration Information System at 703-308-7224. They also are available on the Internet on EPA's gopher server, *GOPHER.EPA.GOV*, or using ftp on *FTP.EPA.GOV*, or using WWW (World Wide Web) on *WWW.EPA.GOV*.

Printed copies of the RED and fact sheet can be obtained from EPA's National Center for Environmental Publications and Information (EPA/NCEPI), PO Box 42419, Cincinnati, OH 45242-0419, telephone 513-489-8190, fax 513-489-8695.

Following the comment period, the furanone RED document also will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about EPA's pesticide reregistration program, the furanone RED, or reregistration of individual products containing furanone, please contact the Special Review and Reregistration Division (7508W), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, between 9:30 am and 7:30 pm Eastern Standard Time, Monday through Friday.

US EPA ARCHIVE DOCUMENT

European Food Safety Authority

EFSA Journal 2011; 9(7):2164

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2):

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 61 flavouring substances in the Flavouring Group Evaluation 10, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The 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 61 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, information on composition of mixture and/or stereoisomerism has not been specified sufficiently.

© European Food Safety Authority, 2011

1 On request from the Commission, Question No EFSA-Q-2011-00128, EFSA-Q-2011-00813, EFSA-Q-2011-00814 adopted on 24 March 2011.

2 Panel members Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle. Correspondence: cef-unit@efsa.europa.eu

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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 61 flavouring substances in the Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 61 flavouring substances belong to chemical groups 9, 13 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The 61 flavouring substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-five of the 61 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 substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition / composition of mixture has not been specified sufficiently.

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

Forty-eight 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 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.

The combined estimated daily *per capita* intake as flavourings of the 54 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.

On the basis of the data available it is concluded that there is no indication that the flavouring substances in the present Flavouring Group Evaluation possess genotoxic potential. However, 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.

It can be anticipated that, at the estimated levels of intake as flavouring substances, 58 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 [FL-no: 02.242, 06.097 and 09.824] of the flavouring substances 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 the 61 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 59 flavouring substances, for which use levels information is available, range from 1600 to 5100 microgram/person/day. For 57 of these substances the mTAMDI is above the threshold of concern of their structural classes and for two substances the mTAMDI is below the threshold. The two 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 59 flavouring substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

In order to determine whether the conclusion for the 61 candidate substances 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 55 flavouring substances. For two substances [FL-no: 06.135 and 08.113] information on solubility is lacking. 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] is an identity test 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 57 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, 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.

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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 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).

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/EFSA/efsa_locale-1178620753812_1211902296182.htm	58
FGE.10Rev2	23 March 2011		61

The present revision of FGE.10, FGE.10Rev2, includes the assessment of three additional candidate substances [FL-no: 08.113, 10.059 and 10.063]. No toxicity or metabolism data were provided for these three substances.

A search in open literature revealed data on metabolism, genotoxicity, repeated dose toxicity as well as reproductive/developmental toxicity for [FL-no: 08.113] but not for [FL-no: 10.059 and 10.063].

FGE.10Rev2 also include additional information submitted by 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], as well as identity information for [FL-no: 06.088 and 06.095].

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

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

1.1. Description

The present Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2), 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 61 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 61 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.

Fourteen 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 and 10.168]; thirty-one 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 and 09.916]; 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 61 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.

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

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-five 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 and 10.168]. In all cases the stereoisomeric composition has been specified.

Due to the presence and the position of a double bond, eight of the 61 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 mixture has not been specified sufficiently. Industry has informed that [FL-no: 10.038 and 10.040] exists as a mixture of (Z)- and (E)-isomers (EFFA, 2010a), however, the composition of the isomeric mixture has to be provided.

1.3. Natural Occurrence in Food

Forty-eight of the 61 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), juice, butter, meat, cheese, 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. These reports include:

- Octane-1,3-diol [FL-no: 02.198]: up to 21 mg/kg in apple and up to 95.1 mg/kg in apple juice.
- Hexadecano-1,5-lactone [FL-no: 10.049]: up to 10.6 mg/kg in butter and up to 1.3 mg/kg in heated lamb and mutton fat.
- Hexadecano-1,4-lactone [FL-no: 10.048]: up to 16.7 mg/kg in heated butter.
- 2-Butoxyethan-1-ol [FL-no: 02.242]: 0.02 mg/kg in mozzarella cheese.
- Hexadecano-1,16-lactone [FL-no: 10.047]: 0.0145 mg/kg in skimmed milk powder.
- Heptano-1,5-lactone [FL-no: 10.045]: up to 0.4 mg/kg in green tea.
- 1-Hydroxypropan-2-one [FL-no: 07.169]: up to 4 mg/kg in coffee.
- 2-Ethyl-4-methyl-1,3-dioxolane [FL-no: 06.088]: up to 2 mg/kg in port wine.
- 4-methyl-2-propyl-1,3-dioxolane [FL-no: 06.095]: up to 2 mg/kg in port wine.
- 1,1,3-Triethoxypropane [FL-no: 06.097]: up to 3 mg/kg in pear brandy and less than 0.8 mg/kg in whisky.

- 2-Isobutyl-4-methyl-1,3-dioxolane [FL-no: 06.135]: up to 2 mg/kg in port wine.
- Nonanedioic acid [FL-no: 08.103]: up to1.5 mg/kg in beer.
- Propyl lactate [FL-no: 09.815]: trace amount in white wine.
- Isobutyl lactate [FL-no: 09.590]: 20 mg/kg in port wine.
- Ethyl 3-hydroxyoctanoate [FL-no: 09.916]: up to 0.05 mg/kg in papaya, 0.02 mg/kg in orange juice and 0.03 mg/kg in grapefruit juice.

According to TNO, 13 of the substances have not been reported in any food items. These substances are listed in Table 1.1 (TNO, 2000; TNO, 2010).

1.1 Candidate substances not reported to occur in nature (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 61 substances have been provided by the Flavouring Industry (EFFA, 2003c; EFFA, 2004ag; Flavour Industry, 2011a) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for 58 of the 61 substances. For one substance [FL-no: 10.063] an identity test is missing and for two substances [FL-no: 06.135 and 08.113] are information on solubility in water and ethanol lacking.

Furthermore, information on geometrical stereoisomerism is needed for four of the candidate substances (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 61 candidate substances from use as flavouring substances in Europe has been reported to be approximately 13160kg (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b). For 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 61 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 of these flavouring 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-

[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.

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 59 of the 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);. 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 59 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 59 candidate substances, for which use levels have been provided, are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

The mTAMDI values for the 53 candidate substances from structural class I, for which use levels have been reported, range from 1600 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 candidate substance from structural class III the mTAMDI is 4100 microgram/person/day.

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

[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

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All*
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All *
03.0	Edible ices, including sherbet and sorbet	All*
04.1	Processed fruits	All*
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All*
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All*
07.0	Bakery wares	All*
08.0	Meat and meat products, including poultry and game	All*
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All* except [FL-no: 08.090]
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, 09.644]
13.0	Foodstuffs intended for particular nutritional uses	All* except [FL-no: 06.095, 09.644]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All*
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All*
15.0	Ready-to-eat savouries	All*
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]

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 (10.059, hexadec-7-en-1,16-lactone and 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, 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 (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto 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 innocous 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 (Dick el al., 1988; Ashley et al., 1994; 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 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.

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 61 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 61 substances are summarised in Table 2a.

Step 1

Fifty-four 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; 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. Therefore, these substances are evaluated via the B-side of the Procedure. The evaluation of the remaining 58 candidate substances proceeds via the A-side of the Procedure.

Step A3

Step A3 applies to 53 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, 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 53 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 58 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/day) the margin of safety would be 5 x 10^8, 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 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 Reference dose (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 61 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 53 candidate substances in structural class I and for which use levels information is available, range from 1600 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 one substance from structural class III the mTAMDI is 4100 microgram/person/day, which is above the threshold of 90 microgram/person/day.

Thus for the 57 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).

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
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
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

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)
02.242	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

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.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b), the combined estimated daily *per capita* intake as flavourings of the 54 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 correspondig structural classes of 1800, 540 and 90 microgram/person/day, respectively.

The 14 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 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 40 candidate substances are structurally related to 32[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 25000 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] 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 32 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. Toxicity

8.1. Acute Toxicity

Data are available for 15 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 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/day and approximately 30 g/kg bw/day are reported (Annex IV, Table IV.1).

Forty-nine 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 body weight (bw) are reported.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute/subchronic/chronic toxicity data are available for five candidate substances, 2-butoxyethanol [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 25 supporting substances of the present Flavouring Group Evaluation (JECFA, 1998a; JECFA, 2000c) (Annex IV, Table IV.2).

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). LOAELs of 69 and 82 mg/kg bw/day (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, 1993a). 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 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) (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 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/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.

[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.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 11 of the candidate substances. These 11 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] and glutaric acid [FL-no: 08.082]. There were genotoxicity data on 26 supporting substances (Annex IV, Table IV.4 and IV.5).

In vitro

Pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168] and methyl acetoacetate [FL-no: 09.634] were reported to be negative in microbial mutagenicity assays.

1-Hydroxypropan-2-one [FL-no: 07.169] was positive in Ames tests using strains TA100 and TA104 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 28 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) 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 this 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 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 TA 97 and TA 102 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.

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

Thirty-five of the 61 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 substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition/composition of mixture has not been specified sufficiently.

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

Forty-eight 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 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.

According to the default MSDI approach, the 61 flavouring substances in this group to which the Procedure has been applied have intakes in Europe from 0.0012 to 48 microgram/*capita*/day which are below the thresholds of concern value for structural class I, II and III substances of 1800, 540 and 90 microgram/person/day, respectively.

The combined estimated daily *per capita* intake as flavourings of the 54 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 14 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 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

5 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 40 candidate substances are structurally related to 32 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 25000 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 25000 microgram/*capita*/day.

On the basis of the data available it is concluded that there is no indication that the candidate substances in the present Flavouring Group Evaluation possess genotoxic potential. However, 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.

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, 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 the 61 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 53 candidate substances in structural class I, for which use levels information is available, range from 1600 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 one substance from structural class III the mTAMDI is 4100, which is above the threshold of 90 microgram/person/day.

