Toxicological profile for

Triethyl citrate

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

Triethyl 2-hydroxypropane-1,2,3-tricarboxylate (PubChem)

1.2. Synonyms

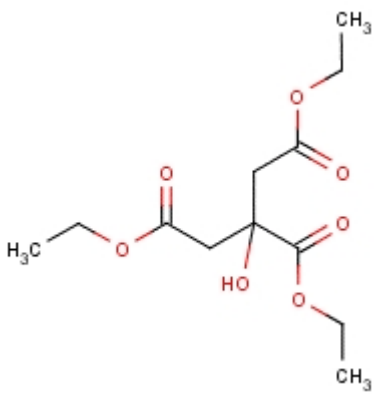
Triethyl citrate; 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester; Citric acid, triethyl ester; Citroflex 2; Ethyl citrate; Eudraflex; EINECS 201-070-7; FEMA No. 3083; 2-Hydroxy-1,2,3-propanetricarboxylic acid, triethyl ester; 4-03-00-01276 (Beilstein Handbook Reference); AI3-00659; BRN 1801199; HSDB 729; Hydragen CAT; NSC 8907; Triaethylcitrat [German]; Triethyl 2-hydroxy-1,2,3-propanetricarboxylate; Triethylester kyseliny citronove [Czech]; UNII-8Z96QXD6UM; 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, 1,2,3-triethyl ester; 2-Hydroxy-1,2,3-propanetricarboxylic acid, delta triethyl ester (ChemIDplus); JECFA 629; INS 1505; CoE 11762

1.3. Molecular formula

C12-H20-O7

1.4. Structural Formula

(ChemIDplus)



1.5. Molecular weight (g/mol)

276.3

1.6. CAS registration number

77-93-0

1.7. Properties

1.7.1. Melting point

(°C): -55 (ChemSpider; EPISuite, 2017; HSDB, 2015); <25 (ChemIDplus; ChemSpider); -46 (ChemSpider)

1.7.2. Boiling point

(°C): 294 (ChemIDplus; ChemSpider; EPISuite, 2017; Merck, 2013); 142 (ChemSpider)

1.7.3. Solubility

About 6.9% in water (Merck, 2013); 65 g/L (ChemIDplus; EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 32, 176 or 230 (ChemSpider); 151 (IPCS, 1999); 155 (closed cup) (HSDB, 2015); 151 (closed cup) (PubChem)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

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

1.7.9. Stability

Stable under recommended storage conditions (HSDB, 2015)

1.7.10. Vapor pressure

(mm Hg at 25°C): 0.000687 (extrapolated) (EPISuite, 2017; HSDB, 2015)

1.7.11. log Kow

0.33 (estimated) (ChemIDplus; EPISuite, 2017); 1.3 at 35°C (CIR, 2012); 1.49 (estimated) (ChemSpider)

2. General information

2.1. Exposure

Cosmetics	Yes	Food	Yes (Burdock, 2010)
Environment	No evidence	Pharmaceuticals	No evidence

No evidence of its presence in tobacco naturally.

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

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	0.02	0.03	Gelatins, puddings	0.01	0.02
Baked goods	0.04	0.12	Hard candy	0.01	0.04
Chewing gum	0.50	0.50	Nonalcoholic beverages	0.01	0.03
Frozen dairy	0.01	0.07	Soft candy	0.03	0.08

Estimated intake from flavouring use: 0.03884 mg/kg bw/day.

As taken from Burdock, 2010.

“Citrate-containing ingredients are allowed as active ingredients in the USA, at a maximum daily dosage of 8 g, in antacid over-the-counter (OTC) products” (CIR, 2012).

Triethyl citrate is used as a “plasticizer for cellulose derivatives and natural resins; plasticizer in pharmaceutical excipients” (CIR, 2012).

Used as a masking, perfuming and plasticizer ingredient in cosmetics in the EU. As taken from

CosIng (Cosmetic substances and ingredients database). Accessed September 2018, available at http://ec.europa.eu/growth/tools-databases/cosing/

“In 2009, the R. J. Reynolds Tobacco Co. released a line of dissolvable tobacco products that are marketed as an alternative to smoking in places where smoking is prohibited. These products are currently available in Indianapolis, IN, Columbus, OH, and Portland, OR. This paper describes the chemical characterization of four such products by gas chromatography-mass spectrometry (GC-MS). The dissolvable tobacco products were extracted and prepared by ultrasonic extraction using acetone, trimethylsilyl derivatization, and headspace solid phase microextraction (SPME). The following compounds were identified in the dissolvables using either ultrasonic extractions or trimethylsilyl derivatization: nicotine, ethyl citrate, palmitic acid, stearic acid, sorbitol, glycerol, and xylitol. The following compounds were identified in the dissolvables using headspace SPME: nicotine, ethyl citrate, cinnamaldehyde, coumarin, vanillin, and carvone. With the exception of nicotine, the compounds identified thus far in the dissolvables are either flavoring compounds or binders. The concentration of free nicotine in the dissolvables was determined from the Henderson-Hasselbalch equation and by measuring the pH and nicotine concentration by GC-MS. The results presented here are the first to reveal the complexity of dissolvable tobacco products and may be used to assess potential oral health effects” (Rainey et al. 2011. Journal of Agricultural and Food Chemistry 59, 2745-2751). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21332188?dopt=AbstractPlus

Triethyl citrate (CAS RN 77-93-0) is listed as an ingredient in inside the home and personal care products by the US Department of Health and Human Services (2018).

Triethyl citrate is listed as a fragrance ingredient by IFRA (2016) and the US EPA (US EPA Inert Finder Database, 2018).

“If, as indicated for ATBC in section 3.1.2, triethylcitrate is extracted more or less as effectively as the phthalates from PVC and the same concentrations are used in the polymers, a migration of up to 10 mg/10cm2/min could be expected from toys when chewed/mouthed by small children. If the released substance is fully hydrolysed this will give a total daily dose of about than 120 microgram/kg ethanol if a child weighing 5 kg chews the toys during 3 hours. Such a dose is without toxicological concern.”

As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

“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 triethyl citrate 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 3, 2015: http://www.epa.gov/cdr/pubs/guidance/cdr_factsheets.html] **PEER REVIEWED**

…..Occupational exposure to triethyl citrate may occur through dermal contact with this compound at workplaces where triethyl citrate is produced or used. Use data indicate that the general population may be exposed to triethyl citrate via ingestion of food and dermal contact with consumer products containing triethyl citrate(SRC).”

As taken from HSDB, 2015

“Used as plasticizer (cellulose acetate, cellulose nitrate, vinyl acetate, natural resins, and hair fixative finishing sprays), softener, agglutinant, perfume base, food emulsifier, and flavor preserving agent; Also used in paint removers and for treatment of bloat in ruminants.”

As taken from Haz-Map, 2017

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Specific Agents by Occupation*

Agent Name	CITRIC ACID, TRIETHYL ESTER		
CAS #	77-93-0		
RTECS #	GE8050000		
Agent Code	X7556		
Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
095	REGISTERED NURSES	3,679	500
096	PHARMACISTS	94	71
235	TECHNICIANS, N.E.C.	105	7
453	JANITORS AND CLEANERS	221	
518	INDUSTRIAL MACHINERY REPAIRERS	62	
684	MISCELLANEOUS PRECISION WORKERS, N.E.C.	71	
725	MISCELLANEOUS METAL AND PLASTIC PROCESSING MACHINE OPERATORS	45	
734	PRINTING MACHINE OPERATORS	379	28
754	PACKAGING AND FILLING MACHINE OPERATORS	1,313	568
756	MIXING AND BLENDING MACHINE OPERATORS	788	59
757	SEPARATING, FILTERING, AND CLARIFYING MACHINE OPERATORS	26	6
766	FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD	45	
768	CRUSHING AND GRINDING MACHINE OPERATORS	34	
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	1,925	1,373
796	PRODUCTION INSPECTORS, CHECKERS, AND EXAMINERS	128	96
849	CRANE AND TOWER OPERATORS	23	
859	MISCELLANEOUS MATERIAL MOVING EQUIPMENT OPERATORS	193	
877	STOCK HANDLERS AND BAGGERS	138	
888	HAND PACKERS AND PACKAGERS	1,451	1,130
889	LABORERS, EXCEPT CONSTRUCTION	777	154
TOTAL		11,496	3,993

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

As taken from NIOSH, available at https://web.archive.org/web/20111024124426/http://www.cdc.gov/noes/noes2/x7556occ.html

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%
triethyl citrate	2517011881	92.46	2420965227	99.28
Total ion chromatogram	2722398873	100	2438465585	100

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

Ingredient CAS Number Formula or structure	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)
Triacetyl citrate CAS 77-93-0	Tri ester	M=276 bp 127 at 1 mm Hg	700	99	Triacetyl citrate Diethyl malonate Ester? Triethyl acetylcitrate 1 unidentified compound	96.2 1.3 0.4 0.2 1.9	340 5 1 0.7 7

2.3. Ingredient(s) from which it originates

“Citrate alkyl esters are typically produced via the condensation of the appropriate alcohol with citric acid” (CIR, 2012).

“By esterification of ethyl alcohol with citric acid” (Burdock, 2010).

“Natural occurrence: Reported found in Morello cherry, sour cherry and red currant. Also reported found in raw cabbage and white wine” (Burdock, 2010)

3. Status in legislation and other official guidance

Food	UK	Yes	EU	Yes	USA	Yes
ADI	Using its procedure for the safety evaluation of flavouring agents, JECFA concluded that triethyl citrate did not represent a safety concern at current estimated levels of intake (2.4 and 3.4 mg/day in the US and Europe respectively). The 1984 JECFA ADI of 20 mg/kg bw was maintained (JECFA, 1999). A website reports that the US Government has approved the use of triethyl citrate as a tobacco additive (Anon).					
Codex Alim.	INS 1505					
C of E no.	1505				FEMA no.	3083
TLV (ACGIH)	Not listed					
Cosmetics (UK)	Listed, Cosmetics Bench Ref.					

FIFRA REQUIREMENTS:

Unless specifically excluded, residues resulting from the use of the following substance as either an inert or an active ingredient in a pesticide chemical formulation, including antimicrobial pesticide chemicals, is exempted from the requirement of a tolerance under FFDCA section 408, if such use is in accordance with good agricultural or manufacturing practices. Citric acid, triethyl ester is included on this list. [40 CFR 180.950 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov] **PEER REVIEWED**

FDA Requirements:

An ingredient whose use in food or food packaging is subject to a prior sanction or approval within the meaning of section 201(s)(4) of the Act is exempt from classification as a food additive. ... Substances classified as plasticizers, when migrating from food-packaging material shall include ... triethyl citrate. [21 CFR 181.27 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov] **PEER REVIEWED**

Substance added directly to human food affirmed as generally recognized as safe (GRAS). [21 CFR

184.1911 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

As taken from HSDB, 2015

No safety concern

“3400 µg/person per day in Europe and 2400 µg/person per day in the United States”

As taken from INCHEM, 2000, WHO FOOD ADDITIVES SERIES: 44; available at http://www.inchem.org/documents/jecfa/jecmono/v44jec10.htm

ADI = 0-20 mg/kg bw (1984)

As taken from WHO, 2018 available at http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3286

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) established in 1979 a temporary ADI of 10 mg/kg bw. This was changed in 1984 to an ADI of 20 mg/kg bw. The Scientific Committee for Food agreed in 1981 and 1990, respectively, to these values (CSTEE/98/17 - Add. 37/b). The Scientific Committee for Food has placed triethyl citrate on its positive list, List 1 of 1995, Substances, e.g. food additives, for which an ADI, a temporary ADI (t-ADI), a MTDI, a PMTDI, a PTWI or the classification “acceptable” has been established by this Committee or by JECFA (CSTEE/98/17 - Add. 37). JECFA at its meeting in June 1999 evaluated the use of triethyl citrate as a flavouring agent according to the Procedure for the Safety Evaluation of Flavouring Agents. Based on estimated intake for Europeans of 3400 microgram/person/day, it was concluded that the intake exceeds the exposure threshold of concern (1800 microgram/person/day), but that there is no safety concern for its use as a flavouring agent (JECFA, 1999).

As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives TRIETHYL CITRATE	
INS:	1505
COE No.:	11762
FEMA No.:	3083
JECFA No.:	629
Chemical names:	TRIETHYL 2-HYDROXY-1,2,3-PROPANETRICARBOXYLATE
Synonyms:	ETHYL CITRATE; TRIETHYL 2-HYDROXY-1,2,3-PROPANE TRICARBOXYLATE
Functional class:	CARRIER SOLVENT; SEQUESTRANT; FLAVOURING AGENT
Latest evaluation:	1999
ADI:	0-20 mg/kg bw (1984)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1984 ADI of 0-20 mg/kg bw was maintained at the fifty-third meeting (1999).
Report:	TRS 896-JECFA 53/67
Specifications:	COMPENDIUM ADDENDUM 11/FNP 52 Add. 11/89 (METALS LIMITS) (2003)
Tox monograph:	FAS 44-JECFA 53/229
Addendum:	FAS 19-JECFA 28/115
Previous status:	2000, COMPENDIUM ADDENDUM 8/FNP 52 Add.8/158. R (FLAVOUR) 1999,

	COMPENDIUM ADDENDUM 7/FNP 52 Add. 7/132. N,T (FLAVOUR) 1984, TRS 710-JECFA 28/19, FNP 31/2-JECFA 28/125 (COMPENDIUM/1543), FAS 14-JECFA 23/93 (1979). 0-20. FU. R 1981, TRS 669-JECFA 25/31. 0-10 (TEMPORARY). TE. S 1979, TRS 648-JECFA 23/18, FNP 12-JECFA 23/115, FAS 14-JECFA 23/93. 0-10 (TEMPORARY). TE. N
6 Feb 04	

As taken from INCHEM, 2004 available at http://www.inchem.org/documents/jecfa/jeceval/jec_2316.htm

MSDI (EU): 2900 ug/capita/day

As taken from Flavouring Group Evaluation 10, Revision 3 (FGE10 Rev3). The EFSA Journal (2012); 10(3): 2563 available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2563/epdf

Included on the FDA's list of Substances Added to Food (formerly EAFUS) as a flavor enhancer, flavoring agent or adjuvant, formulation aid, sequestrant, and solvent or vehicle, and covered under 21 CFR sections 175.300 (Resinous and polymeric coatings), 175.320 (Resinous and polymeric coatings for polyolefin films) and 181.27 (Plasticizers)

As taken from FDA, 2018a,b

Triethyl citrate is generally recognized as safe (GRAS) as a direct human food ingredient under 21 CFR section 184.1911 (FDA, 2018b).

Estimated intake from use as a flavouring is 2900 µg/person/day in the EU (EFSA, 2012) and, in the US, 3400 µg/person/day (JECFA, 1999) or 38.84 µg/kg bw/day (Burdock, 2010).

Triethyl citrate is listed in the US EPA Inert Finder Database (2018) as cleared for food, non-food and fragrance use pesticide products. For food use, it is regulated under 40 CFR Part 180.950e (Tolerances and exemptions for pesticide chemical residues in food: Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2018a).

Triethyl citrate is considered safe for use in animal feed for all animal species at 5 mg/kg complete feed (EFSA, 2013).

Triethyl citrate (CAS RN 77-93-0) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA CDR list (Chemical Data Reporting Rule). The CDR regulation 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://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

There is a REACH dossier on triethyl citrate (ECHA, 2018a).

Triethyl citrate (CAS RN 77-93-0) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2018b).

Included on the US EPA's list of Safer Chemical Ingredients (US EPA, 2018b).

Triethyl citrate is permitted for use as a flavouring in the EU for all categories of flavoured food under Regulation (EU) 872/2012 (European Commission, 2012).

Triethyl citrate has been given GRAS (generally recognized as safe) status by FEMA (Hall RL and Oser BL, 1965).

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0) is included on New Zealand's Inventory of Chemicals and may be used as a single component chemical under an appropriate group standard (NZ EPA, 2006)

Triethyl citrate (E1505) is authorised for use as a food additive in the EU under legislation (EU) nos 1129/2011 and 2015/0647 (European Commission, 2015).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“Rat, mouse and human, liver homogenates cleaved 1 mol triethyl citrate to 1 mol citric acid and 3 mol ethanol, Bruns et al 1962.