Thus, for 59 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. 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 two candidate substances [FL-no: 05.149 and 07.169] which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 61 candidate substances 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 55 flavouring substances. For two substances [FL-no: 06.135 and 08.113] information on solubility is lacking. 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] is an identity test 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 57 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, 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 THE FLAVOURING GROUP EVALUATION 10, REVISION 2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

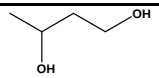
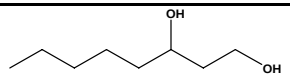
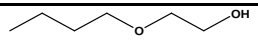
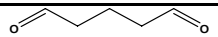
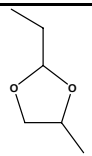
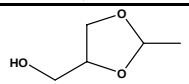
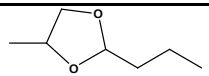
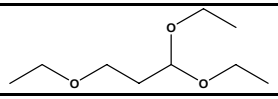
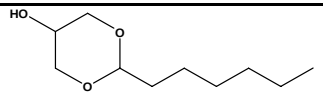
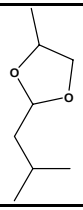
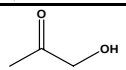
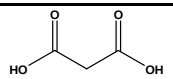
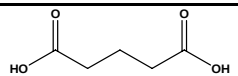
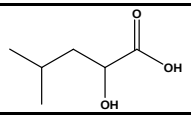
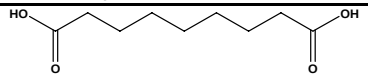
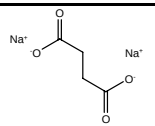
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.132	Butane-1,3-diol		107-88-0	Liquid $C_4H_{10}O_2$ 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) 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	ID 7). Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a). ID test is missing.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

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
06.097	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.	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		4378 18433-93-7	Liquid $C_8H_{16}O_2$ 144.21		150 MS 96 %	n.a. 0.895	SE 8), SW 9). 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		426.03 156.43 IR 60	n.a. n.a.	SE 8), SW 9). Anhydrous when heated to 120°C. Min.assay: Anhydrous 60 %, hydrate 40 %

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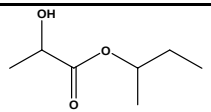
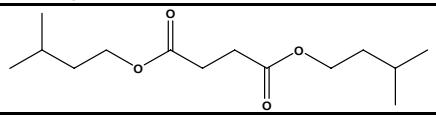
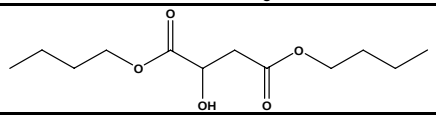
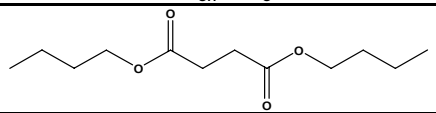
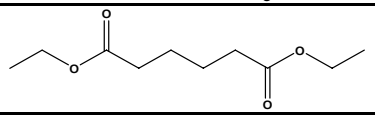
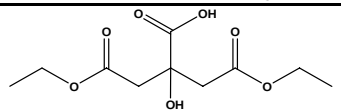
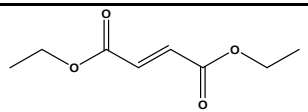
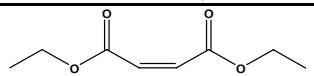
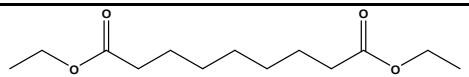
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
								(Fenaroli).
09.333	sec-Butyl lactate		18449-60-0	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).
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	

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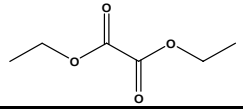
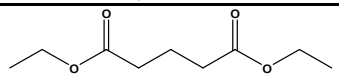
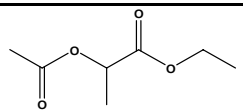
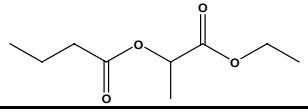
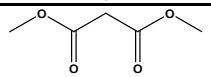
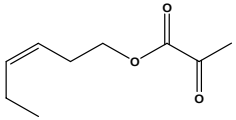
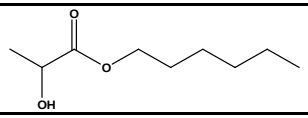
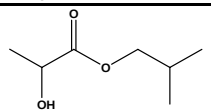
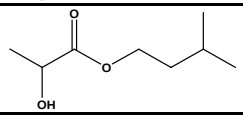
09.353	Diethyl oxalate		95-92-1	Liquid $C_6H_{10}O_4$ 146.14	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.407-1.413 1.076-1.082	
09.354	Diethyl pentanedioate		818-38-2	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	233 MS 95 %	1.421-1.427 1.019-1.025	
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 95 %	1.411-1.417 1.150-1.156	
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.

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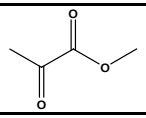
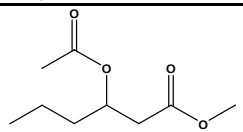
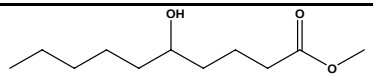
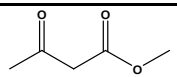
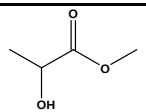
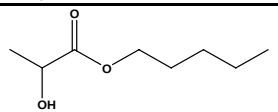
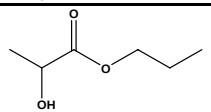
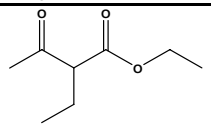
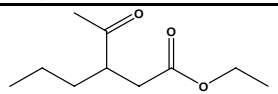
09.626	Methyl 2-oxopropionate		10848 600-22-6	Liquid $C_4H_6O_3$ 120.09	Sparingly soluble Freely soluble	137 MS 95 %	1.401-1.407 1.145-1.151	
09.629	Methyl 3-acetoxyhexanoate		10755 77118-93-5	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	55 (0.7 hPa) MS 95 %	1.420-1.426 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.
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.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

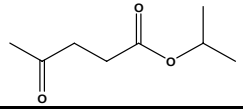
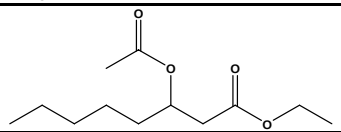
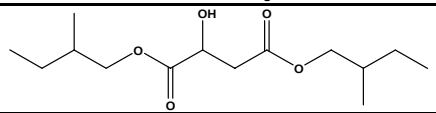
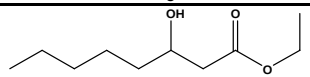
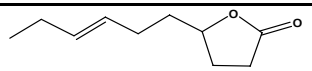
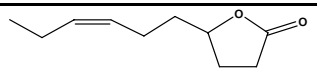
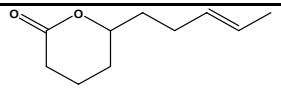
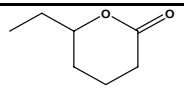
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.833	iso-Propyl 4-oxopentanoate		21884-26-4	Liquid $C_8H_{14}O_3$ 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).
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
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.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

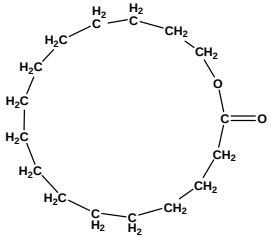
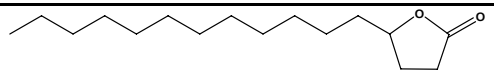
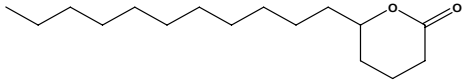
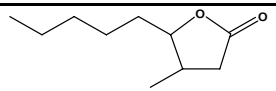
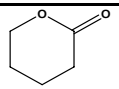
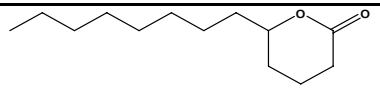
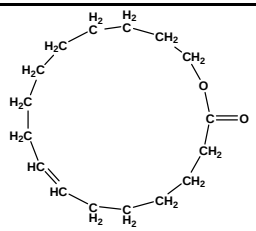
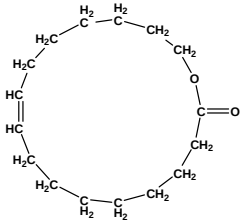
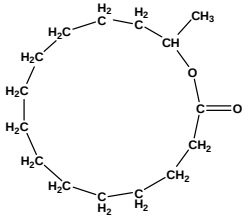
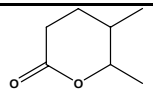
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.047	Hexadecano-1,16-lactone		109-29-5	Solid $C_{16}H_{30}O_2$ 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.
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.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

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
10.063	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).

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.
8) SE: Missing data on solubility in ethanol.
9) SW: Missing data on solubility.

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)

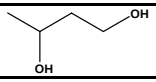
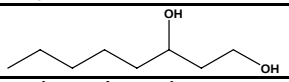
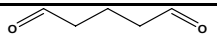
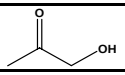
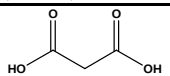
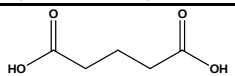
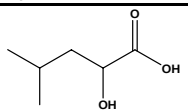
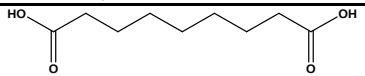
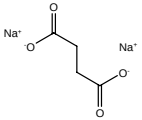
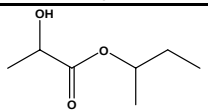
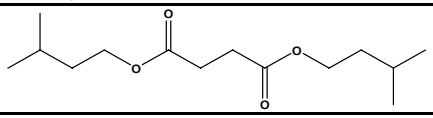
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	a)
02.198	Octane-1,3-diol		0.0012	Class I A3: Intake below threshold	4)	6)	a)
05.149	Glutaraldehyde		0.055	Class I A3: Intake below threshold	4)	6)	a)
07.169	1-Hydroxypropan-2-one		0.22	Class I A3: Intake below threshold	4)	6)	a)
08.053	Malonic acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.082	Glutaric acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.090	2-Hydroxy-4-methylvaleric acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.103	Nonanedioic acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.113	Succinic acid, disodium salt		1500	Class I A3: Intake below threshold	4)	6)	a)
09.333	sec-Butyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	a)
09.345	Di-isopentyl succinate		0.037	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

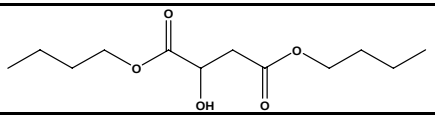
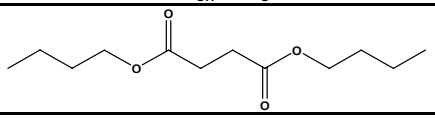
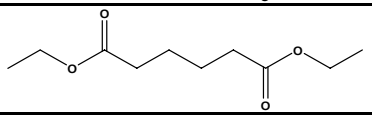
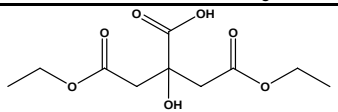
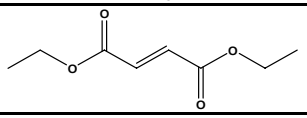
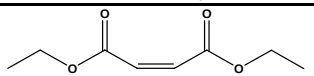
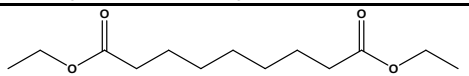
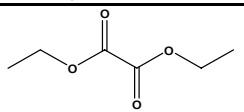
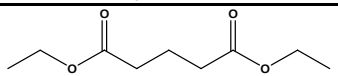
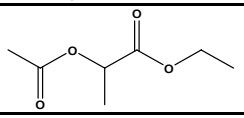
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.346	Dibutyl malate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.347	Dibutyl succinate		0.12	Class I A3: Intake below threshold	4)	6)	a)
09.348	Diethyl adipate		0.027	Class I A3: Intake below threshold	4)	6)	a)
09.349	Diethyl citrate		0.12	Class I A3: Intake below threshold	4)	6)	a)
09.350	Diethyl fumarate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.351	Diethyl maleate		12	Class I A3: Intake below threshold	4)	6)	a)
09.352	Diethyl nonanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.353	Diethyl oxalate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.354	Diethyl pentanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.360	Ethyl 2-acetoxypropionate		4.9	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

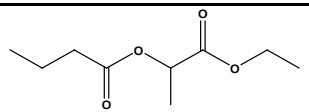
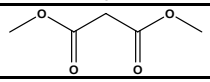
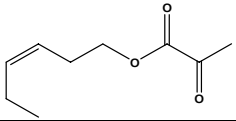
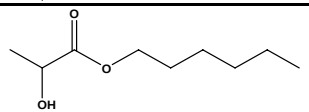
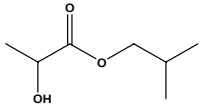
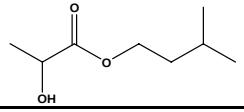
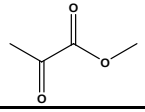
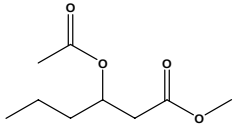
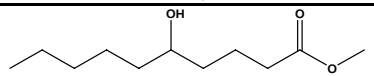
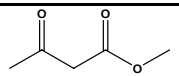
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.502	Ethyl butyryl lactate		0.5	Class I A3: Intake below threshold	4)	6)	a)
09.558	Dimethyl malonate		0.097	Class I A3: Intake below threshold	4)	6)	a)
09.565 1846	Hex-3-enyl 2-oxopropionate		0.74	Class I A3: Intake below threshold	4)	6)	a)
09.580	Hexyl lactate		0.49	Class I A3: Intake below threshold	4)	6)	a)
09.590	Isobutyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	a)
09.601	Isopentyl lactate		7.2	Class I A3: Intake below threshold	4)	6)	a)
09.626	Methyl 2-oxopropionate		0.024	Class I A3: Intake below threshold	4)	6)	a)
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.633	Methyl 5-hydroxydecanoate		0.24	Class I A3: Intake below threshold	4)	6)	a)
09.634	Methyl acetoacetate		0.012	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

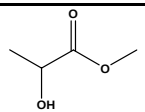
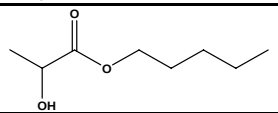
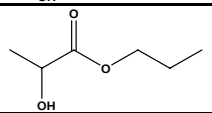
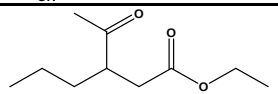
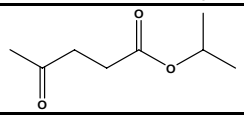
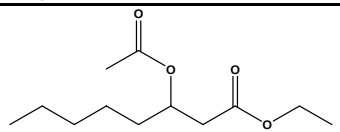
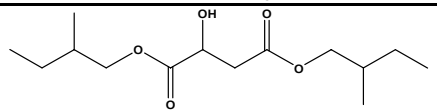
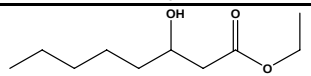
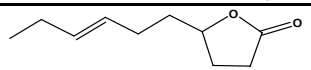
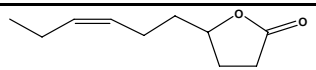
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.644	Methyl lactate		0.34	Class I A3: Intake below threshold	4)	6)	a)
09.683	Pentyl lactate		0.61	Class I A3: Intake below threshold	4)	6)	a)
09.815	Propyl lactate		0.62	Class I A3: Intake below threshold	4)	6)	a)
09.832	Ethyl 3-acetohexanoate		0.33	Class I A3: Intake below threshold	4)	6)	a)
09.833	iso-Propyl 4-oxopentanoate		0.24	Class I A3: Intake below threshold	4)	6)	a)
09.862	Ethyl 3-acetoxy octanoate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.874	Di(2-methylbutyl) malate		0.015	Class I A3: Intake below threshold	4)	6)	a)
09.916	Ethyl 3-hydroxyoctanoate		0.011	Class I A3: Intake below threshold	4)	6)	a)
10.038	Dec-7-eno-1,4-lactone		0.37	Class I A3: Intake below threshold	4)	7)	a)
10.039	cis-Dec-7-eno-1,4-lactone		1.2	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

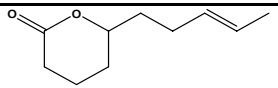
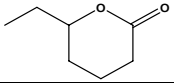
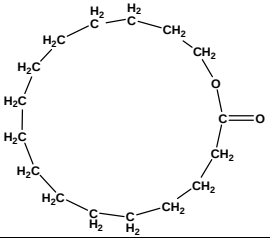
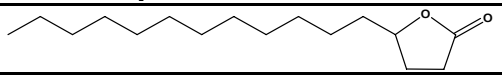
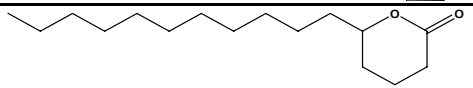
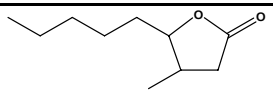
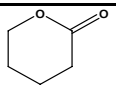
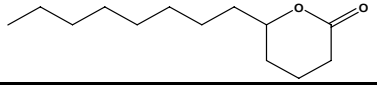
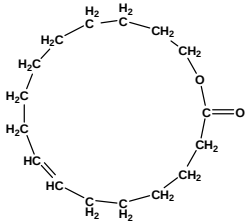
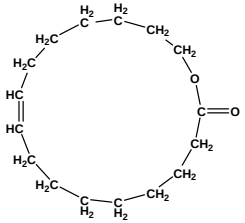
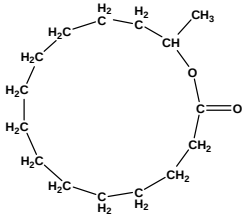
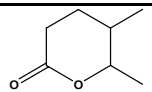
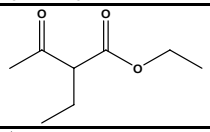
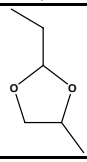
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.040	Dec-8-eno-1,5-lactone		0.011	Class I A3: Intake below threshold	4)	7)	a)
10.045	Heptano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	a)
10.047	Hexadecano-1,16-lactone		0.024	Class I A3: Intake below threshold	4)	6)	a)
10.048	Hexadecano-1,4-lactone		0.0061	Class I A3: Intake below threshold	4)	6)	a)
10.049	Hexadecano-1,5-lactone		0.024	Class I A3: Intake below threshold	4)	6)	a)
10.052	3-Methylnonano-1,4-lactone		0.61	Class I A3: Intake below threshold	4)	6)	a)
10.055	Pentano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	a)
10.058	Tridecano-1,5-lactone		0.61	Class I A3: Intake below threshold	4)	6)	a)

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
10.059	Hexadec-7-en-1,16-lactone		1.9	Class I A3: Intake below threshold	4)	7)	a)
10.063	Hexadec-9-en-1,16 lactone		48	Class I A3: Intake below threshold	4)	7)	a)
10.068	Pentadecano-1,14-lactone		0.9	Class I A3: Intake below threshold	4)	6)	a)
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		1.2	Class I A3: Intake below threshold	4)	6)	a)
09.824	Ethyl 2-acetylbutyrate		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.088	2-Ethyl-4-methyl-1,3-dioxolane		0.0061	Class II A3: Intake below threshold	4)	6)	a)

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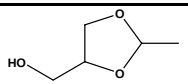
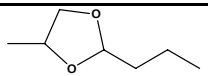
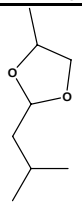
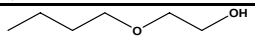
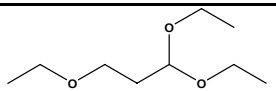
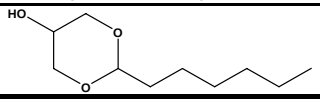
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	a)
06.095	4-Methyl-2-propyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	a)
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		1.2	Class II A3: Intake below threshold	4)	6)	a)
02.242	2-Butoxyethan-1-ol		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.097	1,1,3-Triethoxypropane		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		0.011	Class III A3: Intake below threshold	4)	6)	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) No safety concern at the estimated level of intake based on the MSDI approach.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

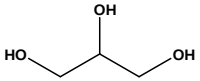
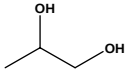
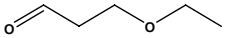
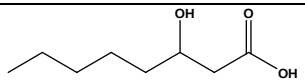
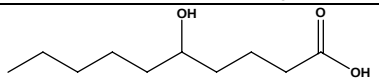
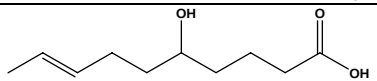
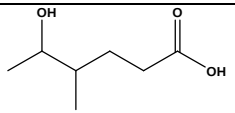
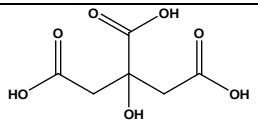
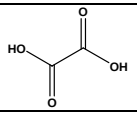
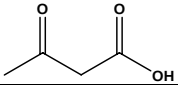
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of “flavouring agent”		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

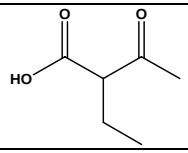
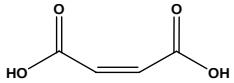
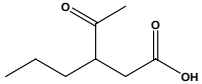
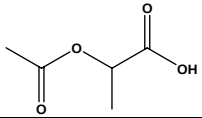
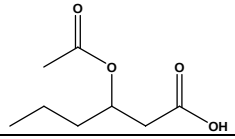
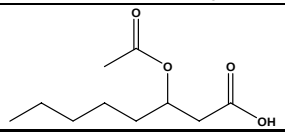
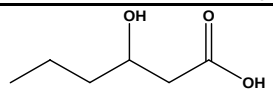
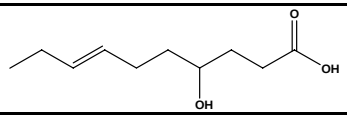
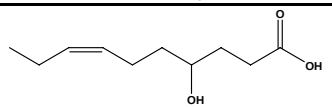
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Acetylbutyric acid		Not evaluated as flavouring substance		Not in EU-Register
	Maleic acid		Not evaluated as flavouring substance		Not in 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-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