Triethyl citrate is an odourless, nearly colourless, oily liquid. No absorption or metabolism studies have been reported, however, it is expected that the compound would rapidly metabolize in the body and liberate the citrate ion which would be handled through the usual biochemical pathways (FASEB, 1976).

It is likely that triethyl citrate will be hydrolyzed to its component parts, citrate and ethanol in vivo.”

As taken from INCHEM, 2000, WHO FOOD ADDITIVES SERIES: 44; available at http://www.inchem.org/documents/jecfa/jecmono/v44jec10.htm

“Samples of freshly collected rat or human serum were spiked with triethyl citrate and the disappearance of the triethyl citrate measured over a 4 hr period. Triethyl citrate was rapidly hydrolysed by rat serum (15 min.), but occurred at a much slower rate in human serum and was not complete at the end of the 4 hr test period (Figdor & Ballinger, 1981).”

“Rat-, mouse- and human-liver homogenates as well as serum enzymes hydrolyse triethyl citrate to 1 mol citric acid and 3 mol ethanol/mol ester (Burns & Werners, 1962). Metabolism"> Comments">”

“Although it seems unlikely that unchanged triethyl citrate would be absorbed, in vitro studies are available to show that both the liver and blood serum have enzyme systems capable of hydrolysing the ester.”

As taken from INCHEM, 1984, WHO FOOD ADDITIVES SERIES: 19; available at http://www.inchem.org/documents/jecfa/jecmono/v19je12.htm

“Data presented to the Scientific Committee for Food in 1990 showed that triethyl citrate is hydrolysed in vivo to citric acid and ethanol, compounds with well-defined, low toxic potential (CSTEE/98/17 - Add. 37/b). Triethyl citrate appeared to be hydrolysed at a slower rate with human serum compared to rat serum (CSTEE/98/17 - Add. 37/d).”

As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

METABOLISM/ METABOLITES:

Triethyl citrate is hydrolyzed in vivo to citric acid and ethanol. Triethyl citrate appeared to be hydrolyzed at a slower rate with human serum compared to rat serum. [European Commission/ Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE). Opinion on the Toxicological Characteristics and Risks of Certain Citrates and Adipates Used as a Substitute for Phthalates as Plasticisers in Certain Soft PVC Products. (September 1999). Available from, as of May 5, 2015: _http://ec.europa.eu/health/scientific_committees/environmental_risks/sctee/index_en.htm
PEERREVIEWED

Samples of freshly collected rat or human serum were spiked with triethyl citrate and the disappearance of the triethyl citrate measured over a 4 hr period. Triethyl citrate was rapidly hydrolysed by rat serum (15 min), but hydrolysis occurred at a much slower rate in human serum

and was not complete at the end of the 4 hr test period. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 19). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

Rat-, mouse- and human-liver homogenates as well as serum enzymes hydrolyse triethyl citrate to 1 mol citric acid and 3 mol ethanol/mol ester. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 19). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

/Triethyl citrate/ is expected to be extensively metabolized by esterases and cytochrome P450 enzymes and break-down in the beta-oxidation or citric acid cycle or in cases subsequent glucuronidation. The substance is assumed to be excreted (if not metabolized completely in beta-oxidation and citric cycle) as metabolites (i.e. conjugates with glucuronic acid) via urine and to a lower extent via bile. [European Chemicals Agency (ECHA); Registered Substances, Triethyl citrate (CAS Number: 77-93-0) (EC Number: 201-070-7) (September 12, 2014). Available from, as of May 6, 2015: http://echa.europa.eu/en/information-on-chemicals] **PEER REVIEWED**

As taken from HDSB, 2015

4.2. Absorption, distribution and excretion

In order to assess the toxicological behavior of triethyl citrate, the available experimental and predicted physico-chemical data have been evaluated. The substance is expected to be absorbed very well. The absorption of any metabolite of the substances of interest is fast and complete. Concerning the absorption after exposure via inhalation, as the chemical has a low vapor pressure, it is clear, that the substance is poorly available after inhalation. Given its lipophilicity (LogPow 1.17) - if absorbed - it is expected to be absorbed directly across the respiratory tract epithelium. The substance is expected to be also poorly absorbed following dermal exposure into the stratum corneum and to a certain extent into the epidermis, due to its molecular weight and its LogPow. In addition, the systemic toxicity via the skin is assumed to be low and this has been proven with the results of the acute dermal study with triethyl citrate, in which a LD50 of 5000 mg/kg bw has been obtained. Concerning the distribution in the body, triethyl citrate is expected to be mainly available in the circulatory system (due to its water solubility). The experimentally determined LogPow value, the water solubility and predicted behavior concerning absorption of the substance triethyl citrate do not indicate a potential for accumulation. [European Chemicals Agency (ECHA); Registered Substances, Triethyl citrate (CAS Number: 77-93-0) (EC Number: 201-070-7) (September 12, 2014). Available from, as of May 6, 2015: http://echa.europa.eu/en/information-on-chemicals] **PEER REVIEWED**

As taken from HSDB, 2015

4.3. Interactions

“Three commonly used flavor industry solvents (propylene glycol, triacetin, and triethyl citrate) were tested for their capacity to interfere with the ability of alpha-, beta-, and gamma-cyclodextrin to form molecular inclusion complexes with flavors. Six flavor compounds (ethyl butyrate, ethyl heptanoate, l-menthol, methyl anthranilate, neral, and geranial) were measured by headspace gas chromatography above 2:1 water/ethanol containing appropriate additions of cyclodextrin and flavor solvent. The smallest and most polar solvent molecule represented by propylene glycol had the least effect on cyclodextrin/flavorant complex formation. In contrast, triacetin, intermediate in size among the three flavor diluents studied, had the greatest effect, even though, based on at least some computed molecular parameters, it appears to be more polar than triethyl citrate. The explanation for this apparent anomaly may lie in differences in the extent to which triacetin and triethyl citrate are able to interact with cyclodextrins by means of partial interaction with the hydrophobic cavities of the latter. Reineccius et al., The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/flavor molecular inclusion complexes.”

As taken from Reineccius et al., The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/flavor molecular inclusion complexes.; J Agric Food Chem. 2005, Jan 26; 53(2):388-92.

“The United States Pharmacopeia (USP) apparatus 3 dissolution procedure was used to study the effects of simulated high fat food, an oil soak, on the release of a model drug, chlorpheniramine maleate, from controlled release ethylcellulose (Aquacoat) coated beads as a function of plasticizer type and concentration and coating level. Drug release was affected by the type and concentration of plasticizer and the level of coating. Beads plasticized with triethyl citrate or dibutyl sebacate had faster drug release rates after soaking in oil. The oil caused films to detach from the bead, producing uneven ridges and cracks in the coating. The glass transition temperature was increased for dibutyl sebacate plasticized films soaked in oil, but was not affected for triethyl citrate plasticized films. Similar results were found for puncture strength, percent elongation, and modulus of elasticity”.

As taken from Williams et al., (1997). In vitro method to investigate food effects on drug release from film coated beads; Pharm. Dev. Technol.; VOL 2 ISS 1 1997, P1-9.

“Triethyl citrate inhibited the transdermal absorption of viprostol, a synthetic prostaglandin E2, through the skin of male hypertensive rats. This effect was demonstrated by the statistically significant decrease in blood radioactivity levels following the topical application of [14C]viprostol in triethyl citrate compared to those found with the use of petrolatum (pet.) or silicone as the vehicle. A comparison of metabolic profiles also demonstrated slower hydrolysis of viprostol to free acid with the use of triethyl citrate as the vehicle” (CIR, 2012).

Triethyl citrate inhibited the transdermal absorption of viprostol, a synthetic prostaglandin E2, through to the skin of male hypersensitive rats. This effect was demonstrated by the statistically significant decrease in blood radioactivity levels following the application of [14C] viprostol in triethyl citrate compared to those found with the use of petrolatum (pet) or silicone as the vehicle. A comparison of metabolic profiles also demonstrated slower hydrolysis of viprostol to free acid with the use of triethyl citrate as the vehicle. [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

As taken from HSDB, 2015

5. Toxicity

5.1. Single dose toxicity

Oral LD50 in the rat – 7ml/kg Finkelstein et al 1959.

Oral LD50 in the cat – 3.5ml/kg Finkelstein et al 1959.

Intraperitoneal LD50 in mouse – 1.75g/kg Meyers et al 1964.

Dermal LD50 in guinea pig - >10ml/kg Fasset 1963.

Dermal LD50 in rabbit - >5g/kg Levenstein 1975.

“The oral LD50 value for triethyl citrate in rats is approximately 7 g/kg (CSTEE/98/17 - Add. 2).” As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source

cat	LD50	oral	3500mg/kg (3500mg/kg)	BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD BEHAVIORAL: ATAXIA GASTROINTESTINAL: NAUSEA OR VOMITING	Food and Cosmetics Toxicology. Vol. 17, Pg. 389, 1979.
guinea pig	LD50	oral	> 25mL/kg (25mL/kg)		German Offenlegungsschrift Patent Document. Vol. #2703360,
mouse	LD50	intraperitoneal	1750mg/kg (1750mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) VASCULAR: OTHER CHANGES	Journal of Pharmaceutical Sciences. Vol. 53, Pg. 774, 1964.
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 17, Pg. 389, 1979.
rat	LC50	inhalation	1300ppm/6H (1300ppm)	LUNGS, THORAX, OR RESPIRATION: ACUTE PULMONARY EDEMA LUNGS, THORAX, OR RESPIRATION: PLEURAL EFFUSION LUNGS, THORAX, OR RESPIRATION: DYSPNEA	"Industrial Hygiene and Toxicology," 2nd ed., Patty, F.A., ed., New York, John Wiley & Sons, Inc., 1958-63Vol. 2, Pg. 1892, 1963.
rat	LD50	intraperitoneal	4gm/kg (4000mg/kg)	BEHAVIORAL: ALTERED SLEEP TIME (INCLUDING CHANGE IN RIGHTING REFLEX) LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Iyakuhin Kenkyu. Study of Medical Supplies. Vol. 16, Pg. 214, 1985.
rat	LD50	oral	5900mg/kg (5900mg/kg)	BEHAVIORAL: ALTERED SLEEP TIME (INCLUDING CHANGE IN RIGHTING REFLEX) LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Iyakuhin Kenkyu. Study of Medical Supplies. Vol. 16, Pg. 214, 1985.
rat	LD50	subcutaneous	6600mg/kg (6600mg/kg)	BEHAVIORAL: ALTERED SLEEP TIME (INCLUDING CHANGE IN RIGHTING REFLEX) LUNGS, THORAX, OR	Iyakuhin Kenkyu. Study of Medical Supplies. Vol. 16, Pg. 214, 1985.

				RESPIRATION: RESPIRATORY DEPRESSION	

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

The corneal reflex in rabbit eyes was temporarily eliminated upon instillation of 3 drops of a 5% suspension of triethyl ... citrate in 3% acacia ... The anesthetic effect was confirmed by the intradermal administration of 0.1 mL of a 2% solution of triethyl ... citrate into an area of the shaved back of guinea pigs. Triethyl citrate resulted in insensitivity to pricking of the area lasting 12 to 20 minutes ...[Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

Symptoms produced by single oral doses of ... triethyl /citrate/ are similar in both rats and cats include signs of weakness, depression and finally hyperirritability with convulsions and respiratory failure. Onset of symptoms was quite rapid ... in some cases symptoms continued for 2 days. [Patty, F. (ed.). Industrial Hygiene and Toxicology: Volume II: Toxicology. 2nd ed. New York: Interscience Publishers, 1963., p. 1892] **PEER REVIEWED**

Intravenous administration of a 100 mg/kg bw dose of triethyl citrate to rabbits produced a marked increase in motor activity and respiration. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 14). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

Non-Human Toxicity Values:

LD50 Guinea pig dermal >10 ml/kg [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]

LD50 Mouse ip 1750 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 3546] **PEER REVIEWED**

LD50 Rat inhalation 3500 pppm [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]LD50 Rat oral 7.0 g/kg [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]

LD50 Rat oral 3.2-6.4 g/kg [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]LD50 Cat oral 35,000 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 3546] **PEER REVIEWED**

As taken from HSDB, 2015

Species	Route	LD50 (mg/kg bw)	Reference
Rat	p.o.	8 000	Finkelstein & Gold, 1955
Cat	p.o.	4 000	Finkelstein & Gold, 1955

As taken from INCHEM, 1979, WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm

LD50 rat = 6990.9 mg/kg, 25.3 mmol/kg (Registry of cytotoxicity data (ZEBET), accessed

September 2018, available at https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/finalrpt/fins7all.pdf)

Inhalation toxicity:

6-hr LC50 rat: 1300-3500 ppm (Fassett, cited in BIBRA, 1998).

Six guinea pigs survived a 6-hr exposure at 1700 ppm vapour (Fassett, cited in BIBRA, 1998)

5.2. Repeated dose toxicity

Young rats were fed triethyl citrate at an initial rate of 1, 2 and 4 g/kg bw for eight weeks. Urinalysis, blood counts and growth measurement, performed periodically, revealed no toxic effects. At necropsy, no gross abnormalities were seen in the thoracic or abdominal organs. Histological sections of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen and kidneys were comparable in appearance to those from the untreated controls. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 14). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

Cats receiving daily doses of 7% of the LD50 (280 mg/kg bw) for eight weeks did not differ from control animals with respect to weight, blood count, hemoglobin, blood sugar and blood nitrogen. However, weakness, ataxia and depression appeared after the fourth or fifth dose and progressed. After treatment was discontinued, the animals recovered within 24-96 days. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 14). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

Subchronic or Prechronic Exposure/ Ethyl /citrate/ esters were fed to rats at levels of 0.5, 1, and 2% for period of 6 wk. No notable effects were seen on wt gain, blood count, blood chemistry, urinalysis or histopathology. [Patty, F. (ed.). Industrial Hygiene and Toxicology: Volume II: Toxicology. 2nd ed. New York: Interscience Publishers, 1963., p. 1892]

Cats tolerated oral dose of 0.25 cc/kg of triethyl citrate ... daily for period of 8 wk but showed mild symptoms of poisoning after fourth & fifth doses, consisting of weakness, ataxia & depression. [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]

Feeding triethyl citrate (highest dose approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs. [European Commission/ Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE). Opinion on the Toxicological Characteristics and Risks of Certain Citrates and Adipates Used as a Substitute for Phthalates as Plasticisers in Certain Soft PVC Products. p.8 (September 1999). Available from, as of May 5, 2015: http://ec.europa.eu/health/scientific_committees/environmental_risks/sctee/index_en.htm **PEER REVIEWED**

Two young adult male and two young adult female beagle dogs were given daily doses of triethyl citrate of 0.05 and 0.25 mL/kg bw for six months. Measurement of body and organ weights, blood and urinalysis and the results of histological examination of tissues revealed no adverse effects. Increasing the daily dose to 2.5 to 3.5 mL/kg bw for seven to 12 weeks resulted in liver pathology in three treated animals. A fourth dog that had previously reacted adversely to a 2 mL/kg bw dose showed no histological changes after receiving 1.5 mL/kg bw daily for an additional month. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 14). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

As taken from HSDB, 2015

“Level causing no toxicological effect - Rat: 4% in the diet (40,000 ppm) equivalent to 2 g/kg body weight.” As taken from INCHEM, 1984, WHO FOOD ADDITIVES SERIES: 19; available at

http://www.inchem.org/documents/jecfa/jecmono/v19je12.htm

Diet of two months to rats: NOEL (mg/kg bw per day) = 4000 [Finkelstein & Gold (1959)]

Gavage of two months to cats: NOEL (mg/kg bw per day) = <285 [Finkelstein & Gold (1959)]

As taken from INCHEM, 2000, WHO FOOD ADDITIVES SERIES: 44; available at http://www.inchem.org/documents/jecfa/jecmono/v44jec10.htm

"Feeding triethyl citrate (highest dose approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs (CSTEE/98/17 - Add 2)."

"No other toxicological data on triethyl citrate have been available to the CSTEE, although the Scientific Committee for Food refers to an older, inadequate long-term study in the rat (CSTEE/98/17 - Add. 37/b)."