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

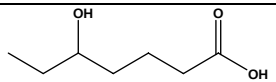
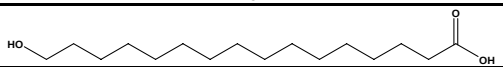
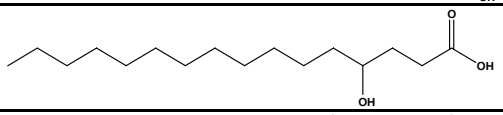
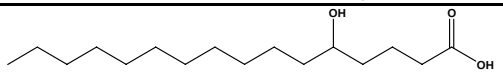
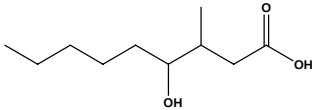
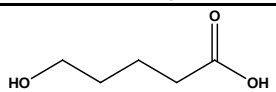
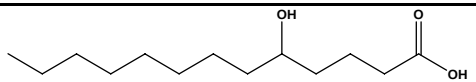
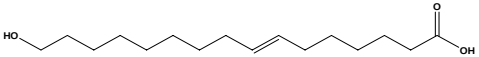
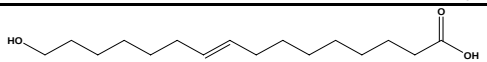
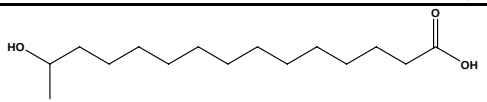
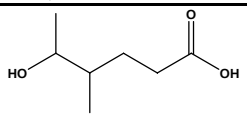
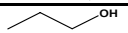
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	5-Hydroxyheptanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadecanoic acid		Not evaluated as flavouring 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
	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
02.002	Propan-1-ol 82		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

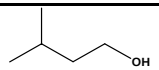
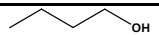
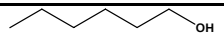
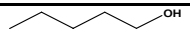
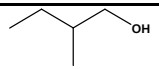
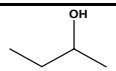
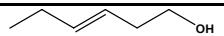
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.004	Butan-1-ol 85		Category 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	
02.040	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 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	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

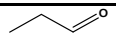
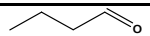
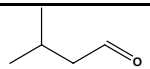
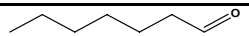
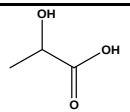
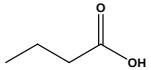
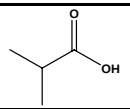
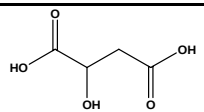
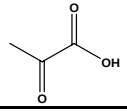
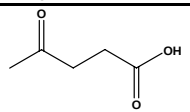
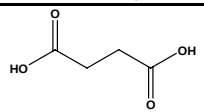
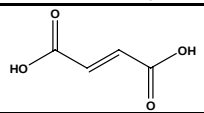
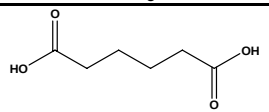
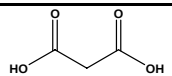
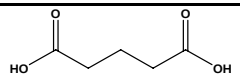
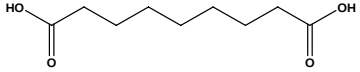
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
05.001	Acetaldehyde 80		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.002	Propanal 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	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	

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

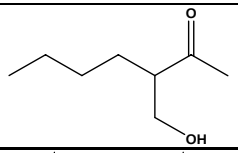
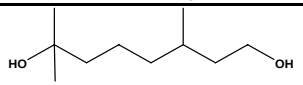
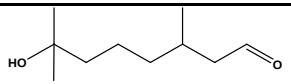
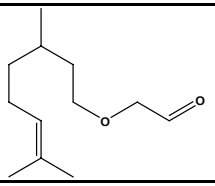
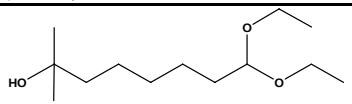
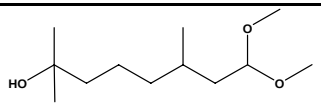
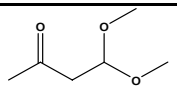
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2001c)	7	No safety concern d) Category B	Not in 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)	

Table 3: Supporting Substances Summary

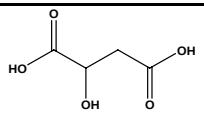
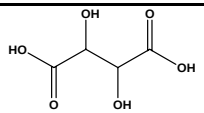
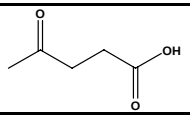
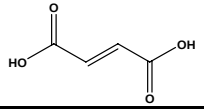
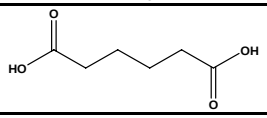
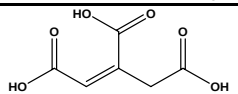
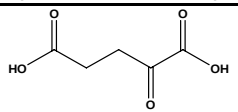
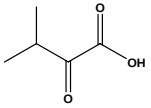
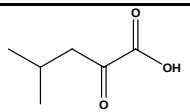
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.017	l-Malic acid		2655 17 6915-15-7	619 JECFA specification (JECFA, 2000d)	13000	No safety concern a) Category A b)	JECFA evaluated l-malic acid (CASrn 97-67-6). (R)- or (S)-enantiomer not specified by CASrn in Register. GrADI: not specified (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-Oxov aleric 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).

Table 3: Supporting Substances Summary

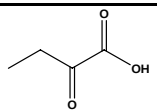
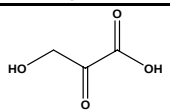
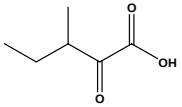
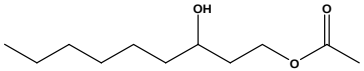
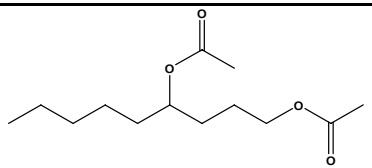
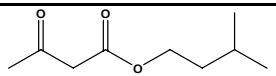
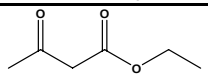
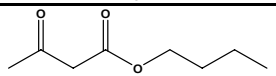
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.066	2-Oxobutyric acid		3723 600-18-0	589 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	
08.086	3-Hydroxy-2-oxopropionic 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: Substances 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)	

Table 3: Supporting Substances Summary

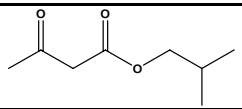
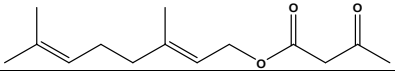
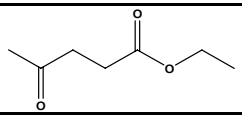
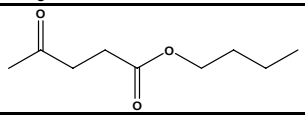
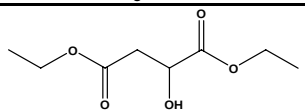
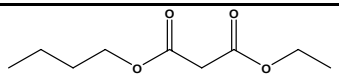
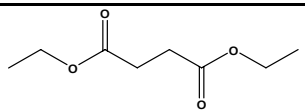
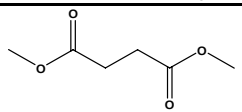
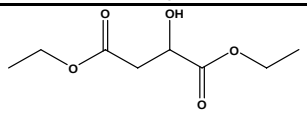
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.404	Isobutyl acetoacetate		2177 242 7779-75-1	597 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
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:Substances 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).

Table 3: Supporting Substances Summary

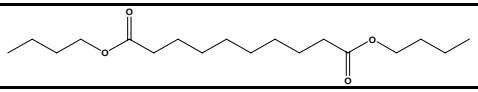
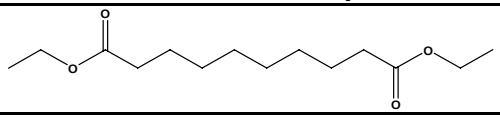
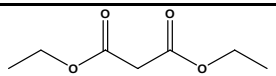
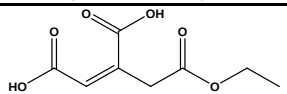
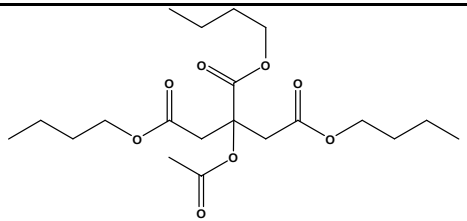
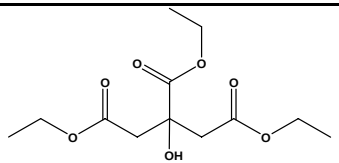
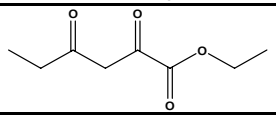
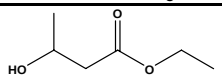
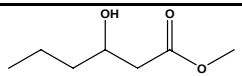
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.474	Dibutyl sebacate		2373 622 109-43-3	625 JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	
09.475	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

Table 3: Supporting Substances Summary

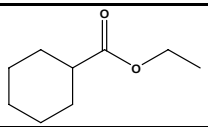
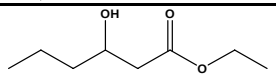
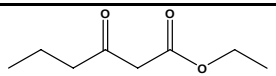
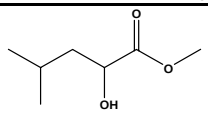
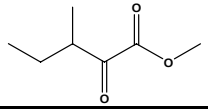
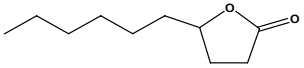
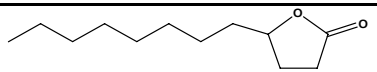
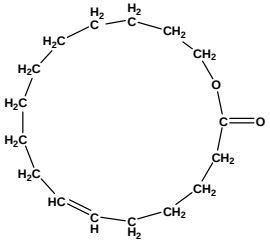
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							not specified by Register CASrn.
09.533	Ethyl brassylate		3543 10571 105-95-3	626 JECFA specification (JECFA, 2002d)	3.0	No safety concern a)	
09.535	Ethyl 3-hydroxyhexanoate		3545 11764 2305-25-1	601 JECFA specification (JECFA, 2002d)	60	No safety concern a)	JECFA evaluated ethyl 3-hydroxyhexanoate (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

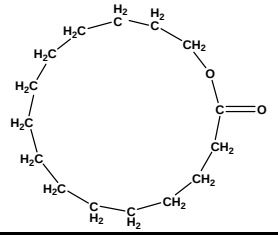
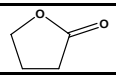
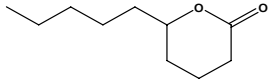
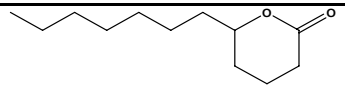
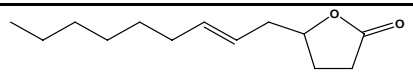
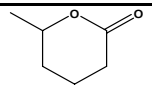
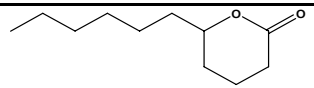
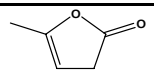
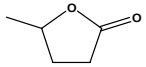
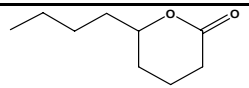
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.004	Pentadecano-1,15-lactone		2840 181 106-02-5	239 JECFA specification (JECFA, 2000d)	73	No safety concern c) Category 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.
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

Table 3: Supporting Substances Summary

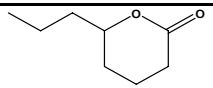
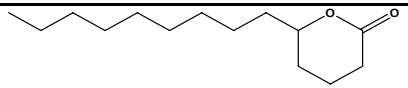
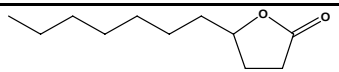
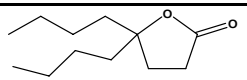
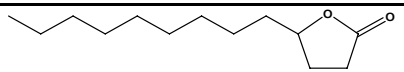
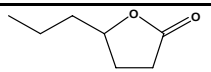
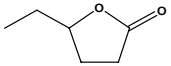
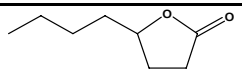
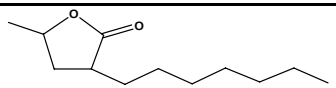
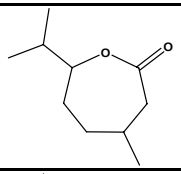
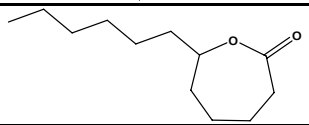
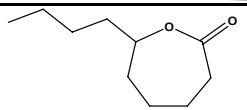
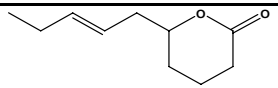
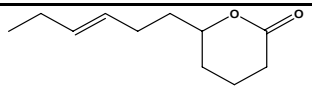
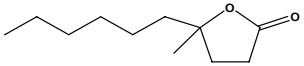
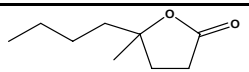
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							racemate.
10.015	Octano-1,5-lactone		3214 2195 698-76-0	228 JECFA specification (JECFA, 2000d)	230	No safety concern 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 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.

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
10.027	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 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).

ND) Not determined.

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).

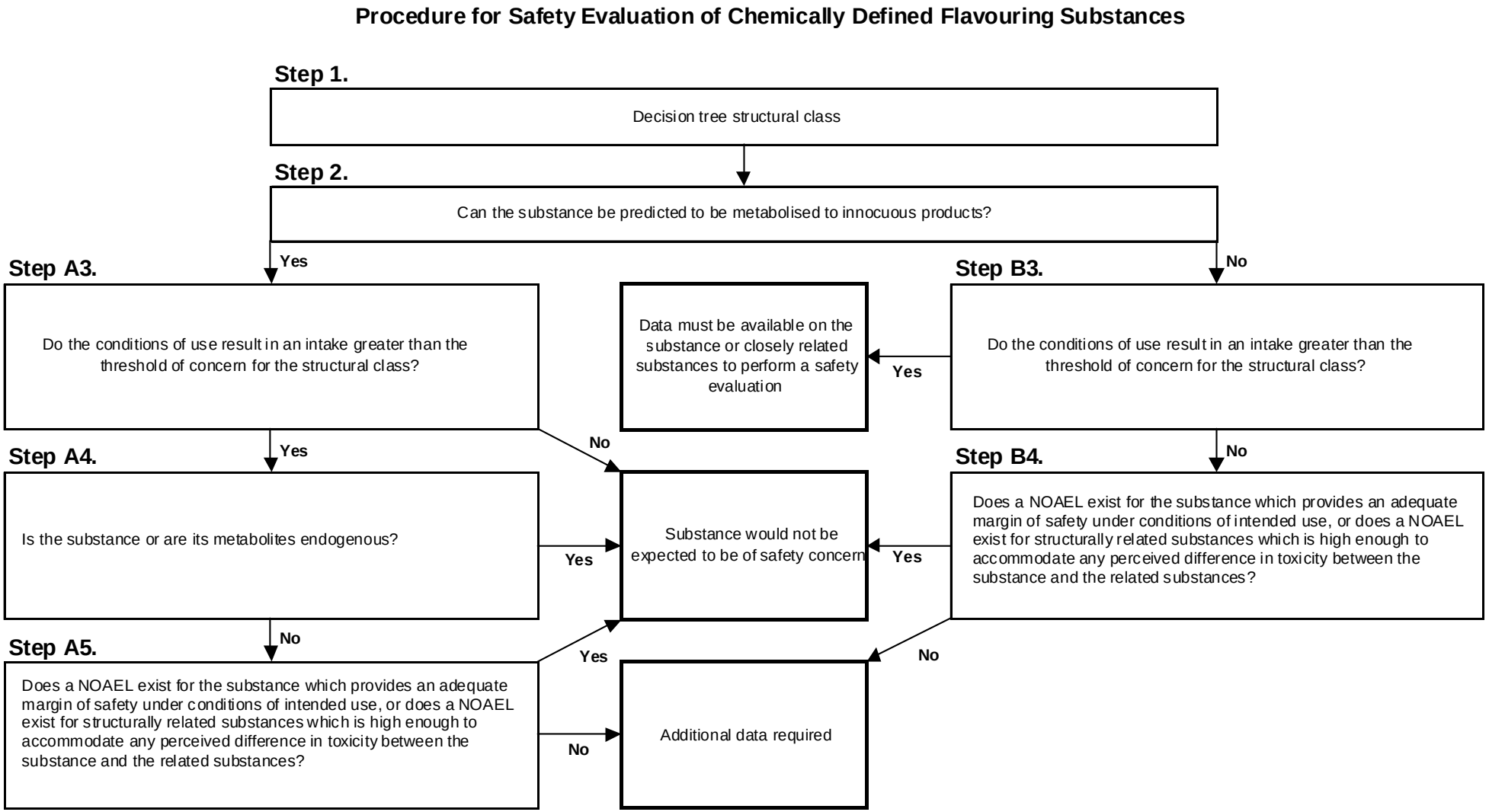


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 59 of the 61 candidate substances in the present Flavouring Group Evaluatioin (Table II.1.2) (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10

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.10

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.10

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
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

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
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
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 59 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). 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
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
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

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).

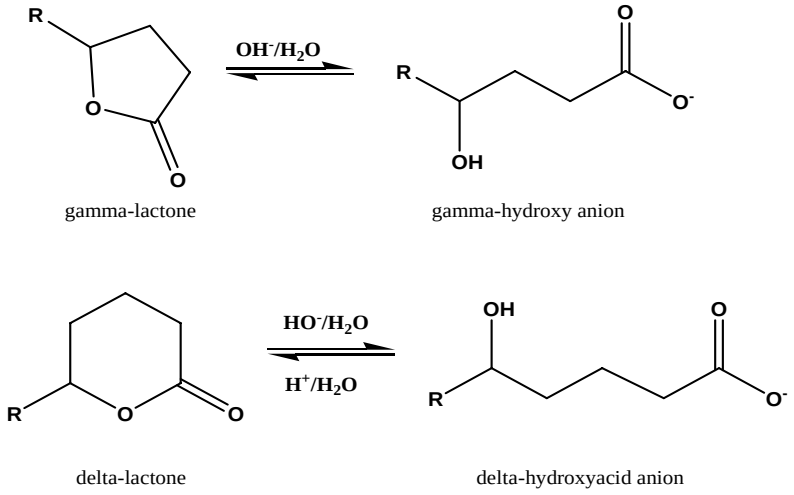


Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion*

III.1.2. Hydrolysis of Aliphatic Lactones

Fourteen 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 and 10.168] 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 been previously 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 utilised 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 (Deisinger et al., 1994; Deuel, 1957; Albro, 1975) 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-one 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 and 09.916]. They are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto 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. Greater than 87 % of the administered radioactivity was eliminated within 24 hours after dosing. At least nine urinary metabolites (59 - 70 %) were detected. Five 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 (K-H Engel, 2003).

Hydroxycitronellal dimethyl acetal similar to the supporting substance hydroxycitronellal diethyl acetal was > 99 % hydrolysed *in vitro* to the terpenoid hydroxycitronellal and ethanol 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 seven candidate substances [FL-no: 09.346, 09.558, 09.634, 09.824, 09.862, 09.874 and 09.916] are precursor of acetoacetic acid or its beta-hydroxy or aldehyde precursor. The latter two 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 been also 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, 08.082, 08.053, 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 and 09.874] 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 deseases, the propan-1,2-diol route is the dominating pathway.

In the fist step acetone is oxidized to 1-hydroxypropan-1,2-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.