As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

Short-term studies:

Mouse

"A group of 20 mice given intraperitoneal doses of 350 mg/kg bw of triethyl citrate daily for 14 days had a slightly lower mean growth rate than control animals. No differences were seen in the two groups in erythrocyte and leucocyte blood cell count, clotting time and haemoglobin levels. Examination of the liver, lung and kidney tissues of two animals at necropsy revealed no pathological cellular changes.

Rat

Young rats were fed triethyl citrate at an initial rate of 1, 2 and 4 g/kg bw for eight weeks (Finkelstein & Gold, 1955). Urinalysis, blood counts and growth measurement, performed periodically, revealed no toxic effects. At necropsy, no gross abnormalities were seen in the thoracic or abdominal organs. Histological sections of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen and kidneys were comparable in appearance to those from the untreated controls.

Cat

Cats receiving daily doses of 7% of the LD50 (280 mg/kg bw) for eight weeks did not differ from control animals with respect to weight, blood count, haemoglobin, blood sugar and blood nitrogen. However, weakness, ataxia and depression appeared after the fourth or fifth dose and progressed. After treatment was discontinued, the animals recovered within 24-96 days (Finkelstein & Gold, 1959).

Dog

Two young adult male and two young adult female beagle dogs were given daily doses of triethyl citrate of 0.05 and 0.25 ml/kg bw for six months. Measurement of body and organ weights, blood and urinalysis and the results of histological examination of tissues revealed no adverse effects (Hodge, 1954). Increasing the daily dose to 2.5 to 3.5 ml/kg bw for seven to 12 weeks resulted in liver pathology in three treated animals. A fourth dog that had previously reacted adversely to a 2 ml/kg bw dose showed no histological changes after receiving 1.5 ml/kg bw daily for an additional month.

Long-term studies:

Rat

Three groups of 15 male and 15 female weanling Sprague-Dawley rats were fed diets containing 0.33, 1.0 and 3.0% triethyl citrate in a two-year feeding study (LaWall & Harrison, 1954). The initial doses were from 0.2 to 2.0 g/kg bw. Weight gain and food intake were reduced below that of the control groups when the level of the compound in the diet was increased. (No specific numbers

were given for these results.) No adverse effects of haematologic, urinalysis, survival, gross or histopathologic parameters could be attributed to triethyl citrate."

As taken from INCHEM, 1979, WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm

Subacute toxicity:

Mice given daily Intraperitoneal doses of 350 mg triethyl citrate for 14 days inhibited weight gain with no changes in blood chemistry or histology, Meyers et al 1964. Rats given triethyl citrate in diet at dose up to 2% caused no observed changes, Finkelstein et al 1959. At 5% in the diet of rats for 12 days one out of eight rats died and body weights were reduced Yoshida et al.

Caused no changes in blood chemistry or histology, Finkelstein et al 1959.

There was some evidence that type of effects produced may have resulted from binding of calcium by release of citrate ion with resultant hypocalcemia. [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]

At ... 296 ppm rats tolerated 6-hr daily exposure for ... 62 days with no reported symptoms. At higher concn, /3500 ppm/ symptoms were ... gasping, weakness & post-mortem examination showed pleural infusion; some pulmonary edema was probably present. [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058]A group of 20 mice given intraperitoneal doses of 350 mg/kg bw of triethyl citrate daily for 14 days had a slightly lower mean growth rate than control animals. No differences were seen in the two groups in erythrocyte and leucocyte blood cell count, clotting time and hemoglobin levels. Examination of the liver, lung and kidney tissues of two animals at necropsy revealed no pathological cellular changes. [WHO/FAO; Expert Committee on Food Additives. Triethyl citrate (WHO Food Additives Series 14). (April 1979). Available from, as of May 5, 2015: http://www.inchem.org/] **PEER REVIEWED**

As taken from HSDB, 2015

Rats exposed at 296 ppm on 6 hr/day for 62 days showed no overt signs of toxicity, whereas higher (unspecified) concentrations caused gasping, weakness and lung damage (Fassett, cited in BIBRA, 1998).

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - mouse	4900 mg/kg/14D (intermittent)	Nutritional and Gross Metabolic - weight loss or decreased weight gain	JPMSAE Journal of Pharmaceutical Sciences. (American Pharmaceutical Assoc., 2215 Constitution Ave., NW, Washington, DC 20037) V.50- 1961- Volume(issue)/page/year: 53,774,1964
TDLo - Lowest published toxic dose	Oral	Mammal - cat	15904 mg/kg/8W (continuous)	Behavioral - somnolence (general depressed activity) Behavioral - muscle weakness Behavioral - ataxia	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 1,283,1959

As taken from RTECS, 1997

Safety Evaluation

Quantitative Risk Type	Quantitative Risk Value	Product Use	Safety Evaluation Owner	POD Method	POD Value	POD Owner
Not calculated	Not calculated	Not specified	COSMOS TTC (NON-CANCER)	NOAEL	284.0 mg/kg bw/day	COSMOS TTC (NON-CANCER)

Critical study: DOG (Chronic Toxicity) Oral exposure for 180 day

NOAEL/LOAEL Owner	Original NOAEL	Original LOAEL	Critical Sites	Critical Effects
US FDA CFSAN	284.0 mg/kg bw/day	Not established		• NO EFFECTS

Safety Evaluation Comments: no comments available.
Source Document: no source document available

As taken from the COSMOS database available at http://www.cosmostox.eu/what/COSMOSdb/

5.3. Reproduction toxicity

"At doses ranging from 0.5 to 10 mg/kg b.w. triethyl citrate was nonteratogenic in the chicken embryo. When injected into the air cell, the LD50 was 1349.86 mg/kg bw (67.49 mg/egg) (Verrett, 1976)."

As taken from INCHEM, 1979, WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm

5.4. Mutagenicity

In vitro					
Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**
Salmonella typhimurium, strains TA1535, TA1537 and TA1538	Details not provided in expert review	Mutation	with and without S9	-ve	Litton Bionetics Inc., 1976 (cited in JECFA, 1979)
Saccharomyces cerevisiae D4 yeast	Details not provided in expert review	Mutation	with and without S9	-ve	Litton Bionetics Inc., 1976 (cited in JECFA, 1979)
+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation					

"Triethyl citrate was not mutagenic in plate and suspension tests using the Ames Salmonella microsome mutagenesis assay in strains TA 1535, TA 1537 and TA 1538 and the Saccharomyces cerevesiae D4 yeast assay with and without tissue homogenate activating systems (Litton Bionetics, Inc., 1976)."

As taken from INCHEM, 1979, WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm

Ames test using triethyl citrate (0.4%-1.6%) on Salmonella typhimurium TA1535, TA1537, TA1538 was negative with and without metabolic activation. /From table/ [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-

safety.org/ingredients] **PEER REVIEWED**

Suspension test using triethyl citrate (0.4%-1.6%) on Salmonella typhimurium TA1535, TA1537, TA1538, and triethyl citrate (0.425%-1.7%) on Saccharomyces cerevisiae D4, with and without metabolic activation was negative /From table/ [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

As taken from HSDB, 2015

5.5. Cytotoxicity

Triethyl citrate inhibited the growth of strain L mouse fibroblasts (Rosenbluth et al. 1967).

5.6. Carcinogenicity

Species	**Test conditions**	**Evidence of carcinogenicity**	**Reference**
Rat, Sprague-Dawley, groups of 15 of each sex	Up to 3% in diet (roughly 1.5 g/kg bw/day) for 2 yr. Only a summary report, inadequate detail on the range of tissues examined	None [This study would be considered inadequate by modern standards, which require that groups of about 50 animals/sex be exposed, on 5-7 days/wk, at several dose levels, for lifetime, and that a comprehensive range of tissues and organs be examined microscopically]	LaWall and Harrison, 1954 (cited in JECFA, 1979). Page

5.7. Irritation/immunotoxicity

Not irritating to rabbit skin full strength, Levenstein 1975. Not irritating to humans at 20% (Epstein 1975)

At 20% no sensitisation reactions in humans (Epstein 1975).

“Triethyl citrate, at concentrations up to 100%, was not an irritant in guinea pigs or rabbits…. Triethyl citrate, applied undiluted during epidermal induction, was a strong sensitizer in a guinea-pig maximization test, but 20% in pet. was not a primary irritant or sensitizer in human studies” (CIR, 2012).

“Triethyl citrate, 33.3%, did produce irritation in rabbit eyes” (CIR, 2012).

“it did not show any evidence of sensitising capacity or skin irritation in humans (CSTEE/98/17 - Adds. 5, 54).”

“Triethyl citrate is a strong sensitiser in guinea pigs using the maximisation test in which the compound was injected adjuvant, although no sensitising capacity for humans was apparent from a repeated insult patch test. Further, it failed to induce irritation in human skin. Thus, triethyl citrate will not readily lead to sensitisation when in contact with normal human skin. However, it cannot be ruled out that it will induce sensitisation when in contact with human skin or mucous membranes that is damaged or affected in such a way that inflammatory responses are present.”

As taken from EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY,

ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, available at http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf

Triethyl citrate 20% in pet (petrolatum) was not a primary irritant or sensitizer in human studies.[Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

The ethyl /citrate/ esters have no effect on skin of guinea pig & are not skin sensitizers. /Ethyl citrate/ [Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 3058] **PEER REVIEWED**

Triethyl citrate, applied undiluted during epidermal induction, was a strong sensitizer in a guinea pig maximization test. [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

Triethyl citrate, at concentrations up to 100%, was not an irritant in guinea pigs or rabbits. [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

Triethyl citrate, 33.3%, did produce irritation in rabbit eyes. [Cosmetic Ingredient Review; Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Esters as Used in Cosmetics. Int J Toxicol 33 (2 suppl): 16S-46S. [Epub ahead of print] (2014) http://www.cir-safety.org/ingredients] **PEER REVIEWED**

As taken from HSDB, 2015

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing citric acid 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 citric acid 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	2081	JTI KB Study Report(s)
In vitro cytotoxicity	2081	JTI KB Study Report(s)

“The CIR Expert Panel (Panel) assessed the safety of citric acid, 12 inorganic citrate salts, and 20 alkyl citrate esters as used in cosmetics, concluding that these ingredients are safe in the present practices of use and concentration..... a number of the citrates are reported to function as skin-conditioning agents but other functions are also reported. The Panel reviewed available animal and clinical data, but because citric acid, calcium citrate, ferric citrate, manganese citrate, potassium citrate, sodium citrate, diammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate are generally recognized as safe direct food additives, dermal exposure was the focus for these ingredients in this cosmetic ingredient safety assessment.” As taken from Fiume MM et al 2014. Int. J. Toxicol. 33(2 suppl), 16S-46S. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24861367

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

Rat

“In Wistar rats dose intraperitoneally at 400 mg/kg bw triethyl citrate produced a loss of the righting reflex, an effect reversible within 15 minutes.

Rabbit

Intravenous administration of a 100 mg/kg bw dose of triethyl citrate to rabbits produced a marked increase in motor activity and respiration (Meyer et al., 1964).”

As taken from INCHEM, 1979, WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm

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

Triethyl citrate blocked nerve conduction in the rat and produced cord depression , temporarily abolished the corneal reflex in the rabbit eye, exhibited local anaesthetic activity in the guinea pig, and decreased blood pressure in rabbits and cats, causing smooth muscle depression or cardiac depression, Meyers et al 1964.

“The corneal reflex in rabbit eyes was temporarily eliminated upon instillation of 3 drops of a 5% suspension of triethyl or tributyl citrate in 3% acacia; the number of animals used was not stated. The anesthetic effect was confirmed by the intradermal administration of 0.1 ml of a 2% solution of triethyl or tributyl citrate into an area of the shaved back of guinea pigs; again, the number of animals used was not stated. Triethyl citrate resulted in insensitivity to pricking of the area lasting 12-20 min” (CIR, 2012).

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 triethyl citrate 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 triethyl citrate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	819	Baker et al., 2004a
	6.5 780	JTI KB Study Report(s)
	1440	Roemer et al., 2014
In vitro genotoxicity	819	Baker et al., 2004c
	6.5	Renne et al., 2006
	6.5 600	JTI KB Study Report(s)
	1440	Roemer et al., 2014
In vitro cytotoxicity	819	Baker et al., 2004c
	6.5 600	JTI KB Study Report(s)
	1440	Roemer et al., 2014
Inhalation study	30	Gaworski et al., 1998
	819	Baker et al., 2004c
	6.5	Renne et al., 2006
	6.5 600	JTI KB Study Report(s)
	1440	Schramke et al., 2014
Skin painting	6.5	JTI KB Study Report(s)
In vivo genotoxicity	1440	Schramke et al., 2014

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following data:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]: Bond Method :	6.39E-010 atm-m3/mole (6.48E-005 Pa-m3/mole)
Group Method:	Incomplete
Exper Database:	3.84E-09 atm-m3/mole (3.89E-004 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.549E-009 atm-m3/mole (2.583E-004 Pa-m3/mole) VP: .000198 mm Hg (source: MPBPVP) WS: 2.82E+004 mg/L (source: WSKOWWIN)]

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]: Log Kow used:	0.33 (KowWin est)
Log Kaw used:	-6.804 (exp database)
Log Koa (KOAWIN v1.10 estimate):	7.134
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):	0.9546 0.9999 2.7971 (weeks) 3.9826 (days) 1.2373 0.9889 0.9086
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01): Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]: Vapor pressure (liquid/subcooled):	0.0916 Pa (0.000687 mm Hg))
Log Koa (Koawin est):	7.134
Kp (particle/gas partition coef. (m3/ug)):Mackay model: Octanol/air (Koa) model:	3.28E-0053.34E-006
Fraction sorbed to airborne particulates (phi): Junge-Pankow model:	0.00118
Mackay model:	0.00261
Octanol/air (Koa) model:	0.000267
Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction: OVERALL OH Rate Constant =	7.3255 E-12 cm3/molecule-sec
Half-Life =	1.460 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	17.521 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.0019 (Junge-Pankow, Ma avg) 0.000267 (Koa method)Note: the sorbed fraction may be resista atmospheric oxidation	
Soil Adsorption Coefficient (KOCWIN v2.00): Koc :	21.02 L/kg (MCI method)
Log Koc:	1.323 (MCI method)
Koc :	3.673 L/kg (Kow method)
Log Koc:	0.565 (Kow method)
Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Total Kb for pH > 8 at 25 deg C:	1.349E-002 L/mol-sec
Kb Half-Life at pH 8:	1.628 years

Kb Half-Life at pH 7:	16.279 years

(Total Kb applies only to esters, carbmates, alkyl halides)

Volatilization from Water: Henry LC: 3.84E-009 atm-m3/mole (Henry experimental database) Half-Life from Model River:	2.534E+005 hours (1.056E+004 days)
Half-Life from Model Lake:	2.765E+006 hours (1.152E+005 days)

Removal In Wastewater Treatment:

Total removal:	1.86 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.76 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount(percent)	Half-Life(hr)	Emissions(kg/hr)
Air	0.0756	35	1000
Water	26.6	360	1000
Soil	73.3	720	1000
Sediment	0.0721	3.24e+003	0

Persistence Time: 664 hr

The Ecological Categorization Results from the Canadian Domestic Substances List state that 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0) is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.9889
TOPKAT probability of biodegradation	0.996
EPI Predicted hydrolysis half-life (days)	5.95E+003
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	1.46

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

10.2. Aquatic toxicity

According to the Ecological Categorization List from the Canadian Domestic Substances List, 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	27.068988
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	327.2
Toxicity to fish (LC50 in mg/l) as predicted by Aster	27.068988
Toxicity to fish (LC50 in mg/l) as predicted by PNN	84.67954
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	7,262.123
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in cosar v0.99g	2.54E+003

Data accessed August 2017on the OECDwebsite: http://webnet.oecd.org/CCRWeb/Search.aspx

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 77-93-0:

Values used to Generate ECOSAR Profile:

Log Kow: 0.334 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 6.5E+004 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations
Esters

ECOSAR Class		Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters	:	Fish	96-hr	LC50	347.109
Esters	:	Daphnid	48-hr	LC50	862.461
Esters	:	Green Algae	96-hr	EC50	477.933
Esters	:	Fish		ChV	37.044
Esters	:	Daphnid		ChV	952.216
Esters	:	Green Algae		ChV	71.326
Esters	:	Fish (SW)	96-hr	LC50	584.769
Esters	:	Mysid	96-hr	LC50	1307.163
Esters	:	Fish (SW)		ChV	62.101
Esters	:	Mysid (SW)		ChV	2.87e+006 *