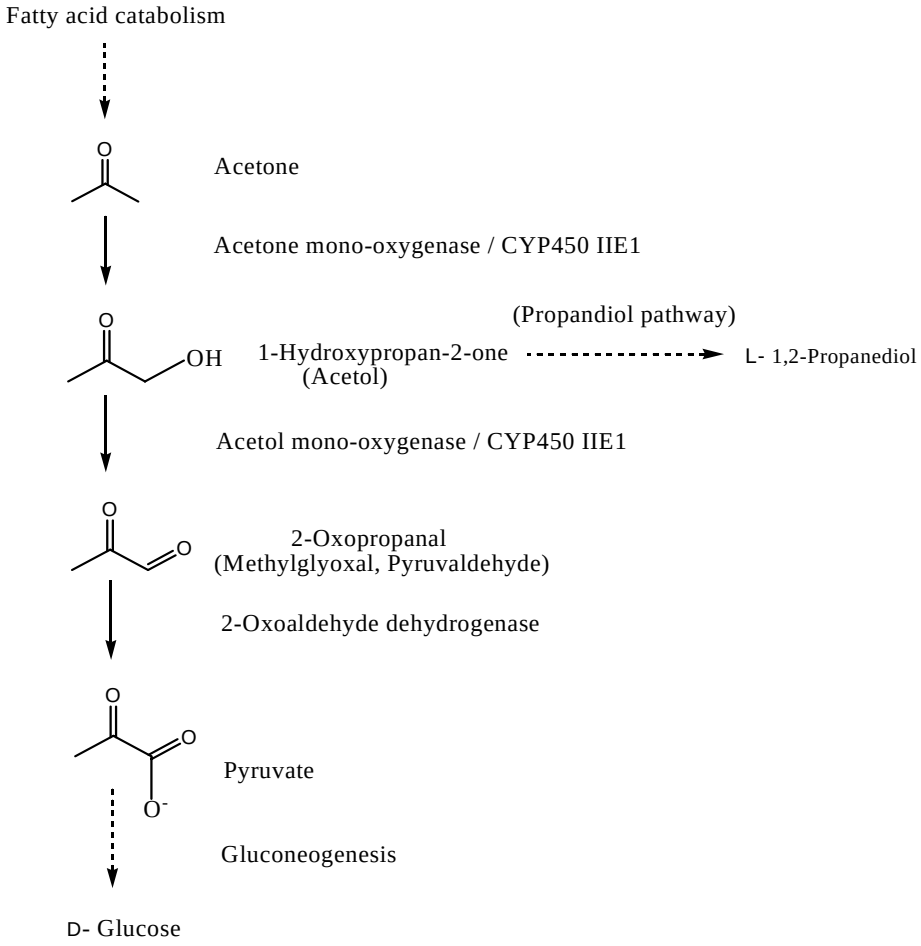


Figure III.2. *Acetone metabolism (methylglyoxal pathway)*

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 that indicate 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 in the 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 ia 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 silphate) (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 hour 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 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 Petersen, 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 then are 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 (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy- or keto-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 innocous 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 structually 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, 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 15 candidate substances of the present Flavouring Group Evaluation of 58 substances from chemical groups 9, 13 and 30 and for 49 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)
Isobutyl lactate [09.590]	Rat	NR	Oral	> 2000	(Riebeek, 1989)
(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, 1972a)
	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, 1977d)
(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)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat	M, F	Gavage	1600 – 5000	(Piccirillo and Hartman, 1980a)
(Mixture of 5-Hydroxy-2-decenoic acid delta-lactone, 5-Hydroxy-2-dodecenoic acid delta-lactone, and 5-Hydroxy-2-tetradecenoic acid delta–lactone)	Rat	M, F	Gavage	3363	(Reagan and Becci, 1984a)
(Dodec-6-eno-1,4-lactone [10.009])	Rat	M, F	Oral	> 5000	(Watanabe and Morimoto, 1990)
(Hexadec-6-eno-1,16-lactone [10.003])	Rat	NR	Oral	> 5000	(Wohl, 1974a)
(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)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(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)
(Ethyl 2,4-dioxohexanoate [09.514])	Rat	M, F	Gavage	6450	(Wolven and Levenstein, 1969)
2-Butoxyethan-1-ol [02.242]	Rat	M	Gavage	1480	(Smyth et al., 1941)
	Rat	NR	Oral	1174	(BASF, 1956)
	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, 1973a)
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, 1976a)
	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)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(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)
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, 1953a)
	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 LD50 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 23 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)	a)
(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)	a)
(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)	a)
	Rat; M, F 1/20	Diet	2 years	50[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)	a)
(Decano-1,5-lactone [10.007])	Rat; M, F 1/NR	Diet	49 weeks	150[2]	(Fassett, 1961)	a)
	Dog; M, F 1/NR	Diet	38 weeks	250[2]	(Fassett, 1961)	a)
(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)	a)
	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)	a)
(Dodecano-1,4-lactone [10.019])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
(Dodecano-1,5-lactone [10.008])	Rat; M, F 1/NR	Diet	49 weeks	300[2]	(Fassett, 1961)	a)
	Dog; M, F	Diet	38 weeks	150[2]	(Fassett, 1961)	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
	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)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat; M, F 1/30	Diet	90	12.1[2]	(Cox et al., 1974h)	a) A carefully performed one dose study not in compliance with a specific testing guideline but of sufficient quality to accept the data. "Reliable with restriction" according to (Klimisch et al., 1997).
(5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [10.023])	Rat; M, F 1/30	Diet	90	M: 1.29[2] ; F: 1.47[2]	(Posternak et al., 1969)	a)
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Rat; M, F 6/8-16	Diet	13 - 52 weeks	46[2]	(Munday and Kirkby, 1973; Munday and Kirkby, 1971a)	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).
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

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
						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 2/3	Diet	16	1000[2]	(Tischer et al., 1942)	a)
(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

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
						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 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, 1953a)	a)
	Rat; M 5/16	Diet	2 years	6250[2]	(Smith, 1953a)	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/developmentaltoxicity 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.

12Animals 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).
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)	
	Rabbit; F	Gavage	3/15	Developmental toxicity: Gestation	Maternal: 15; Fetal: 15	(Hellwig, 1991b)	

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
				days 7 – 19			
(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/developmentaltoxicity 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 F0 and F1 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 24 supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Supporting 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)	
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Formation of 32P-labelled DNA fragment (test on isolated DNA).	*p53* tumour suppression gene	1mM (128 µg/ml)	Negative[7]	(Yamashita et al., 1998)	
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)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Rec-assay	*B. subtilis*; H17, M45	20µl/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 - 1600µg/plate	Positive[8]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20µl/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 - 20µl/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.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	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 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 24hours) 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 currunt 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, 1984)	
	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)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	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)	
Diethyl maleate [09.351]	Forward mutation assay	Mouse lymphocytes L5178Y TK+/-	2.250 – 9.750 x 10^{-3} 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 TA102, TA104 and TA 102 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, A102, 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 international (US-FDA, US-EPA, UK, Japan) GLP Guidelines. Negative result not discussed in

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						view of positive results in other studies. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	15.4 µg/plate[2, 15] 51.6 µg/plate[3]	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be 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.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
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.
	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)	
(Ethylene 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, TA 102	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 substance 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.
2-Butoxyethan-1-ol [02.242] continued	*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: 1375mg/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: 0,55ml/kg (males) of a 5% solution	Negative	(Putman, 1987)	Time points of investigation: single dose: 8,12 hou rs. Repeated dose: 12hrs. 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 OECD477.
	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 OECD477.
(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	(Single 1460 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.

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, D.H., Caldwell, J., Paulson, G.D. (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, W.B. (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.

Cox GE, Bailey DE and Morgareidge K, 1974h. 90-day feeding study in rats with compound 14807 (5-hydroxy-2,4-decadienoic acid-lactone). Lab. No. 2107o. December 30, 1974. Unpublished report submitted by EFFA to SCF.

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, 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.

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, 2011a. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. Specifications Succinic acid. A-10rev2.

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, W.B. (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., 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 63*rd* 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, 1976a. Acute oral toxicity (rats-5 gms./kg. Body weight dose). Dermal toxicity (rabbits-5 gms./kg. Body weight dose). Ethyl caprate, Assay no. 62990; Ethyl isovalerate, Assay no. 62992. Leberco Laboratories, Inc. May 18, 1976. Unpublished report submitted by EFFA to SCF.

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, D. H., Caldwell, J., Paulson, G.D. (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, 1972a. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Citonellyl butyrate. MB Research Laboratories, Inc. Project no. MB 72-5x. November 1, 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, 1977d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Acetyl butyryl, project no. MB 77-1744, August 18, 1977. Acetyl methyl carbinol, project no. MB 77-1691, June 20, 1977. Acetyl propionyl, MB 76-1445, January 25, 1997. MB Research Laboratories, Inc. 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. Ppomalus; 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. 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.

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.

Munday R and Kirkby WW, 1971a. Biological evaluation of a flavor cocktail. 2. 13-Week study in rats. Research report PCW 71 1624. Unpublished data submitted by EFFA to SCF.

Munday R and Kirkby WW, 1973. Biological evaluation of a flavor cocktail. 3. One-year feeding study in rats. Research report PCW73 1103. Unpublished data submitted by EFFA to SCF.

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 frinking 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, F.A., 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, 1973a. Acute oral toxicity (rats - 5 gms/kg body weight dose). Dermal toxicity (rabbits - 5 gms/kg body weight dose). Geranyl isobutyrate. Food and Drug Research Laboratories, Inc. IBL no. 12207-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, W.B., (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.

Wohl AJ, 1974a. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Amyl vinyl carbinol. Biological Science Laboratories. April 2, 1974. Unpublished data submitted by EFFA to SCF.

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.

Wolven AM and Levenstein I, 1969. Acute oral toxicity reports on rats. Diethylmalonate. Leberco Laboratories. Assay no. 23460. July 31, 1962. 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.

Yamashita N, Murata M, Inoue S, Hiraku Y, Yoshinaga T and Kawanishi S, 1998. Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper (II). Mutat. Res. 397, 191-201.

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.

ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADI	Acceptable Daily Intake
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
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
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

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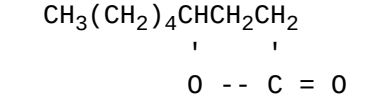
TOXICOLOGICAL EVALUATION OF SOME FLAVOURING SUBSTANCES AND NON-NUTRITIVE SWEETENING AGENTS

Geneva, 21-28 August 1967

The Eleventh Report of the Joint FAO/WHO Expert Committee on Food Additives is published as FAO Nutrition Meetings Report Series, 1967, No. 44; Wld Hlth Org. techn. Rep. Ser., 1968, 383. This Report contains general considerations, including the principles adopted for the evaluation, and a summary of the results of the evaluations of a number of food additives. Additional information, such as biological data and a toxicological evaluation, considered at that meeting, is to be found in this document.

Food and Agriculture Organization of the United Nations
World Health Organization
1967

GAMMA-NONALACTONE

Chemical name	Gamma-Nonalactone
Empirical formula	$C_9H_{16}O_2$
Structural formula	
Molecular weight	156.23

Definition	gamma-Nonalactone contains not less than 97 per cent. $C_9H_{16}O_2$.
Description	A nearly colourless to yellow liquid having a strong odour suggestive of coconut.

Biological Data

Biochemical aspects

This lactone was found to open the ring on incubation with rat liver homogenate, and 62-88 per cent. was hydrolysed (Oser, 1967).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body-weight)	References
Rat	oral	9780	Jenner et al., 1964
Guinea-pig	oral	3440	Jenner et al., 1964

Short-term studies

Rat. In a 12-week feeding study on 15 males and 15 females, using mixed lactones, a slight reduction in the growth rate was noted at a level of 32.7 mg/kg body-weight/day (Oser, 1967). A 90-day study on 15 male and 15 female rats fed 0 or 67.5 mg/kg body-weight/day showed no adverse effect (Oser et al., 1965).

Long-term studies

Rat. Groups of 20 male and 20 female rats were fed diets containing 0, 0.1 and 0.5 per cent. of nonalactone for 2 years without any adverse effects being noted (Baer & Griepentrog, 1967).

Comments

The long-term studies in rats compensate for the inadequate information on the metabolic fate of this compound, and serve as basis

for the evaluation. Nevertheless, as only one species has been investigated, metabolic studies and studies in other species are desirable.

EVALUATION

Level causing no toxicological effect

Rat. 0.5 per cent. (= 5000 ppm) of the diet, equivalent to 250 mg/kg body-weight/day.

Estimate of acceptable daily intake for man

	mg/kg body-weight
Unconditional acceptance	0-1.25

REFERENCES

Baer, F. & Griepentrog, F. (1967) Med. Ehrnährung, 8, 244

Jenner, P. M., Hagan, E. C., Taylor, J. M., Cook, E. L. & Fitzhugh, O. C. (1964) Fd Cosmet. Toxicol., 2, 327

Oser, B. L., Carson, S. & Oser, M. (1965) Fd Cosmet. Toxicol., 3, 563

Oser, B. L. (1967) Unpublished report

See Also:

Toxicological Abbreviations

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

gamma-NONALACTONE

COE No.:	178
FEMA No.:	2781
JECFA No.:	229
Chemical names:	4-NONANOLIDE; 5-PENTYLDIHYDRO-2(3H)-FURANONE
Synonyms:	NONA-1,4-LACTONE; ALDEHYDE C-18 (SO-CALLED)
Functional class:	FLAVOURING AGENT
Latest evaluation:	1997
ADI:	0-1.25 mg/kg bw (1967)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1967 ADI of 0-1.25 mg/kg bw was maintained at the forty-ninth meeting (1997).
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. R 1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/40, FAS 68.33/NMRS 44A-JECFA 11/67. 0-1.25. FU. N

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See Also:
Toxicological Abbreviations

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

Roger A. Renne
Battelle, Toxicology Northwest, Richland, Washington, USA

Hiroyuki Yoshimura
Japan Tobacco, Inc., Tokyo, Japan

Kei Yoshino
Japan Tobacco, Inc., Kanagawa, Japan

George Lulham
JTI Macdonald Corp., Toronto, Canada

Susumu Minamisawa
Japan Tobacco, Inc., Tokyo, Japan

Albrecht Tribukait
Japan Tobacco, Inc., Germany, Cologne, Germany

Dennis D. Dietz, Kyeonghee Monica Lee, and R. Bruce Westerberg
Battelle, Toxicology Northwest, Richland, Washington, USA

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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Address correspondence to Roger Renne, PO Box 999, Richland, WA 99352, USA. E-mail: renne@battelle.org

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.

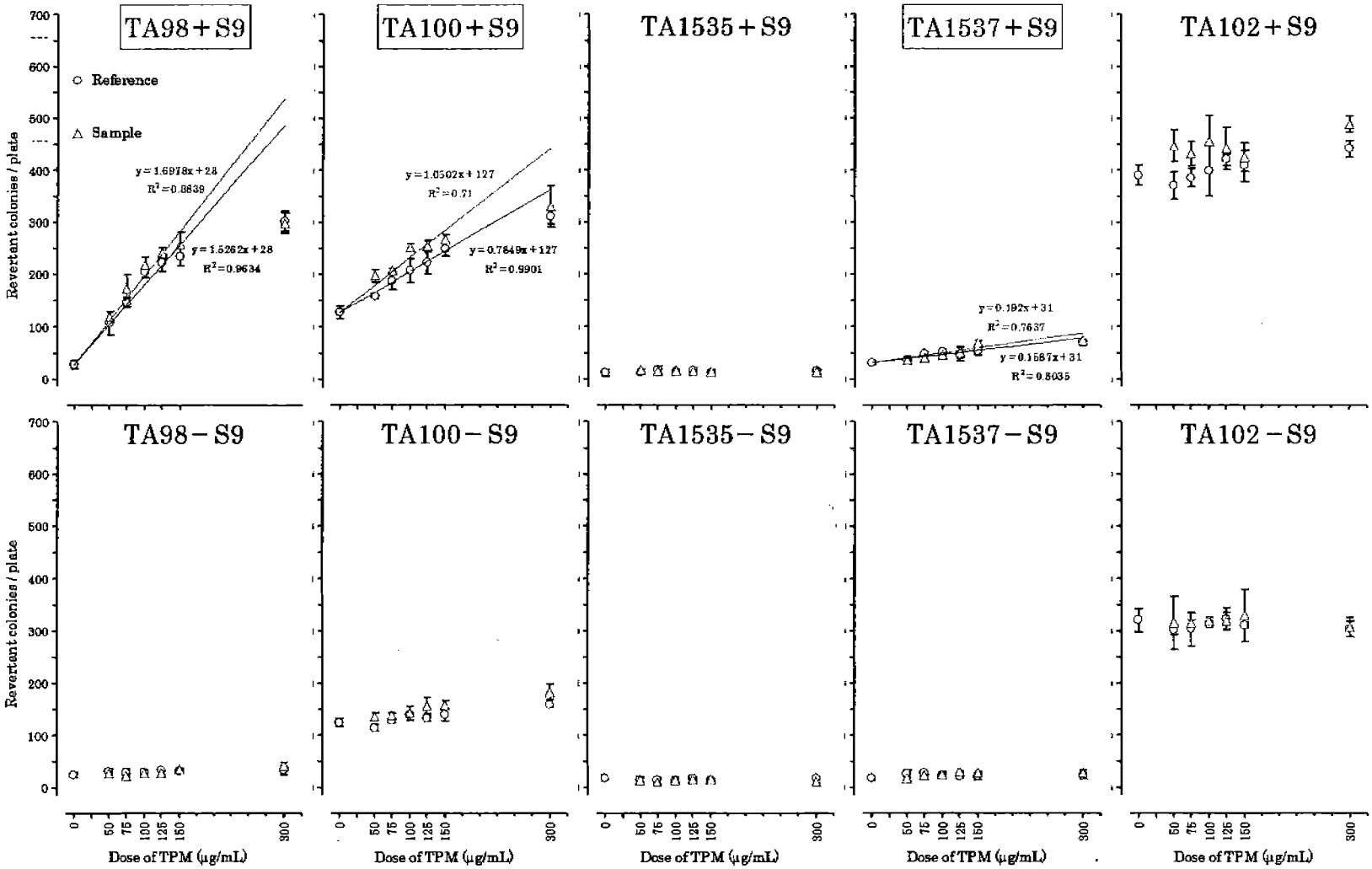
Body Weights and Clinical Observations

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.

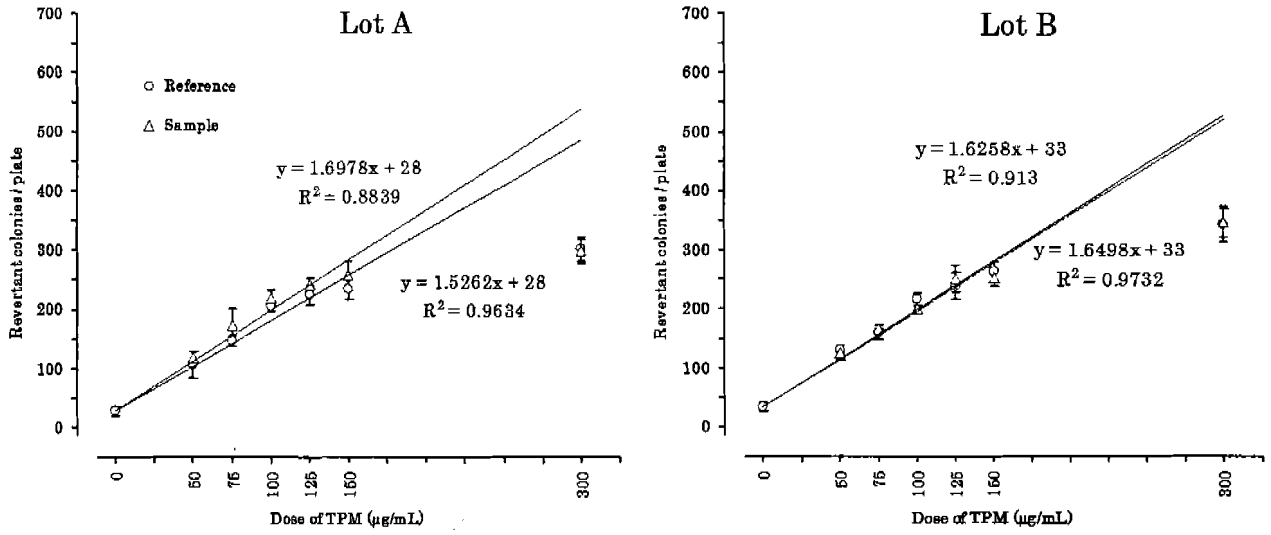
Organ Weights

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

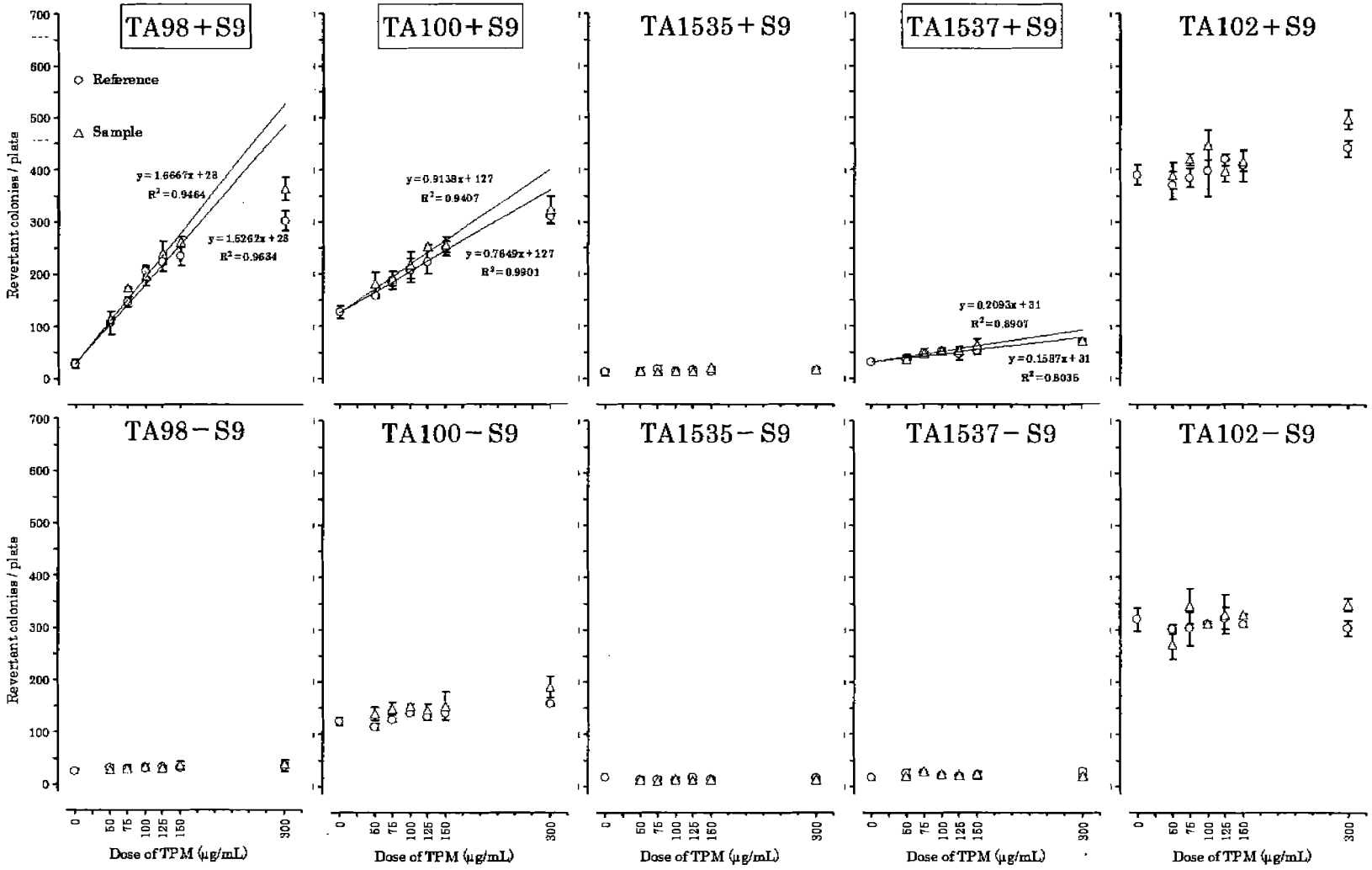
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 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