Neutral Organic SAR :	Fish	96-hr	LC50	7116.854
(Baseline Toxicity) :	Daphnid	48-hr	LC50	3464.793
	Green Algae	96-hr	EC50	1366.275
	Fish		ChV	580.205
	Daphnid		ChV	220.212
	Green Algae		ChV	254.013

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

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 77-93-0:

Values used to Generate ECOSAR Profile:

Log Kow: 0.334 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 6.5E+004 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations
Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters	Earthwor	14-day	LC50	12408.355

:	m			

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01): Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-3.8667 days (HL = 0.0001359 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.030 (BCF = 0.9342)
Log BAF Arnot-Gobas method (upper trophic):	-0.030 (BCF = 0.9342)
log Kow used:	0.33 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List state that 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0) is not bioaccumulative in the environment:

Log Kow predicted by KowWin	0.33
Log BAF T2MTL predicted by Gobas	0.0301788598698057
Log BCF 5% T2LTL predicted by Gobas	0.0244981718843084
Log BCF Max predicted by OASIS	1.10626438336973
Log BCF predicted by BCFWIN	0.5

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

11. References for conventional products

- Anon [undated]. Ingredients added to tobacco in the manufacture of cigarettes by the six major American cigarette companies. 599 ingredient list published 1994. Available at http://www.free-cigarettes.com/cigarette-additives.html
- Baker 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.
- BIBRA (1998). Toxicity Profile: Triethyl citrate. BIBRA International Ltd.
- Bruns F H et al (1962). Zum Stoffwechsel van Triathylcitrat und Acetyltriathylcitrat. Klin. Wschr. 40,1169.
- Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.
- ChemIDplus. Accessed September 2018. Available at https://chem.nlm.nih.gov/chemidplus/
- ChemSpider. Record for triethyl citrate (CAS RN 77-93-0). Undated, accessed September 2018. Available at http://www.chemspider.com/Chemical-Structure.13850879.html
- CIR (2012). Cosmetic Ingredient Review. Final report on the safety assessment of citric acid, inorganic citrate salts, and alkyl citrate esters as used in cosmetics. Available at http://www.cir-safety.org/sites/default/files/citric032012FR.pdf
- CosIng (Cosmetic substances and ingredients database). Record for triethyl citrate. Undated, accessed September 2018. Available at http://ec.europa.eu/growth/tools-databases/cosing/
- COSMOS Database. Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity

of COSMetics to Optimise Safety. Database version 1.0. Record for triethyl citrate (CAS RN 77-93-0). Accessed September 2018. Available at http://www.cosmostox.eu/what/COSMOSdb/

- 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 (2018a). European Chemicals Agency. Information on Chemicals. Record for triethyl citrate. Last updated 28 September 2018. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances
- ECHA (2018b). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 28 September 2018. Available at https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR (undated). Record for 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0). Accessed August 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. EFSA Journal 10(3):2563. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2563/epdf
- EFSA (2013). EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes (chemical group 1) when used as flavourings for all animal species EFSA Journal 11(4), 3169. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3169/epdf
- Eilers H and Alexeyev OA (2016). Effect of GT-peptide and triethyl citrate on P. Acnes biofilm formation, viability and dispersion. J. Drugs Dermatol. 15(6), 778-81. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27272091
- EPISuite (undated). Record for 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0). Accessed August 2017. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EPISuite (2017). Record for 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0). 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
- Epstein W L (1975). Report to RIFM, 15 August.
- EUROPEAN COMMISSION, SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE), Brussels, 28/9/1999, http://ec.europa.eu/health/ph_risk/committees/sct/documents/out45_en.pdf
- European Commission (2012). Database of food flavourings. Last modified 17 September 2012. Accessed September 2018. Available at https://webgate.ec.europa.eu/foods_system/
- European Commission (2015). Database of food additives. Last modified 25 February 2015. Accessed September 2018. Available at https://webgate.ec.europa.eu/foods_system/
- Fassett D W (undated). Unpublished data cited in Patty, 1963 (cited in BIBRA, 1998).
- FDA (2018a). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 5 September 2018. Accessed September 2018. Available at https://www.accessdata.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2018b). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Current as of 27 September 2018. Accessed September 2018. Available at https://www.ecfr.gov/cgi-bin/ECFR?page=browse

- Finkelstein M et al (1959). Toxicology of the citric acid esters: … triethyl citrate … Toxic. appl. Pharmac. 1, 283.
- Fiume MM et al (2014). Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Citrate Esters as Used in Cosmetics. Int. J. Toxicol. 33(2 suppl), 16S-46S. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24861367
- 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.
- 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.%20GRAS%20Substances(2001-3124)_0.pdf
- Haz-Map (2017). Record for triethyl citrate (CAS RN 77-93-0). Last updated October 2017. Accessed September 2018. Available at https://hazmap.nlm.nih.gov/
- HSDB (2015). Record for triethyl citrate. Hazardous Substances Databank Number: 729. Last updated 23 December 2015. Accessed September 2018. Available at https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm
- IFRA (2016). International Fragrance Association. IFRA Volume of Use Survey 2016: Transparency List. Accessed September 2018 Available at http://www.ifraorg.org/en/ingredients#
- INCHEM (1979). WHO FOOD ADDITIVES SERIES: 14; TRIETHYL CITRATE; available at http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm
- INCHEM (1984). WHO FOOD ADDITIVES SERIES: 19; available at http://www.inchem.org/documents/jecfa/jecmono/v19je12.htm
- INCHEM (2000). WHO FOOD ADDITIVES SERIES: 44; available at http://www.inchem.org/documents/jecfa/jecmono/v44jec10.htm
- INCHEM (2004). Record for triethyl citrate. 6 February 2004. Available at http://www.inchem.org/documents/jecfa/jeceval/jec_2316.htm
- IPCS (1999). Triethyl citrate. International Chemical Safety Card 1350. Peer reviewed 18 October 1999. Accessed September 2018. Available at http://www.inchem.org/documents/icsc/icsc/eics1350.htm
- JECFA (1979). 23rd Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series No. 14. http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm.
- JECFA (1999). 53rd Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series No. 44. http://www.inchem.org/documents/jecfa/jecmono/v44jec10.htm.
- JTI KB Study Report (s).
- JTI Study Report (s).
- LaWall and Harrison (1954). Consultants, Philadelphia. Unpublished report prepared for Fleischmann Laboratories (cited in JECFA, 1979).
- Levenstein I (1975). Report to RIFM, 30 May.
- Litton Bionetics Inc. (1976). FDA-75-10, LBI Project No. 2468. Unpublished data submitted to the FDA (cited in JECFA, 1979).
- Merck (2013). The Merck Index. An encyclopaedia of chemicals, drugs and biologicals. Fifteenth edition. O'Neil MJ et al. ed. Royal Society of Chemistry. ISBN 978-1-84073-670-1
- Meyers D B et al (1964). Toxicity of plastics used in medical practice. II. J. pharm. Sci. 53, 774.
- NIOSH. National Institute for Occupational Safety and Health. National Occupational Exposure Survey (1981-1983). Record for citric acid, triethyl ester (CAS RN 77-93-0). Accessed September 2018. Available at https://web.archive.org/web/20111024124426/http://www.cdc.gov/noes/noes2/x7556occ.html
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Record for 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0). Date added to inventory: 1 December 2006. Accessed September 2018. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/14982

- OECD (undated). Organisation for Economic Co-operation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS RN 77-93-0). Accessed August 2017. Available via http://webnet.oecd.org/CCRWeb/Search.aspx
- PubChem (2018). Record for triethyl citrate (CAS RN 77-93-0). Created 26 March 2005. Last modified 29 September 2018. Available at https://pubchem.ncbi.nlm.nih.gov/compound/6506
- Rainey CL et al. (2011). Chemical characterization of dissolvable tobacco products promoted to reduce harm. Journal of Agricultural and Food Chemistry 59, 2745-2751. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/21332188?dopt=AbstractPlus
- Registry of cytotoxicity (RC) data (ZEBET). Accessed September 2018. Available at https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/finalrpt/fins7all.pdf
- Reineccius et al. The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/flavor molecular inclusion complexes.; J Agric Food Chem. 2005, Jan 26; 53(2):388-92.
- 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.
- 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.
- Rosenbluth S A et al (1967). Growth changes in mammalian cell cultures by plastic additives (effect of triethyl citrate). J. biomed. Mater. Res. 1, 197.
- RTECS (1997). Registry of Toxic Effects of Chemical Substances. Record for citric acid, triethyl ester (CAS RN 77-93-0). Last updated January 1997. Accessed September 2018.
- 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.
- US Department of Health and Human Services (2018). Household Products Database. Last updated June 2018. Accessed September 2018. Available at https://hpd.nlm.nih.gov/index.htm
- US EPA (2018a). US Environmental Protection Agency. Electronic Code of Federal Regulations (eCFR). Current as of 27 September 2018. Accessed September 2018. Available at http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- US EPA (2018b). Safer Chemical Ingredients List. Last updated 27 September 2018. Accessed September 2018. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA 2012 CDR list (Chemical Data Reporting Rule). Accessed September 2018. Available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA Inert Finder Database (2018). Last updated 27 August 2018. Accessed September 2018. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA TSCA inventory. Accessed September 2018. Available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- WHO (2018). Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Record for triethyl citrate. World Health Organization. Available at http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3286
- Williams et al. (1997). In vitro method to investigate food effects on drug release from film coated beads; Pharm. Dev. Technol.; VOL 2 ISS 1 1997, P1-9.

12. Other information

Jin Y et al. (2014). Application of Triethyl Citrate to Filters in Virginia Type Cigarettes. Beiträge zur Tabakforschung 26(4), 176-182. Available at http://www.degruyter.com/view/j/cttr.2015.26.issue-4/cttr-2015-0005/cttr-2015-0005.xml?rskey=Axfq06&result=1

13. Last audited

October 2018

Substance	*ID Code*	*Rpt No.*	*Year*	*Conclusion**	*21 CFR Section*
Triethyl citrate	77-93-0	84	1977	1	184.1911

SCOGS Opinion:

The citrate ion is widely distributed in plants and animals and is a naturally occurring component of the diet. It is a common metabolite in oxidative metabolism and an important component of bone. Exogenous citrate administered to infants and adults as a component of commonly consumed diets is considered completely metabolizable. The addition of citric acid to foods is considered equivalent to adding citrate salts except in foods of very high acidity. The amount of citrate added to foods by foods processors is about 500mg per person per day. This amount occurs naturally in 2 ounces of orange juice and does not constitute a significant addition to the total body load. Although data on acute and chronic effects of orally administered sodium citrate, calcium citrate and potassium citrate are limited, no biological effects of the citrate-containing substances evaluated in this report cause concern about the safety of these GRAS substances used in reasonable amounts and in accordance with prescribed tolerances and limitations.

In light of the foregoing, the Select Committee concludes that: There is no evidence in the available information on citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when used at levels that are now current or that might reasonably be expected in the future.

**** denotes Type of Conclusion 1, 2, 3, 4, or 5. Definitions of conclusion types can be found at the end of this report..***

TRIETHYL CITRATE

Explanation

The citrate ion is widely distributed in plant and animal tissues and is a naturally occurring component of man's diet. It is a common metabolic intermediate in oxidative metabolism. Citrate was evaluated by the ninth session of the JECFA and was given an ADI not limited.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Triethyl citrate is an odourless, nearly colourless, oily liquid. No absorption or metabolism studies have been reported, however, it is expected that the compound would rapidly metabolize in the body and liberate the citrate ion which would be handled through the usual biochemical pathways (FASEB, 1976).

TOXICOLOGICAL STUDIES

Acute toxicity

Species	Route	LD_{50} (mg/kg bw)	Reference
Rat	p.o.	8 000	Finkelstein & Gold, 1955
Cat	p.o.	4 000	Finkelstein & Gold, 1955

Short-term studies

Mouse

A group of 20 mice given intraperitoneal doses of 350 mg/kg bw of triethyl citrate daily for 14 days had a slightly lower mean growth rate than control animals. No differences were seen in the two groups in erythrocyte and leucocyte blood cell count, clotting time and haemoglobin levels. Examination of the liver, lung and kidney tissues of two animals at necropsy revealed no pathological cellular changes.

Rat

Young rats were fed triethyl citrate at an initial rate of 1, 2 and 4 g/kg bw for eight weeks (Finkelstein & Gold, 1955). Urinalysis, blood counts and growth measurement, performed periodically, revealed no toxic effects. At necropsy, no gross abnormalities were seen in the thoracic or abdominal organs. Histological sections of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen and kidneys were comparable in appearance to those from the untreated controls.

Cat

Cats receiving daily doses of 7% of the LD_{50} (280 mg/kg bw) for eight weeks did not differ from control animals with respect to weight, blood count, haemoglobin, blood sugar and blood nitrogen. However, weakness, ataxia and depression appeared after the fourth or fifth dose and progressed. After treatment was discontinued, the animals recovered within 24-96 days (Finkelstein & Gold, 1959).

Dog

Two young adult male and two young adult female beagle dogs were given daily doses of triethyl citrate of 0.05 and 0.25 ml/kg bw for six months. Measurement of body and organ weights, blood and urinalysis and the results of histological examination of tissues revealed no adverse effects (Hodge, 1954). Increasing the daily dose to 2.5 to 3.5 ml/kg bw for seven to 12 weeks resulted in liver pathology in three treated animals. A fourth dog that had previously reacted adversely to a 2 ml/kg bw dose showed no histological changes after receiving 1.5 ml/kg bw daily for an additional month.

Long-term studies

Rat

Three groups of 15 male and 15 female weanling Sprague-Dawley rats were fed diets containing 0.33, 1.0 and 3.0% triethyl citrate in a two-year feeding study (LaWall & Harrison, 1954). The initial doses were from 0.2 to 2.0 g/kg bw. Weight gain and food intake were reduced below that of the control groups when the level of the compound in the diet was increased. (No specific numbers were given for these results.) No adverse effects of haematologic, urinalysis, survival, gross or histopathologic parameters could be attributed to triethyl citrate.

Special studies on reproduction and teratology

At doses ranging from 0.5 to 10 mg/kg b.w. triethyl citrate was nonteratogenic in the chicken embryo. When injected into the air cell, the LD_{50} was 1349.86 mg/kg bw (67.49 mg/egg) (Verrett, 1976).

Special studies on mutagenesis

Triethyl citrate was not mutagenic in plate and suspension tests using the Ames Salmonella microsome mutagenesis assay in strains TA 1535, TA 1537 and TA 1538 and the Saccharomyces cerevesiae D4 yeast assay with and without tissue homogenate activating systems (Litton Bionetics, Inc., 1976).

Special studies on neurological activity

Rat

In Wistar rats dose intraperitoneally at 400 mg/kg bw triethyl citrate produced a loss of the righting reflex, an effect reversible within 15 minutes.

Rabbit

Intravenous administration of a 100 mg/kg bw dose of triethyl citrate to rabbits produced a marked increase in motor activity and respiration (Meyer et al., 1964).

Comments

Citrate was evaluated by the ninth session of JEFCA (1966)[1] and ADI not limited was given. It is likely that triethyl citrate will be hydrolyzed to its component parts, citrate and ethanol in vivo. Data from two-year feeding studies suggest that rats can tolerate up to 2.0 g/kg. Dogs tolerated up to 0.25 ml/kg bw for six months without effects.

Triethyl citrate was not mutagenic in several microbiological assays.

EVALUATION

Level causing no toxicological effect

Rat: 2 g/kg bw

Estimate of temporary acceptable daily intake for man

0-10 mg/kg bw

FURTHER WORK OR INFORMATION

Required by 1981.

Repeat metabolic studies in several species, preferably including man.

---

[1] Changed to 1973 on draft which was seventeenth session.

REFERENCES

FASEB (1976) SCOGS, 84, Contract No. FDA 223-75-2006, submitted to FDA, Washington, D.C.