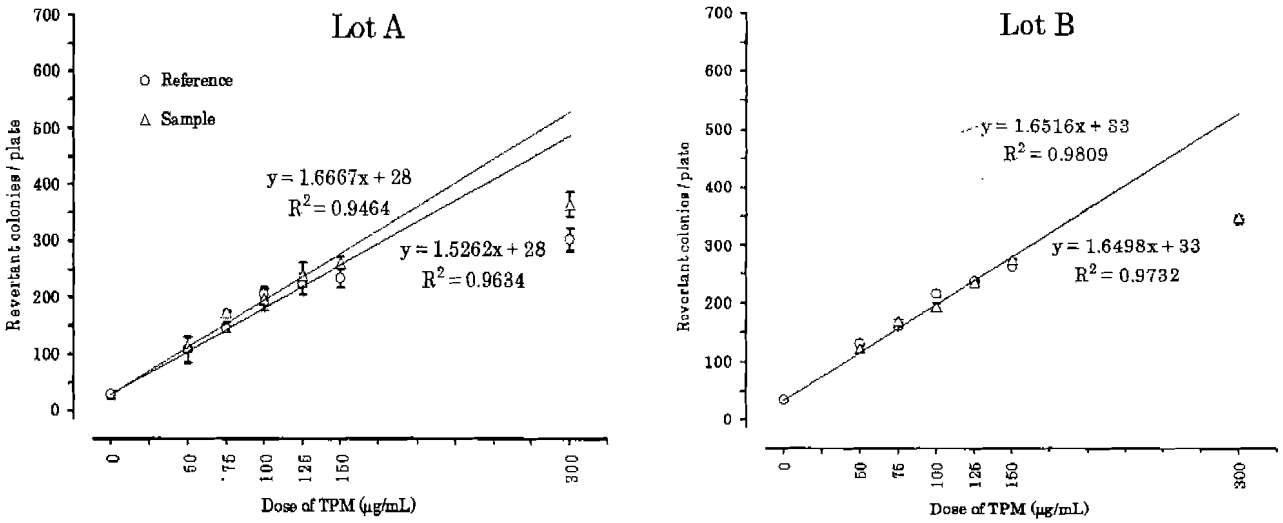
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

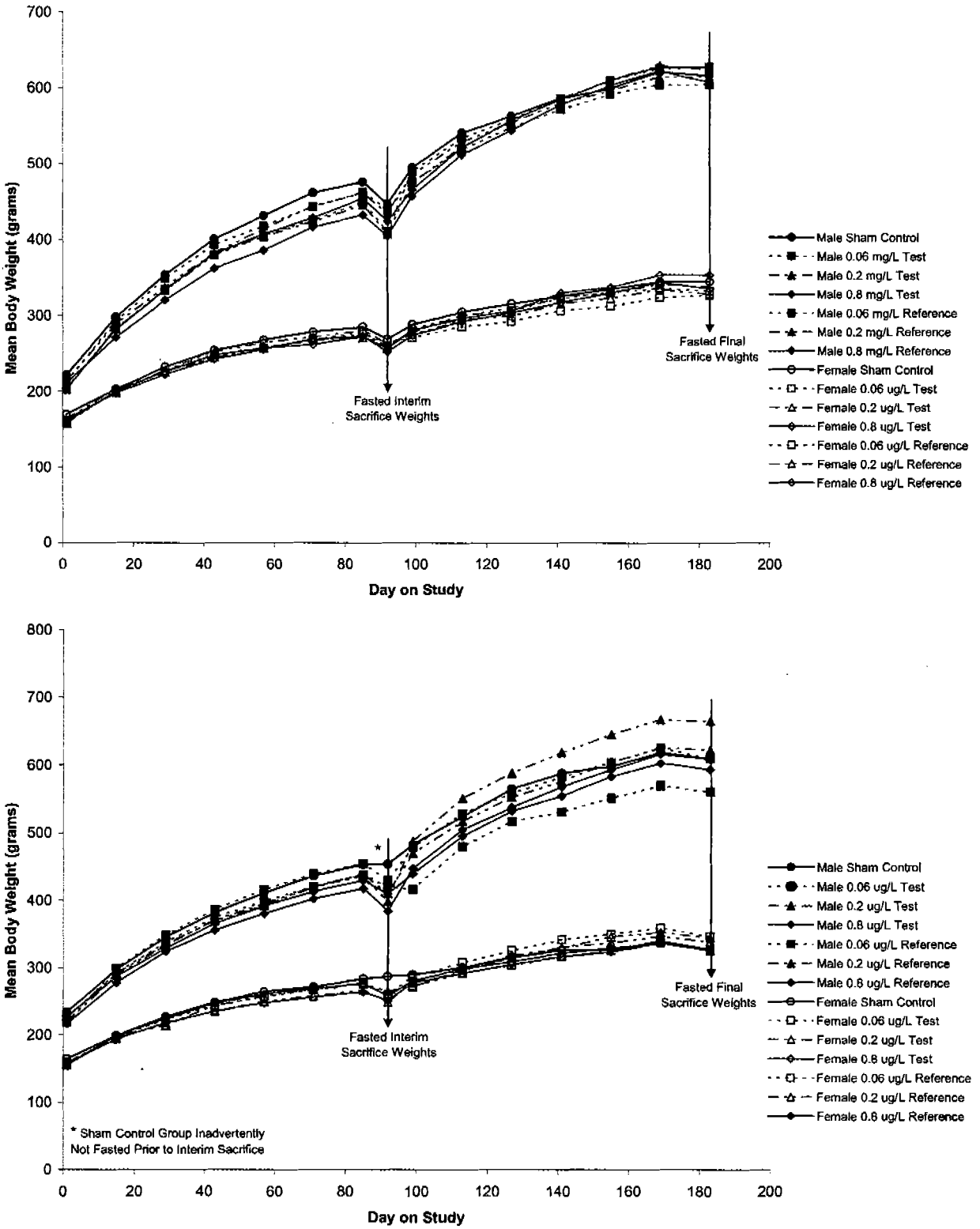


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.3
Revision Date 16.06.2022
Print Date 08.11.2022
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	:	γ-Nonalactone γ-Nonalactone
Product Number	:	44542
Brand	:	Sigma-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.	:	104-61-0

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	:	Sigma-Aldrich Chemie GmbH Industriestrasse 25 CH-9471 BUCHS
Telephone	:	+41 81 755 2511
Fax	:	+41 81 756 5449
E-mail address	:	technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+41 43-508-2011 (CHEMTREC) +41 44-251-5151 (Tox-Zentrum) 145(Tox Info Suisse)

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.

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SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	:	$C_9H_{16}O_2$
Molecular weight	:	156,22 g/mol
CAS-No.	:	104-61-0
EC-No.	:	203-219-1

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
Consult a physician. Show this material safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

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
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

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SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.
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

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

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
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

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The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Body Protection
Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	liquid
b)	Color	colorless
c)	Odor	No data available
d)	Melting point/freezing point	No data available
e)	Initial boiling point and boiling range	121 - 122 °C at 8 hPa
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	113 °C - closed cup
i)	Autoignition temperature	No data available
j)	Decomposition temperature	No data available
k)	pH	No data available
l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	No data available
n)	Partition coefficient: n-octanol/water	No data available
o)	Vapor pressure	No data available
p)	Density	0,976 g/cm3

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	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agentsStrong oxidizing agents, Strong bases

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 - male - 6.600 mg/kg
(OECD Test Guideline 401)
Inhalation: No data available
Dermal: No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
Eyes - Rabbit
Result: No eye irritation - 24 h
(OECD Test Guideline 405)

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available
Test Type: Mouse

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Test system: lymphocyte
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 476
Result: negative

Species: Mouse

Application Route: Oral
Method: OECD Test Guideline 474
Result: negative

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: LU3675000
Irritation, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 52 mg/l - 48 h
Toxicity to algae	Growth inhibition EC50 - Pseudokirchneriella subcapitata (green algae) - 63,5 mg/l - 72 h (OECD Test Guideline 201)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 28 d Result: 71 % - Readily biodegradable. (OECD Test Guideline 301D)

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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
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

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.

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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.

International Chemical Weapons Convention (CWC) Schedules of Toxic Chemicals and Precursors :

REACH - Restrictions on the manufacture, placing on the market and use of certain dangerous substances, preparations and articles (Annex XVII) :

Regulation (EC) No 649/2012 of the European Parliament and the Council concerning the export and import of dangerous chemicals :

REACH - Candidate List of Substances of Very High Concern for Authorisation (Article 59). :

This product contains a substance listed on Annex XIV of the REACH Regulation (EC) Nr. 1907/2006.

Listed substance / Sunset Date :

After the sunset date the use of this substance requires either an authorization or can only be used for exempted uses, e.g. use in scientific research and development which includes routine analytics or use as intermediate.

Regulation (EC) No 1005/2009 on substances that deplete the ozone layer :

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Further information

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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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