Finkelstein, M. & Gold, H. (1959) Tox. Appl. Pharmacol., 1, 283

Hodge, H. C. (1954) Unpublished data submitted to FASEB

LaWall, (?) & Harrison, (?) (1954) Unpublished, prepared for Fleishmann Laboratories, Standard Brands, Inc., Stamford, Conn., USA

Litton Bionetics, Inc. (1976) FDA-75-10 LBI Project No. 2468, unpublished data submitted to the FDA

Meyer, D., Aulian, J. & Guess, W. L. (1964) J. Pharm. Sci., 53, 776

Smith, H. et al. (1976) Health Physics, 30, 318

Verret, M. J. (1976) Unpublished data, Food and Drug Administration

See Also:

- Toxicological Abbreviations
- Triethyl citrate (ICSC)
- Triethyl citrate (WHO Food Additives Series 19)
- TRIETHYL CITRATE (JECFA Evaluation)

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

WHO FOOD ADDITIVES SERIES: 44

Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

World Health Organization, Geneva, 2000
IPCS - International Programme on Chemical Safety

ALIPHATIC PRIMARY ALCOHOLS, ALDEHYDES, CARBOXYLIC ACIDS, ACETALS, AND ESTERS CONTAINING ADDITIONAL OXYGENATED FUNCTIONAL GROUPS

First draft prepared by Dr P.J. Abbott

Australia New Zealand Food Authority, Canberra, Australia

1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 47 flavouring agents that includes aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups (see Table 1) using the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, p. 122).

The Committee previously evaluated eight members of this group for other functional uses. Fumaric acid (No. 618) was first considered by the Committee at its tenth meeting (Annex 1, reference 13), and at its thirty-fifth meeting (Annex 1, reference 88) the Committee established a group ADI of 'not specified'[1] for fumaric acid and its salts. Triethyl citrate (No. 629) was first considered by the Committee at its twenty-third meeting (Annex 1, reference 50), and at

its twenty-eighth meeting (Annex 1, reference 66) the Committee established an ADI of 0–20 mg/kg bw. Diethyl tartrate (No. 622) was first considered by the Committee at its twenty-third meeting (Annex 1, reference 50), but an evaluation was not possible on the basis of the data available at that time. As no additional data were available to the Committee at its twenty-fifth meeting (Annex 1, reference 56), no ADI was allocated. The Committee also evaluated related terpenoid flavouring agents, including linalool, linalyl acetate, citronellol, citral, and geranyl acetate, and established a group ADI of 0–0.5 mg/kg bw at its twenty-third meeting (Annex 1, reference 50).

1.2 Estimated daily per capita intake

The estimated *per capita* intake of these agents, modified to calculate intake of flavouring agents (see p. 121), was derived from surveys in Europe and the United States. The total annual production of the 47 substances in this group is 200 tonnes in Europe and 1700 tonnes in the United States, which is equivalent to a total estimated daily *per capita* intake of 28 mg in Europe and 300 mg in the United States.

Fumaric acid (No. 618) and (–)-malic acid (No. 619) account for approximately 59% of the total daily *per capita* intake of these 47 substances in Europe and 88% in the United States. The estimated total daily consumption of fumaric acid resulting from its use as a flavouring agent is approximately 0.9 mg/person in Europe and 219 mg/person in the United States. The total daily consumption of (–)-malic acid is estimated to be 16 mg/person in Europe and 58 mg/person in the United States.

Of the 47 substances evaluated, 25 have been detected as natural components of traditional foods (Maarse et al., 1994).

1.3 Metabolism

Studies on the absorption, metabolism, and elimination of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters with additional oxygenated functional groups show that these substances are readily hydroly-sed and absorbed and are completely metabolized. Many of these substances or their metabolites are endogenous in humans.

---

[1] ADI 'not specified' is a term applicable to a food component of very low toxicity which, on the basis of the available chemical, biological, toxicological, and other data, the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason and for those stated in the evaluation, the establishment of an ADI expressed in numerical form is deemed unnecessary.

Many of the substances in this group are esters or diesters and are expected to undergo hydrolysis to their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols or branched-chain hydroxy or keto alcohols). The presence of a second oxygenated functional group has little if any effect on the hydrolysis of these esters. ß-Keto acids and derivatives such as acetoacetic acid easily undergo decarboxylation and, with alpha-keto and alpha-hydroxyacids, yield breakdown products which are incorporated into normal biochemical pathways. The gamma-keto acids and related substances may undergo complete or partial ß-oxidation to yield metabolites, which are eliminated in the urine. The omega-substituted derivatives are readily oxidized and/or excreted in the urine. The simple aliphatic di-and tricarboxylic acids either occur endogenously in humans or are structurally related to endogenous substances. These substances are metabolized through the fatty acid ß-oxidation pathway or the tricarboxylic acid cycle.

1.4 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, p. 122) to the above-mentioned aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups, the Committee assigned all 47 substances to structural class I (Cramer et al., 1978).

Step 2. Metabolic data on individual members of the group are limited, but the common structural features and common pathways of metabolism allow some general conclusions to be drawn on the likely metabolic fate of these agents. Fourteen substances are found normally in human metabolism, and 28 substances in the group are esters or diesters that would be expected to be metabolized to innocuous products. There was evidence that the other substances in the group, including acetals, derivatives of beta-keto and beta-hydroxy acids, gamma-keto and gamma-hydroxy acids, and aliphatic di-and tricarboxylic acids, are also metabolized to innocuous products. For all substances in this group, therefore, the evaluation should proceed via the left-hand side of the

decision-tree.

Table 1. Summary of results of the safety evaluation of 47 aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups

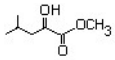
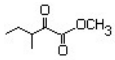
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
2-Oxobutyric acid 	589	600-18-0	No	N/R	N/R	No safety concern
Methyl 2-hydroxy-4-methylpentanoate 	590	40348-72-9	No	N/R	N/R	No safety concern
Methyl 2-oxo-3-methyl-pentanoate 	591	3682-42-6	No	N/R	N/R	No safety concern

Table 1. (continued)

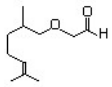
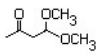
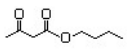
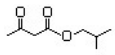
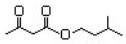
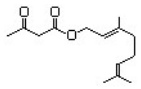
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Citronelloxyacetaldehyde 	592	7492-67-3	No	N/R	N/R	No safety concern
3-Oxobutanal dimethyl acetal 	593	5436-21-5	No	N/R	N/R	No safety concern
Ethyl 3-hydroxybutyrate 	594	5405-41-4	No	N/R	N/R	No safety concern
Ethyl acetoacetate 	595	141-97-9	Yes	Yes[b]	N/R	No safety concern

Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Butyl acetoacetate 	596	591-60-6	No	N/R	N/R	No safety concern
Isobutyl acetoacetate 	597	7779-75-1	No	N/R	N/R	No safety concern
Isoamyl acetoacetate	598	2308-18-1	No	N/R	N/R	No safety concern



Geranyl acetoacetate	599	10032-00-5	No	N/R	N/R	No safety concern



Methyl 3-hydroxyhexanoate	600	21188-58-9	No	N/R	N/R	No safety concern

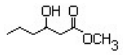
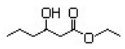
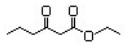


Table 1. (continued)

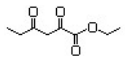
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Ethyl 3-hydroxyhexanoate	601	2305-25-1	No	N/R	N/R	No safety concern



Ethyl 3-oxohexanoate	602	3249-68-1	No	N/R	N/R	No safety concern



Ethyl 2,4-dioxohexanoate	603	13246-52-1	No	N/R	N/R	No safety concern



3-(Hydroxymethyl)-2-heptanone	604	65405-68-7	No	NR	N/R	No safety concern

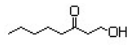
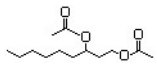


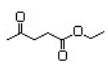
Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
1,3-Nonanediol acetate (mixed esters)	605	1322-17-4	No	N/R	N/R	No safety concern



Laevulinic acid	606	123-76-2	No	N/R	N/R	No safety concern

Ethyl laevulinate	607	539-88-8	No	N/R	N/R	No safety concern



Butyl laevulinate	608	2052-15-5	No	N/R	N/R	No safety concern

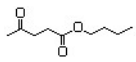


Table 1. (continued)

Substance	JECFA	CAS No.	Step A3[a]	Step A4	Step A5	Conclusion based

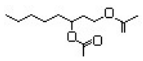
and structure	No.		Does intake exceed the threshold for human intake?	Is the substance or are its metabolites endogenous?	Adequate NOEL for substance or related substance?	on current intake
1,4-Nonanediol diacetate 	609	67715-81-5	No	N/R	N/R	No safety concern
Hydroxycitronellol 	610	107-74-4	No	N/R	N/R	No safety concern
Hydroxycitronellal 	611	107-75-5	No	N/R	N/R	No safety concern
Hydroxycitronellal dimethyl acetal 	612	141-92-4	No	N/R	N/R	No safety concern

Table 1. (continued)

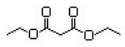
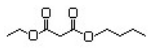
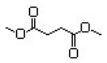
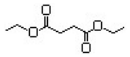
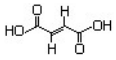
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Hydroxycitronellal diethyl acetal 	613	7779-94-4	No	N/R	N/R	No safety concern
Diethyl malonate 	614	105-53-3	No	N/R	N/R	No safety concern
Butyl ethyl malonate 	615	17373-84-1	No	N/R	N/R	No safety concern
Dimethyl succinate 	616	106-65-0	No	N/R	N/R	No safety concern

Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Diethyl succinate 	617	123-25-1	No	N/R	N/R	No safety concern
Fumaric acid[c] 	618	110-17-8	Yes	Yes[d]	N/R	No safety concern

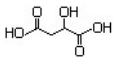
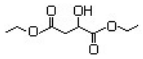
(-)-Malic acid 	619	97-67-6	Yes	Yes[d]	N/R	No safety concern
Diethyl malate 	620	7554-12-3	No	N/R	N/R	No safety concern

Table 1. (continued)

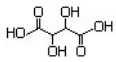
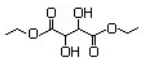
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Tartaric acid (+-, --, ±-, meso-) 	621	87-69-4	Yes	No	Yes. NOEL was 1200 mg/kg bw per day in a two-year study in rats	No safety concern
Diethyl tartrate 	622	87-91-2	No	N/R	N/R	No safety concern

Table 1. (continued)

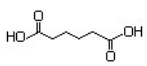
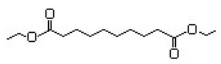
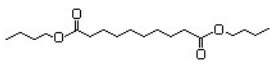
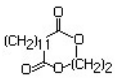
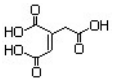
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Adipic acid 	623	124-04-9	Yes	No	Yes. The NOEL for the structurally related compound, dibutyl sebacate, was 6200 mg/kg bw per day in a two-year study in rats	
Diethyl sebacate 	624	110-40-7	No	N/R	N/R	No safety concern
Dibutyl sebacate 	625	109-43-3	No	N/R	N/R	No safety concern

Table 1. (continued)

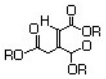
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Ethylene brassylate	626	105-95-3	No	N/R	N/R	No safety concern



Aconitic acid	627	499-12-7	No	N/R	N/R	No safety concern



Ethyl aconitate (mixed esters)	628	-	No	N/R	N/R	No safety concern



Triethyl citrate[c]	629	77-93-0	Yes	Yes[d]	N/R	No safety concern

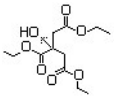
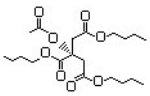
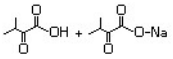


Table 1. (continued)

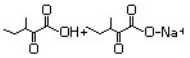
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Tributyl acetylcitrate	630	77-90-7	No	N/R	N/R	No safety concern



3-Methyl-2-oxobutanoic acid and sodium salt	631	759-05-7 3715-29-6	No	N/R	N/R	No safety concern



3-Methyl-2-oxopentanoic acid and sodium salt	632	1460-34-0 3715-31-9	No	N/R	N/R	No safety concern



4-Methyl-2-oxopentanoic acid and sodium salt	633	816-66-0 4502-00-5	No	N/R	N/R	No safety concern

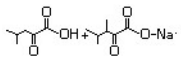
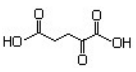
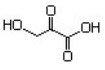


Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
2-Oxopentandioic acid	634	328-50-7	No	N/R	N/R	No safety concern



3-Hydroxy-2-oxopropionic acid	635	1113-60-6	No	N/R	N/R	No safety concern



All of the substances in the group are in structural class I, the human intake threshold of which is 1800 µg per person per day, and all of the substances in the group are metabolized to innocuous products.

a The threshold for human inatke of substances in class I is 1800 µg per day.
b Ethyl acetoacetate is expected to be hydrolysed to acetoacetic acid, which is endogenous in humans.
c The ADI for this substance was maintained.
d Fumaric acid, (-)-malic acid, and triethyl citrate are components of the tricarboxylic acid cycle.

Step A3. The estimated daily *per capita* intakes in Europe and the United States of 41 of the substances in this group are below the threshold of concern for substances in class I (1800 µg), indicating that they would not raise concern for safety. The intakes of six substances, namely, ethyl acetoacetate (No. 595; 1900 µg/person per day in Europe and 3900 µg/person per day in the United States), fumaric acid (No. 618; 220 000 µg/person per day in the United States); (-)-malic acid (No. 619; 16 000 µg/person per day in Europe and 58 000 µg/person per day in the United States), tartaric acid (No. 621; 4400 µg/person per day in Europe and 14 000 µg/person per day in the United States), adipic acid (No. 623; 18 000 µg/person per day in the United States), and triethyl citrate (No. 629; 3400 µg/person per day in Europe and 2400 µg/person per day in the United States), are greater than the threshold for human intake for class I (1800 µg). The evaluation of the safety of these six substances therefore proceeds to step A4.

Step A4. Four of the six substances for which the intake exceeds the threshold of concern for class I are endogenous in humans. Three of these four substances, namely, fumaric acid (No. 618), (-)-malic acid (No. 619), and triethyl citrate (No. 629), are components of the tricarboxylic acid cycle. The fourth substance, ethyl acetoacetate (No. 595), is expected to be hydrolysed to acetoacetic acid, which is endogenous in humans and is formed from the condensation of two acetyl coenzyme A units in the fatty acid pathway. For tartaric acid and adipic acid, the evaluation should proceed to step A5.

Step A5. The NOEL for tartaric acid in a two-year study of toxicity in rats was 1200 mg/kg bw per day, the highest dose tested, which provides adequate margins of safety (> 10 000 and > 1000) for the known levels of intake (74 and 230 µg/kg bw per day in Europe and the United States, respectively). No NOEL was available for adipic acid, but the NOEL for the structurally related material, dibutyl sebacate, in a two-year study in rats was 6200 mg/kg bw per day, which provides adequate margins of safety (> 100 000 000 and > 10 000 times) for the known levels of intake of adipic acid (0.2 and 300 µg/kg bw per day in Europe and the United States, respectively). These substances would not therefore be expected to raise concern.

Table 1 summarizes the stepwise evaluation of the 47 aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups used as flavouring agents.

1.5 Consideration of combined intake

All of the 47 aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups that were evaluated would be efficiently metabolized by common biochemical pathways to innocuous substances.

In the unlikely event that foods containing all 47 substances were consumed simultaneously on a daily basis, the total estimated daily per capita intake of these substances in Europe and the United States would exceed the threshold for human intake of substances in class I. The Committee considered that such intake would not give rise to perturbations outside the physiological range.

1.6 Conclusions

The Committee concluded that the safety of flavouring agents in this group would not raise concern when they were used at he current levels of estimated intake.

No data on toxicity were available for application of the Procedure to 45 of the 47 substances in this group. For the remaining two substances, tartaric acid (No. 621) and adipic acid (No. 623), the data on toxicity were consistent with the results of the safety evaluation made with the Procedure.

The ADIs for fumaric acid and its salts and for triethyl citrate were maintained at the present meeting.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

Forty-seven aliphatic primary alcohols, aldehydes, carboxylic

acids, acetals, and esters containing additional oxygenated functional groups are included in this group of flavouring agents (see Table 1). The substances were selected on the basis of the criteria that all members of the group are simple aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters and contain additional oxygenated functional groups. Eight substances in this group (Nos 589, 591, 603, 631-635) are alpha-keto acids, esters, or related substances; five substances (Nos 590, 619-622) are alpha-hydroxy acids, esters, or related substances; 12 substances (Nos 593-602, 614, 615) are beta-keto or beta-hydroxy alcohols, aldehydes, carboxylic acids, and related acetals and esters; five substances (Nos 605-609) are gamma-keto acids, esters, or related substances; four substances (Nos 610-613) are omega-substituted alcohols, aldehydes, or acetals; and 22 substances (Nos 614-631) are simple, aliphatic di-and tricarboxylic acids or their esters.

2.2 Additonal considerations on intake

The total annual production of each of the 47 substances in this group is shown in Table 2.

2.3 Biological data

2.3.1 Absorption, metabolism, and elimination

2.3.1.1 Ester and diesters

Twenty-eight substances in this group (Nos 590, 591, 594-603, 605, 607-609, 614-617, 620, 622, 624-626, and 628-630) are esters or diesters, including one cyclic diester, which are expected to undergo hydrolysis to their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols or branched-chain hydroxy or keto alcohols) and acid components (alpha, beta-, or gamma-keto or hydroxy acids or simple aliphatic acids, diacids, or triacids), which would be further metabolized. Hydrolysis occurs in the intestinal tract, blood, and liver and in most tissues and is catalysed by carboxylesterases or esterases, the most important of which are the B-esterases (Anders, 1989; Heymann, 1980). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters.

Evidence for hydrolysis of these esters has come from various experiments. Incubation of aqueous methyl 2-oxo-3-methylpentanoate (No. 591) with a 2% pancreatin solution (pH 7.5) resulted in virtually complete hydrolysis (> 98%) within 80 min (Leegwater & Van Straten, 1979). Dibutyl sebacate (No. 625) in 10% acacia solution was also hydrolysed *in vitro* in a 10% crude pancreatic lipase solution (Smith, 1953). ^{14}C-Tributylacetyl citrate (No. 630) administered to male Sprague-Dawley rats by gavage at a dose of 70 mg/kg bw was rapidly absorbed (half-life, 1 h) and partially hydrolysed. More than 87% of the radiolabel was eliminated within 24 h of dosing. At least nine urinary metabolites representing 59-70% of the dose were detected. Five were identified as the partially hydrolysed mono-, di-, and trialkylesters of citric acid. Three metabolites representing 25-26% of the dose were identified in the faeces. Approximately 2% was eliminated as $^{14}CO_2$ (Hiser et al., 1992). Hydrolysis of the cyclic diester ethylene brassylate (No. 626) would be expected to occur on the basis of the hydrolysis of structurally related lactones like omega-6-hexadecenlactone. In simulated intestinal fluid, omega-6-hexadecenlactone underwent nearly complete hydrolysis (92%) to its open-chain form within 15 min (Morgareidge, 1962a).

The alcohol, aldehyde, and acid components of these esters, diesters, and cyclic diester are completely metabolized. At higher concentrations, they may be conjugated with glucuronic acid and excreted.

2.3.1.2 alpha-Keto-and alpha-hydroxy acids and their esters

alpha-Keto-and alpha-hydroxyacids and their esters (Nos 589-591, 603, 631-635) would be expected to be metabolized in the same way as endogenous alpha-ketoacids formed from oxidative deamination of amino acids, such as isoleucine, methionine, and valine, *in vivo*. 2-Oxobutyric acid (alpha-ketobutyric acid, No. 589) is produced endogenously in humans as a product of methionine degradation and undergoes alpha-decarboxylation to yield propionyl-coenzyme A, which ultimately enters the tricarboxylic acid cycle as succinyl-coenzyme A. Nos 631-635 are intermediates formed endogenously from the oxidative deamination of valine, isoleucine, leucine, glutamic acid, and serine, respectively (Voet & Voet, 1990).

2.3.1.3 Acetals

Three substances in this group are acetals (Nos 593, 612, and 613), which are likely to undergo uncatalysed hydrolysis *in vivo* to yield their component aldehydes and alcohols. 3-Oxobutanal dimethyl acetal (No. 593) would be expected to undergo hydrolysis to yield methanol and acetoacetaldehyde, which may be oxidized to acetoacetic acid. More than 99% of hydroxycitronellal dimethyl acetal (No. 612) was hydrolysed to the terpenoid hydroxycitronellal and methanol in simulated gastric juice (pH 2.1) after 1 h, and > 6% was hydrolysed

in intestinal fluid (pH 7.5) after 2 h (Morgareidge, 1962b). Hydroxy-citronellal diethyl acetal (No. 613) would be expected to undergo similar metabolism.

2.3.1.5 beta-Keto-and beta-hydroxy acids and their esters

Esters of beta-keto or beta-hydroxy acids (Nos 594-603, 605) are hydrolysed to acetoacetic acid or its beta-hydroxy or aldehyde precursor. The last two can be oxidized *in vivo* to acetoacetic acid, which is endogenous in humans and is formed from the condensation of two acetyl coenzyme A 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 coenzyme A and is completely metabolized. When the endogenous levels are high, beta-ketoacids may undergo non-enzymatic decarboxylation, which for acetoacetic acid yields acetone and carbon ioxide (Voet & Voet, 1990).

2.3.1.6 gamma-Keto and gamma-hydroxy acids and their esters

Small amounts of gamma-hydroxy and gamma-keto acids and related substances (Nos 606-609) are expected to be completely metabolized to carbon dioxide. With greater exposure, the ketone function may be reduced to the corresponding secondary alcohol (Bosron & Ting-Kai, 1980) and excreted as the glucuronic acid conjugate (Williams, 1959). Products of partial beta-oxidation or glucuronic acid conjugation have been identified in the urine. For example, a 1-g dose of the structurally related substance gamma-hydroxybutyrate was excreted in human urine unchanged and as S-3,4-dihydroxybutyrate and glycolate (Lee, 1977).

2.3.1.7 omega-Substituted derivatives

omega-Substituted derivatives (Nos 610-613) may undergo complete oxidation or conjugation with glucuronic acid and are then excreted primarily in the urine. Products of incomplete oxidation and reduction have also been observed. In rabbits, orally administered hydroxycitronellal (No. 611) is reduced to hydroxy-citronellol (No. 610) and oxidized to hydroxycitronellic acid, both of which are excreted in the urine (Ishida et al., 1989).

2.3.1.8 Aliphatic di- and tricarboxylic acids and their esters

The simple aliphatic di- and tricarboxylic acids either occur endogenously in humans (Nos 618, 619, 627, and 634) or are structurally related to endogenous substances (Nos 621-626, and 630). The esters of these acids (616, 617, 620, 628, and 629) are hydrolysed, as discussed above. Succinic acid, derived from the esters (Nos 616 and 617), fumaric acid (No. 618), (-)-malic acid (No. 619), aconitic acid (No. 627), citric acid derived from triethyl citrate (No. 629), and 2-oxopentandioic acid (No. 634) 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), and citric, tartaric, malic, aconitic, fumaric, and adipic acids are present in adult human urine (Osteux & Laturaze, 1954). alpha-Ketoglutaric acid is an intermediate metabolite of citric acid, fumaric acid, and succinic acid and is formed by alpha-oxidation (Krebs et al., 1938; Simola & Krusius, 1938).

Simple aliphatic di-and tricarboxylic acids and their esters (Nos 614-635) are metabolized (after hydrolysis in the case of esters) in the fatty acid beta-oxidation pathway or tricarboxylic acid cycle. When ^{14}C-labelled (-)-malic acid (No. 619) was administered to male albino Wistar rats by gavage at a dose of 2.5 mg/kg bw, 93% of the radiolabel was recovered in expired air, urine, and faeces (Daniel, 1969). Radiolabelled adipic acid fed to rats by stomach tube at a dose of 200-300 mg/kg bw was partially or completely metabolized, and the radiolabelled 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 would be oxidized in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidized to their corresponding carboxylic acids (Bosron & Ting-Kai, 1980; Levi & Hodgson, 1989; Feldman & Weiner, 1972). The resulting carboxylic acids would be metabolized in the fatty acid pathway and tricarboxylic acid cycle (Voet & Voet, 1990). Branched-chain diols or keto alcohols may undergo oxidation to their corresponding aldehydes and carboxylic acid, which would be further metabolized or excreted.

2.4 Toxicological studies

2.4.1 Acute toxicity

The available data on this group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters which contain additional oxygenated functional groups demonstrate that they have little acute toxicity when given orally. Oral LD_{50} values have been reported for 29 of the 47 substances in the group; these range from 1628 to > 34 000 mg/kg bw in male and female rats and from 1900 to > 31 000 mg/kg bw in male and female mice (Smyth et al., 1949, 1951; Smith, 1953; Smyth et al., 1954; Horn et al., 1957; Finkelstein &

Gold, 1959; Wolven & Leverstein, 1962; Jenner et al., 1964;

Levenstein, 1969; Smyth et al., 1969; Hart & Wong, 1971; Levenstein, 1973; Moreno, 1973; Pellmont, 1973; Shelanski & Moldovan, 1973; Lawrence et al., 1974; Moreno, 1976, 1977; Vernot et al., 1977; Moreno, 1978; Pellmont, 1978; Moreno et al., 1979; Moreno, 1980; Levenstein, 1981; Hoechst, 1995).

2.4.2 Short-term and long-term studies of toxicity

The results of short-term and long-term studies of the toxicity of the substances in this group are shown in Table 3. Details of the studies which were critical to the evaluation of the safety of tartaric acid and adipic acid are given below.

2.4.2.1 Tartaric acid (No. 621)

Rats

The toxicity of fumaric, tartaric, oxalic, and maleic acids was compared in groups of 12 weanling Osborne-Mendel rats of each sex, with 24 of each sex in the control group. The animals were given diets containing tartaric or fumaric acid at concentrations of 0, 0.1, 0.5, 0.8, or 1.2%, equivalent to 100, 500, 800, or 1200 mg/kg bw per day. The mortality rates in treated groups were not different from those of controls, and there was no statistically significant difference in body-weight gain or weekly food consumption. Necropsy performed on most animals at two years did not reveal any macroscopic changes. Histopathological examination of a wide range of tissues revealed no treatment-related changes. The NOEL was 1200 mg/kg bw per day (Fitzhugh & Nelson, 1947).

Rabbits

In a study of the toxicity of citric, fumaric, and tartaric acids, 15 New Zealand rabbits (sex not specified) weighing 1-3 kg were given the sodium salt of tartaric acid in the diet at a concentration of 7.7% for 150 days, equivalent to 2300 mg/kg bw per day. A control group was fed ground diet alone. Each animal was examined daily, and food intake and body weights were determined weekly. Haematological and urinary analyses were performed after 60 days of treatment on five treated and six control rabbits. Two animals were examined grossly 30 days after treatment, and one animal was examined after 60 days. The testis was examined histologically. At 100 days, half of the surviving rabbits were examined grossly, and the liver, kidney, and testis were examined microscopically. At the end of the study at 150 days, all animals were killed and examined grossly and histologically. Haematological and urinary analyses showed no changes. No significant gross or histopathological changes attributable to tartaric acid were observed (Packman et al., 1963).

Table 3. Results of short-term and long-term studies of the toxicity of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters with additional oxygenated functional groups

No.	Substance	Species	Sex	No. test groups[a]/no. per test group[b]	Route	Duration	NOEL (mg/kg bw per day)	Reference
595	Ethyl acetoacetate	Rat	M/F	3/32	Diet	28-29 days	300	Cook et al. (1992)
606	Laevulinic acid	Rat	NR	2/3	Diet	16 days	1000	Tischer et al. (1942)
611	Hydroxycitronellal	Rat	M/F	2/20, 2/60	Diet	2 years	250	Bar & Griepentrog (1967)
614	Diethyl malonate	Rat	M/F	2/20	Diet	13 weeks	< 500[c,d]	Posternak (1964)
614	Diethyl malonate	Rat	M/F	2/20-32	Diet	90 days	406	Posternak et al. (1969)
618	Fumaric acid	Rat	M/F	8/12	Diet	2 years	1200	Fitzhugh & Nelson (1947)
618	Fumaric acid	Rat	NR	2/14, 14/20	Diet	2 years	1380	Levey et al. (1946)
618	Fumaric acid	Guinea-pig	M/F	NR	Diet	1 year	400	Levey et al. (1946)
618	Fumaric acid[e]	Rabbit	NR	3/15	Diet	150 days	2070	Packman et al. (1963)
621	Tartaric acid	Rat	M/F	8/12	Diet	2 years	1200	Fitzhugh & Nelson (1947)
621	Tartaric acid[e]	Rabbit	NR	3/15	Diet	150 days	2300[c]	Packman et al. (1963)
621	Tartaric acid	Dog	NR	1/4	Oral	90-114 days	< 990[c]	Krop et al. (1945)

Table 3. (continued)

No.	Substance	Species	Sex	No. test groups[a]/no. per test group[b]	Route	Duration	NOEL (mg/kg bw per day)	Reference
624	Diethyl sebacate	Rat	M/F	2/10	Diet	17-18 or 27-28 weeks	1000	Hagan et al. (1967)
625	Dibutyl sebacate	Rat	M	4/10	Diet	1 year	1250	Smith (1953)
625	Dibutyl sebacate	Rat	M	5/16	Diet	2 years	6250	Smith (1953)
629	Triethyl citrate	Rat	M/F	3/7	Diet	2 months	4000	Finkelstein & Gold (1959)
629	Triethyl citrate	Cat	NR	1/6	Gavage	2 months	< 285	Finkelstein & Gold (1959)
630	Tributyl acetylcitrate	Rat	M/F	2/4	Diet	2 months	5000	Finkelstein & Gold (1959)
630	Tributyl acetylcitrate	Cat	NR	1/2	Gavage	2 months	< 5700[c]	Finkelstein & Gold (1959)

M, male; F, female; NR, not reported

[a] Number of test groups does not include controls.

[b] Number per test group comprises male and female animals.

[c] Only one dose tested

[d] Changes in relative liver weight and glomerular and renal tubular histological appearance observed

[e] Administered as the sodium salt

Dogs

As part of a comparison of the toxicity of hydroxyacetic acid, citric acid, and tartaric acid, four dogs (sex not specified) received tartaric acid daily in a gelatin capsule at a dose of 990 mg/kg bw per day for periods of 90 to 114 days. The changes in body weight varied from a 30% gain to a 32% loss. Haematological and urinary parameters were examined. Urinary casts (gelled protein) were observed in all dogs and were graded as hyaline (clear) in three dogs. Blood chemical parameters remained normal except in one dog which showed azotaemia (increased concentrations of urea in the blood) and died at 90 days, according to the authors due to nephrotoxicity. There was no NOEL (Krop et al., 1945).

2.4.2.2 Diethyl sebacate (No. 624)

Rats

In a study of the toxicity of about 50 flavouring agents, groups of five weanling Osborne-Mendel rats of each sex were fed diethyl sebacate (referred to in the paper as ethyl sebacate) at a dietary concentration of 1000 mg/kg for 27-28 weeks or 10 000 mg/kg for 17-18 weeks, equivalent to 100 and 1000 mg/kg bw per day. A group of 10 males and 10 females served as controls. Body weights, food intake, and general condition were recorded weekly, and haematological examinations were performed at the end of the study. All tissues were examined grossly at necropsy. The livers, kidneys, spleens, hearts, and testes from six controls and eight animals at the high dose, evenly divided by sex, were weighed and examined microscopically. There was no difference in growth rate or food consumption between test and control animals, and haematological examination revealed normal values. No macroscopic or microscopic changes were observed in the tissues. The NOEL was 1000 mg/kg bw per day (Hagan et al., 1967).

2.4.2.3 Dibutyl sebacate (No. 625)

Rats

Groups of 10 male Sprague-Dawley rats, five weeks old, were fed dibutyl sebacate at dietary concentrations of 0, 0.01, 0.05, 0.25, or 1.25%, equivalent to 0, 10, 50, 250, and 1250 mg/kg bw per day, for one year. Body weight and food intake were measured periodically throughout the study. Measurement of haematological parameters and microscopic examination at necropsy revealed no adverse effects (Smith, 1953).

Groups of 16 five-to six-week-old male Sprague-Dawley rats were given dibutyl sebacate in the diet at concentrations of 0 (two control groups), 0.01, 0.05, 0.25, 1.25, or 6.25%, equivalent to 0, 10, 50, 250, 1250, and 6250 mg/kg bw per day, for two years. Administration of dibutyl sebacate did not adversely affect the growth or survival of the animals. Body weight and food intake were measured periodically throughout the study. Measurement of haematological parameters and microscopic examination at necropsy revealed no adverse effects. The lesions observed in older control and treated rats at necropsy included inflammatory changes in the lungs, enlarged and discoloured

kidneys, and fatty changes in the liver. The incidence of these gross lesions was not considered to be associated with the administration of dibutyl sebacate. The NOEL was 6250 mg/kg bw per day (Smith, 1953).

2.4.4 Genotoxicity

The results of tests for the genotoxicity of substances in this group are shown in Table 4.

2.4.5 Other relevant studies

2.4.5.1 Adipic acid (No. 623)

In a study of teratogenicity, groups of 20-24 pregnant rats were given adipic acid by oral intubation on days 6-15 of gestation at doses of 0, 3, 13, 62, or 288 mg/kg bw per day. A sixth group of 24 pregnant females was given aspirin at a dose of 250 mg/kg bw per day as a positive control. The maternal parameters evaluated included clinical signs of toxicity, body weight, and food consumption. The fetuses were removed surgically from all dams on day 20. The numbers of implantation sites, resorption sites, and live births were counted, and the body weights of live pups and external, visceral, and skeletal abnormalities were evaluated. Administration of adipic acid had no adverse effect on the maternal parameters evaluated, nor did it adversely affect fetal survival or the number of abnormalities in soft or skeletal tissues (Morgareidge, 1973).

In a study of potential peroxisome proliferation, male Fischer 344 rats were fed adipic acid at a dietary concentration of 2%, equivalent to about 2000 mg/kg bw per day, for three weeks. Control animals received powdered Purina rat chow alone. No effect on hepatic peroxisomes or their associated enzymes was observed in treated animals (Moody & Reddy, 1978).

2.4.5.2 Tartaric acid (No. 621)

The potential immunotoxicity of tartaric acid was evaluated in a rapid screening protocol in which groups of 10-20 female CD1 or $B6C3F_1$ mice were given the material orally at doses up to 3000 mg/kg bw per day (doses not specified) for five days. A group of control animals was also evaluated. The animals received an infectious challenge on day 3 of dosing and immunization on day 5, and the antibody plaque-forming cell response was measured four days later. Deaths and survival were monitored for 10 days after infection. There were no statistically significant differences in spleen weight, thymus weight, spleen cellularity, anti-sheep red blood cell or plaque-forming cell response, or death due to *Listeria* infection between test and control animals (Vollmuth et al., 1989).

Table 4. Results of studies of the genotoxicity of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters with additional oxygenated functional groups

No.	Substance	End-point	Test system	Concentration	Results	Reference
595	Ethyl acetoacetate	Gene mutation	B. subtilis H17, M45 $rec^{+/-}$	20 mg/disc	Negative	Oda et al. (1978)
595	Ethyl acetoacetate	Gene mutation	B. subtilis H17, M45 $rec^{+/-}$	20 ml/disc	Positive	Yoo (1986)
595	Ethyl acetoacetate	Gene mutation	E. coli WP2 uvrA	25-320 mg/plate	Positive	Yoo (1986)
595	Ethyl acetoacetate	Gene mutation	B. subtilis H17, M45 $rec^{+/-}$ (test tube)	10-20 ml/ml	Weakly positive	Kuroda et al. (1984)
595	Ethyl acetoacetate	Chromosomal aberration	Chinese hamster cells	2 mg/ml	Negative	Ishidate et al. (1984)
595	Ethyl acetoacetate	Gene mutation	S. typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98 (preincubation protocol)	25 mg/plate	Negative[a]	Ishidate et al. (1984)
595	Ethyl acetoacetate	Gene mutation	S. typhimurium TA97, TA102 (preincubation protocol)	0.01-10 mg/plate	Negative[a]	Fujita & Sasaki (1987)
610	Hydroxycitronellol	Gene mutation	S. typhimurium TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Negative[a]	Wild et al. (1983)
610	Hydroxycitronellol	Micronucleus formation	Mouse	1204 mg/kg bw	Negative	Wild et al. (1983)
610	Hydroxycitronellol	Gene mutation	D. melanogaster	10 mmol/L	Negative	Wild et al. (1983)
611	Hydroxycitronellal	Gene mutation	S. typhimurium TA1535,	3.6 mg/plate	Negative[a]	Wild et al.

			TA100, TA1537, TA1538, TA98			(1983)

Table 4. (continued)

No.	Substance	End-point	Test system	Concentration	Results	Reference
611	Hydoxycitronellal	Micronucleus formation	Mouse	861 mg/kg bw	Negative	Wild et al. (1983)
611	Hydoxycitronellal	Gene mutation	D. melanogaster	37 mmol/L	Negative	Wild et al. (1983)
612	Hydroxycitronellal dimethyl acetal	Gene mutation	S. typhimurium TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Negative[a]	Wild et al. (1983)
612	Hydroxycitronellal dimethyl acetal	Micronucleus formation	Mouse	763 mg/kg bw	Negative	Wild et al. (1983)
612	Hydroxycitronellal dimethyl acetal	Gene mutation	D. melanogaster	25 mmol/L	Negative	Wild et al. (1983)
614	Diethyl malonate	Gene mutation	S. typhimurium TA98, TA100, TA1535, TA1537	3 mmol/plate (480 mg/plate)[b]	Negative[a]	Florin et al. (1980)
616	Dimethyl succinate	Gene mutation	S. typhimurium TA100, TA1535, TA1537, TA98	20 000 mg/plate	Negative[a]	Andersen & Jensen (1984)
616	Dimethyl succinate	Gene mutation	S. typhimurium TA97, TA98, TA102, TA104, TA1535, TA1538	10 mg/plate	Negative[a]	Zeiger et al. (1992)
618	Fumaric acid	Gene mutation	S. typhimurium TA100	1000 mg/plate	Negative[a]	Rapson et al. (1980)
618	Fumaric acid	Gene mutation	S. typhimurium TA98, TA100, TA1535, TA97 (preincubation protocol)	2000 mg/plate	Negative	Zeiger et al. (1988)
619	(-)-Malic acid	Gene mutation	S. typhimurium TA97, TA98, TA100, TA104	2000 mg/plate	Negative[a]	Al-Ani & Al-Lami (1988)

Table 4. (continued)

No.	Substance	End-point	Test system	Concentration	Results	Reference
623	Adipic acid	Gene mutation	E. coli WP2 uvrA	5000 mg/plate	Negative[a]	Shimizu et al. (1985)
623	Adipic acid	Gene mutation	S. typhimurium TA100, TA98,	5000 mg/plate	Negative[a]	Shimizu et al. (1985)
623	Adipic acid	Gene mutation	D. melanogaster	4000 ppm	Negative	Ramel & Magnusson (1979)
625	Dibutyl sebacate	Gene mutation	S. typhimurium TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Negative[a]	Wild et al. (1983)
625	Dibutyl sebacate	Micronucleus formation	Mouse	2829 mg/kg bw	Negative	Wild et al. (1983)
625	Dibutyl sebacate	Gene mutation	D. melanogaster	19 mmol/L	Negative	Wild et al. (1983)
626	Ethylene brassylate	Gene mutation	S. typhimurium TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Negative[a]	Wild et al. (1983)
627	Aconitic acid	Gene mutation	S. typhimurium TA100, TA1535, TA1537, TA98	20 000 mg/plate	Negative[a]	Andersen & Jensen (1984)

[a] With and without metabolic activation
[b] Calculation based on relative molecular mass of 160.17

3. REFERENCES

Al-An, 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.

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*, New York: Taylor &

Francis, 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, 283-288.

Bar, V.F. & Griepentrog, F. (1967) Where we stand concerning the evaluation of flavouring substances from the viewpoint of health. *Med. Ernahr.*, 8, 244-251.

Bosron, W.F. & Ting-Kai, L. (1980) Alcohol dehydrogenase. In: Jacoby, W.B., ed., *Enzymatic Basis of Detoxification*, Vol. 1, New York: Academic Press, pp. 231-248.

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, 567-573.

Cramer, G.M., Ford, R.A. & Hall, R.L. (1978) Estimation of toxic hazard: A decision tree approach. *Food Cosmet. Toxicol.*, 16, 255-276.

Daniel, J.W. (1969) The metabolism of l-and dl-malic acids by rats. *Food Cosmet. Toxicol.*, 7, 103-106.

Feldman, R.I. & Weiner, H. (1972) Horse liver aldehyde dehydrogenase. I. Purification and characterization. *J. Biol. Chem.*, 247, 260-266.

Finkelstein, M. & Gold, H. (1959) Toxicology of the citric acid esters: Tributyl citrate, acetyltributyl citrate, triethyl citrate, and acetyltriethyl citrate. *Toxicol. Appl. Pharmacol.*, 1, 283-298.

Fitzhugh, O.G. & Nelson, A. (1947) The comparative chronic toxicities of fumaric, tartaric, oxalic, and maleic acids. *J. Am. Pharm. Assoc.*, 36, 217-219.

Florin, I., Rutberg, L., Curvall, M. & Enzell, C.R. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicologist*, 15, 219-232.

Fujita, H. & Sasaki, M. (1987) Mutagenicity test of food additives with *Salmonella typhimurium* TA97 and TA102. II. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, 38, 423-430.

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, 141-157.

Hart, E.R. & Wong, L.C.K. (1971) Acute oral toxicity studies in rats, acute dermal toxicity and primary skin irritation studies in rabbits of fragrance materials. Unpublished report by Bionetics Research Laboratories. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Heymann, E. (1980) Carboxylesterases and amidases. In: Jacoby, W.B., ed., *Enzymatic Basis of Detoxication*, 2nd Ed., New York, Academic Press, pp. 291-323.

Hise,r 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 for 3-hydroxy-2-oxopropionic acid. Unpublished document submitted to WHO by the Flavor and Extract Manufacturers' Association.

Horn, H.J., Holland, E.G. & Hazleton, L.W. (1957) Safety of adipic acid as compared with citric and tartaric acid. *J. Agric. Food Chem.*, 5, 759-762.

International Organization of the Flavour Industry (1975) European inquiry on volume of use. Unpublished report submitted to WHO by the Flavor and Extract Manufacturers' Association.

Ishida, R., Toyota, M. & Asakawa, Y. (1989) Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal, (+/-)-7-hydroxycitronellal, citral, (-)-perillaldehyde, (-)-myrtenal, cuminaldehyde, thujone, and (+/-)-carvone in rabbits. *Xenobiotica*, 19, 843-855.

Ishidate, M., Jr, Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. & Matsu, A. (1984) Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.*, 22, 623-636.

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

Krebs, H.A., Salvin, E. & Johnson, W.A. (1938) The formation of citric acid and alpha-ketoglutaric acids in the mammalian body. *Biochem. J.*, 32, 113-117.

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. (1984) Rec-assay of food additives. *Nippon Kosnu Eisei Zasshi*, 31, 277-281.

Lawrence, W.H., Malik, M., & Autian, J. (1974) Development of a toxicity evaluation program for dental materials. 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. & Van Straten, S. (1979) *in vitro* digestion test on methyl-2-keto-3-methyl valerate. Unpublished report from Central Institute for Nutrition and Food Research. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Levenstein, I. (1969) Acute oral toxicity reports on rats. Unpublished report from Leberco Laboratories. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Levenstein, I. (1973) Acute oral toxicity reports on rats. Unpublished report from Leberco Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Levenstein, I. (1981) Acute oral toxicity reports on rats. Unpublished report from Leberco Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

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, 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*, London: Taylor & Francis, pp. 119-138.

Maarse, C.A. Visscher, L.C., Willemsens, L.M., Nijssen, M.H. & Boelens, M.H., eds (1994) *Volatile Components in Food*, 6th Ed., Suppl. 5, Zeist: TNO Nutrition and Food Research.

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.

Moody, D.E. & Reddy, J.K. (1978) Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds. *Toxicol. Appl. Pharmacol.*, 45, 497-504.

Moreno, O.M. (1973) Acute toxicity studies on rats and rabbits. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Moreno, O.M. (1976) Acute toxicity studies in rats, mice, rabbits and guinea pigs. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Moreno, O.M. (1977) Acute toxicity study in rats, rabbits and guinea pigs. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Moreno, O.M. (1978) Acute, toxicity studies in rats, mice, rabbits and guinea pigs. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Moreno, O.M. (1980) Acute toxicity studies. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Moreno, O.M., Moreno, M.T. & Altenbach, E.J. (1979) Acute oral toxicity study in rats with methyl-2-oxo-3-methylpentanoate. Unpublished report from MB Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Morgareidge, K. (1962a) *in vitro* digestion of four lactones. Unpublished report from the Food and Drug Research Laboratories. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Morgareidge, K. (1962b) *in vitro* digestion of four acetals. Unpublished report from the Food and Drug Research Laboratories. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Morgareidge, K. (1973) Teratologic evaluation of adipic acid in rats. Unpublished report from the Food and Drug Research Laboratories. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

National Academy of Sciences (1989) 1987 Poundage and technical effects update of substances added to food. Washington DC: Committee on Food Additive Survey Data.

Oda, Y., Hamono, Y., Inoue, K., Yamamoto, H., Niihara, T. & Kunita, N. (1978) Mutagenicity of food flavours in bacteria. *Shokuhin Eisei Hen*, 9, 177-181.

Osteux, R. & Laturaze, J. (1954) Paper chromatography of the organic acids found in urine. *C.R. Acad. Sci. (Paris)*, 239, 512-513.

Packman, E.W., Abbott, D.D. & Harrisson, W.E. (1963) Comparative subacute toxicity for rabbits of citric, fumaric, and tartaric acids. *Toxicol. Appl. Pharmacol.*, 5, 163-167.

Pellmont, B. (1973) Acute oral toxicity of ethyl-3-oxohexanoate. Unpublished report. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Pellmont, B. (1978) Acute oral toxicity in mice with methyl-2-hydroxy-4-methyl-pentanoate. Unpublished report. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Posternak, J.M. (1964) Diethyl malonate. Unpublished report from Firmenich & Co. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

Posternak, J.M., Linder, A. & Vodoz, C.A. (1969) Summaries of toxicological data. Toxicological tests on flavouring matters. *Food Cosmet. Toxicol.*, 7, 405-407.

Ramel, C. & Magnusson, J. (1979) Chemical induction of nondisjunction in *Drosophila*. *Environ. Health Perspectives*, 31, 59-66.

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

Rusoff, I.I., Balldwin, R.R., Dominues, F.J., Monder, C., Ohan, W.J. & Thiessen, R., Jr (1960) Intermediary metabolism of adipic acid. *Toxicol. Appl. Pharmacol.*, 2, 316-330.

Shelanski, M.V. & Moldovan, M. (1973) Acute oral and dermal toxicity studies. Unpublished report from Food and Drug Research Laboratories. Submtted to WHO by the Flavor and Extract Manufacturers' Association.

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

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

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

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

Smyth, H.F., Carpenter, C.P. & Weil, C.S. (1951) Range-finding toxicity data. List IV. *Arch. Ind. Hyg. Occup. Med.*, 4, 119-122.

Smyth, H.F., Carpenter, C.P., Weil, C.S. & Pozzani, U.C. (1954) Range-finding toxicity data. List V. *Arch. Ind. Hyg.*, 10, 61-68.

Smyth, H.F., Carpenter, C.P., Weil, C.S., Pozzani, V.C., Striegel, J.A. & Nycum, J.S. (1969) Range-finding toxicity data. List VII. *Am. Ind. Hyg. Ass. J.*, 30, 470-476.

Tischer, R.G., Fellers, C.R. & Doyle, B.J. (1942) The non-toxicity of levulinic acid. *J. Am. Pharm. Assoc.*, 31, 217-220.

Vernot, E.H., MacEwen, J.D., Huan, 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, 417-423.

Voet, D. & Voet, J.G., eds (1990) *Biochemistry*, New York: John Wiley & Sons, pp. 506-527, 632-633, 690.

Vollmuth, T.A., Heck, J.D., Ratajczak, H.V. & Thomas, P.T. (1989) Immunotoxicity assessment of flavouring ingredients using a rapid and economical screen. *Toxicologist*, 9, 206.

Wild, D., King, M.T., Gocke, E. & Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, basc and micronucleus test. *Food Chem. Toxicol.*, 21, 707-719.

Williams, R.T., ed. (1959) *Detoxication Mechanisms. The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds*, 2nd Ed., London: Chapman & Hall, pp. 119-120.

Wolven, A. & Leverstein, I (1962) Acute oral toxicity study of diethyl malonate in mice. Unpublished report from Givaudan Corporation. Submitted to WHO by the Flavor and Extract Manufacturers' Association.

Yoo, Y.S. (1986) Mutagenic and antimutagenic activities of flavouring agents used in foodstuffs. *J. Osaka City Med. Center*, 34, 267-288.

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 (Suppl. 21), 2-141.

See Also:
Toxicological Abbreviations

SCOGS-84

EVALUATION OF THE HEALTH ASPECTS OF CITRIC ACID, SODIUM CITRATE, POTASSIUM CITRATE, CALCIUM CITRATE, AMMONIUM CITRATE, TRIETHYL CITRATE, ISOPROPYL CITRATE, AND STEARYL CITRATE AS FOOD INGREDIENTS

1977

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20014

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO. FDA/BF-78/96	2.	3. Recipient's Accession No. PB280954
4. Title and Subtitle: Evaluation of the Health Aspects of Citric Acid, Sodium Citrate, Potassium Citrate, Calcium Citrate, Ammonium Citrate, Triethyl Citrate, Isopropyl Citrate, and Stearyl Citrate as Food Ingredients			5. Report Date]977 6.
7. Author(s)			8. Performing Organization Rept. No. SCOGS-84
9. Performing Organization Name and Address: Life Sciences Research Office, Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, Md. 20014			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) FDA 223-75-2004 (G)
12. Sponsoring Organization Name and Address: Food and Drug Administration, 200 C Street, S.W., Washington, D.C. 20204			13. Type of Report & Period Covered: Final]920-]977 14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

This report, by a group of qualified scientists designated the Select Committee on GRAS Substances (SCOGS), provides an independent evaluation of the safety of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate, when used in food, at present or projected levels of use.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

APPROVED FOR UNLIMITED RELEASE

William L. Jackson DATE 5/16/78

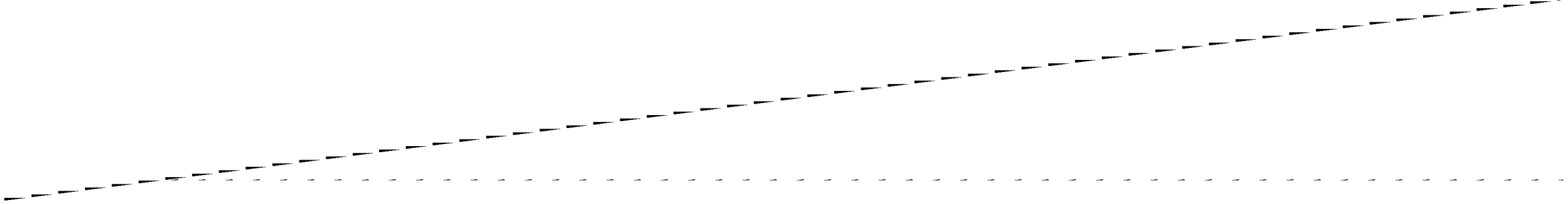
WILLIAM L. JACKSON
DIRECTOR, FDA/DRDC (NTIS)

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21
Release unlimited	20. Security Class (This Page)	22. Price A03 - A01

(See ANSI-Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce



SCOGS-84

EVALUATION OF THE HEALTH ASPECTS OF CITRIC ACID, SODIUM CITRATE, POTASSIUM CITRATE, CALCIUM CITRATE, AMMONIUM CITRATE, TRIETHYL CITRATE, ISOPROPYL CITRATE, AND AND STEARYL CITRATE AS FOOD INGREDIENTS

1977

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004

Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014

NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independendently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

CONTENTS

I. INTRODUCTION

This report concerns the health aspects of using citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. It has been based partly on the information contained in two scientific literature reviews (monographs) furnished by FDA (1, 2), which summarize the world's scientific literature from 1920 through 1973/4.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of September 2, 1977 (42 FR 44284-44285) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation, or in lieu of an oral presentation, submit a written statement of data, information and views on the health aspects of using citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. The Select Committee received no requests for a public hearing but received one statement on ammonium citrate (dibasic) from Pfizer, Incorporated, New York, New York.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (3) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

---

*The documents (PB-223 850/9 and PB-241 967/9) are available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee, is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Citric acid, 2-hydroxy-1, 2, 3, -propanetricarboxylic acid, and its salts are natural constituents and common metabolites of plants and animals. Citric acid is an intermediary compound in the Krebs cycle linking oxidative metabolism of carbohydrate, protein and fat. The concentration of naturally occurring citrate is relatively higher in fruits, particularly citrus fruits and juices, than in vegetables and animal tissues (4-6). Typical concentrations, fresh weight, are about 1 percent in orange juice and up to 8 percent in unripe lemon juice as compared to less than 0.1 percent in peas, corn, and cabbage and about 0.1 percent in human milk.

Citric acid [21 CFR 182.6033 and 182.1033], calcium citrate [21 CFR 182.6195 and 182.1195], potassium citrate [21 CFR 182.6625 and 182.1625], and sodium citrate [21 CFR 182.6751 and 182.1751] are GRAS substances listed in the Code of Federal Regulations (3) as sequestrants and as multiple purpose GRAS food substances; calcium citrate [21 CFR 182.5195] is listed also as a nutrient and/or dietary supplement. Identity standards provide for the addition of citric acid, calcium citrate, potassium citrate and sodium citrate as optional ingredients to certain cheeses [21 CFR 133], ice cream [21 CFR 135], jellies and preserves [21 CFR 150], canned vegetables [21 CFR 155.156], nonalcoholic beverages [21 CFR 165], and dressings [21 CFR 169].

Under provisions of the Code of Federal Regulations (7), citric acid is used to increase the effectiveness of antioxidants in lard or shortening at 0.01 percent; in dry sausage at 0.001 percent; and in fresh pork sausage and dried meats at 0.01 percent. It may be used to protect the flavor of oleomargarine and to flavor chili con carne at levels sufficient for that purpose. Citric acid or sodium citrate may be used as a curing accelerator in combination with a curing agent and as an anticoagulant at 0.2 percent with beef blood. Sodium citrate, potassium citrate and ammonium citrate are prior sanctioned food ingredients that may be used as stabilizers in manufacturing food packaging material [21 CFR 181.29] (3). Such uses do not contribute significantly to total citrate intake from all sources.

Food grade specifications limit citric acid, potassium citrate and sodium citrate to 3 ppm arsenic and 10 ppm heavy metals (as Pb); calcium citrate may contain 3 ppm arsenic and 20 ppm heavy metals (as Pb) but is limited to 10 ppm lead (8, 9). These citrates are water soluble, white or colorless powders or crystalline solids that are most frequently used in foods for pH control and as flavoring agents or flavoring enhancers (10). The metal ion complexing properties of citrates make them useful as sequestrants, antioxidants, and preservatives.

Food grade specifications are not given for all forms of the citrate salts. The form of potassium citrate described in the Food Chemicals Codex (8) is $C_6H_5K_3O_7 \cdot H_2O$; specifications are not given for monopotassium citrate. In the case of sodium citrate, specifications are given for trisodium citrate, $C_6H_5Na_3O_7 \cdot 2H_2O$, and not for disodium citrate. For diammonium citrate, $C_6H_{14}N_2O_7$, no food grade specifications are listed.

The isopropyl, stearyl, and ethyl esters of citric acid are evaluated separately in this report, although these esters share some of the properties of the citrate salts and yield citrate by hydrolysis. The Code of Federal Regulations (3) lists as GRAS: monoisopropyl citrate [21 CFR 182.6511], stearyl citrate [21 CFR 182.6851] with a tolerance of 0.15 percent, and isopropyl citrate [21 CFR 182.6386] with a tolerance of 0.02 percent as sequestrants. The Code lists triethyl citrate [21 CFR 182.1911] in dried egg whites with a tolerance of 0.25 percent as a multiple purpose GRAS food substance. Monoisopropyl citrate, mono-, di-, and tristearyl citrate, and triethyl citrate [21 CFR 181.27] may be used as plasticizers in the manufacture of food packaging materials. The standard of identity for margarine and oleomargarine [21 CFR 166.110] permits the use of up to 0.15 percent stearyl citrate, or up to 0.02 percent isopropyl citrate mixture.

Triethyl citrate is odorless, nearly colorless, oily liquid and the food grade material may contain no more than 3 ppm arsenic and 10 ppm heavy metals as lead (8). Extremely low concentrations of triethyl citrate occur naturally in sour cherries and red currants (11). Expressed as a weighted mean, its usual level of addition to certain foods by food category in 1970 was: baked goods, 36 ppm; frozen dairy products, 6 ppm; soft candy, 32 ppm; gelatin and puddings, 7 ppm; nonalcoholic beverages, 12 ppm; alcoholic beverages, 15 ppm; hard candy, 9 ppm; and chewing gum, 502 ppm (10). Maximum average levels of addition are known to be higher in most instances, for example, maximum average level of addition to baked goods was 125 ppm (12).

The composition of commercially available isopropyl citrate is 65 to 80 percent monoisopropyl, 15 to 30 percent diisopropyl, and 5 to 10 percent triisopropyl citrate (13). In 1970, it was added to one category of foods, the category of fats and oils; the weighted mean level of addition of isopropyl citrate ranged from a usual level of 33 ppm to a maximum of 100 ppm, which is half of the permitted level of 200 ppm (10).

Stearyl citrate is also a mixture; it contains 10 to 15, 70 to 80, and 10 to 15 percent, respectively, of the monostearyl, distearyl, and tristearyl derivatives (13). Its use in food was not reported in a 1970 survey of the food industry (10).

Food grade specifications for isopropyl citrate and stearyl citrate are not given in the Food Chemicals Codex (8).

III. CONSUMER EXPOSURE DATA

A National Research Council (NRC) subcommittee surveyed the 1970 industrial food use of GRAS substances and calculated possible average daily intakes for each GRAS substance resulting from its addition to processed foods (10). This calculation was based on Market Research Corporation of America data on the mean frequency of eating foods by food category, U.S. Department of Agriculture data on mean portion size of foods in these categories and the assumption that all food products within a given category contained the GRAS substance when it was added to any product in that category. The NRC subcommittee suggested these calculated possible intakes often represent considerable overestimates of the actual average daily intakes, and the Select Committee believes this is true for the case of the citrates evaluated in this report.

For the age group 2 to 65+ years, the calculated possible average intakes for citric acid, sodium citrate, potassium citrate, triethyl citrate, calcium citrate, and isopropyl citrate were 3100, 1600, 280, 7.4, 6, and 0.6 mg per day, respectively. The actual average daily intakes are probably nearer to the quantities used by the food industry expressed on a per capita basis as shown in Table I. These per capita estimates are about tenfold smaller and are derived from NRC survey data which represents the poundage used in 1970 by the survey respondents. If the per capita figures are expressed as citrate ion, about 480 mg of citrate was added per capita to foods by industrial processing. Thus, the addition of these compounds to foods represents only a fraction of the daily citrate intake for most individuals, e.g. one 8-ounce glass of orange juice provides about 2 g of citric acid.

For adults, citric acid and sodium citrate are the major sources of added citrate. They were added to at least one food product in nearly all of the food categories used in the NRC survey. The level of addition was usually below 0.5 percent, expressed as a weighted mean (10). Potassium citrate was added at similar levels but was used in fewer food categories. Calcium citrate was added to foods in only two categories; it was used as a firming agent in gelatins, puddings, and fillings, and as a nutrient supplement in baby formulas.

Ammonium citrate was not included in the list of GRAS substances utilized by the National Research Council in their 1970 survey of industry. However, one or more of the respondents to the survey indicated that the substance was added to foods (10). The Select Committee has been informed that ammonium citrate is used in the media for cheese cultures where it functions as a buffer and fermentation aid resulting in a usual level in cheese and whey of 0.00295 percent with a corresponding maximum level of

TABLE I

Quantity of Citrates Added Annually to Foods and Per Capita Daily Intake Calculated Therefrom (10)

Substance	Relative quantities added[a] 1970/1960	Total quantity added 1970[b] kg	Per capita daily "intake"[c] mg
Citric acid	1	27,000,000	360
Sodium citrate	6	12,000,000	160
Potassium citrate	5	440,000	5.9
Isopropyl citrate	1	30,000	0.4
Calcium citrate	4	14,000	0.2
Triethyl citrate	1	8,000	0.1

[a] Based only on the reports of those respondents to the National Research Council (NRC) survey submitting information for both 1960 and 1970.
[b] Recalculated to 100 percent from data estimated to represent 60 percent of actual usage.
[c] Based on a U.S. population of 205 million.

0.00516 percent (14). These levels of use appear consistent with levels reported for ammonium salts previously reviewed by the Select Committee (15).

The NRC subcommittee's calculated possible average daily intakes of added citrates for the 0 to 5 month age group are for citric acid, 610 mg; potassium citrate, 560 mg; and calcium citrate, 330 mg. The relative contribution of these compounds to the intake of added citrates differs from that of the 2 to 65+ year age group in part from the formulation of certain baby formulas with potassium and calcium citrate. Citric acid is used in many categories of baby foods at a weighted mean level of addition below 0.5 percent. There was no reported addition of the isopropyl, stearyl and ethyl esters of citric acid to baby foods.

Consumer exposure to the citrate esters appears to be small, based on 1970 usage data. Stearyl citrate was included in the NRC survey and no addition of this compound to foods was reported. The calculated possible average daily intake of isopropyl citrate for persons over age 2 years was about 0.6 mg and the per capita industrial usage was about 0.4 mg per day. Fewer than four industrial firms reported adding isopropyl citrate to any product in the category of fats and oils. Triethyl citrate was used in a greater variety of foods than isopropyl citrate but the per capita industrial usage was less, 0.1 mg daily.

IV. BIOLOGICAL STUDIES

This report emphasizes biological studies in which citric acid, citrates, and citrate esters were given orally. Results obtained by parenteral administration of these compounds such as the use of citrate as an anticoagulant are not relevant to an evaluation of the safety of citrates and citric acid as food ingredients. The major physiological effects from large amounts of citric acid taken orally are related to its strong carboxylic acid nature and to its ion chelating properties, particularly in binding calcium ions.

A. Citric acid and its sodium, potassium, calcium and ammonium salts

Absorption and metabolism

The biochemical reactions involved in the biosynthesis and metabolism of citric acid are well established because of its involvement in the Krebs cycle (6). The human body contains about 80 g of citrate, most of this as a component of bone. Whole blood citrate concentration is about 2 mg per dl, and 0.2 to 1.0 g of citrate is excreted daily in urine (16, 17). Orally administered citric acid is well absorbed and largely metabolized. Exogenous as well as endogenous citric acid can be completely metabolized and serve as a source of energy, furnishing 2.47 kcal per g. Infants demonstrate efficient metabolism of citric acid and the kidney tubules reabsorb most (about 90 percent) of the filtered load of citric acid (18, 19).

Acute toxicity

The acute oral LD_{50} of citric acid (produced by Candida sp fermentation of normal paraffin) in mice was about 5 g per kg body weight and 12 g per kg body weight in rats (20). The oral LD_{50} of sodium citrate in mice was 7.1 g per kg, all mice tested at a dose of 4.8 g per kg survived (21). The signs of acute toxicity from orally administered citric acid in mice and rats are those of organic acidosis and of calcium deficiency. Animals given citric acid orally in lethal doses demonstrated hemorrhage of the gastric mucosa at necropsy.

Acute oral toxicity studies of citric acid in man have not been reported. On the basis of tolerated chronic oral doses of citric acid in dogs and rabbits, Nazario (22) estimated that an adult 70 kg man should be able to tolerate 53 g of citric acid daily without damage to health. However, he reviewed one report of a young woman who vomited and almost died after ingesting 25 g of citric acid as a single dose.

Short-term studies

Six young rats weighing about 75 g were fed a diet supplemented with 2.5 percent citric acid (about 2 g per kg body weight) for 9 days (23). The experimental group showed weight loss or no appreciable gain in weight during the first few days and then recovered their expected rate of growth.

Rogers et al. (24) found no benefit to the growth of male, weanling rats (50 to 60 g) during a 2-week period from the addition of 2.47 percent diammonium citrate (2.5 g per kg per day) to a basal diet containing 16 percent amino acids when the diet contained the necessary level of indispensable amino acids and the total dispensable amino acid nitrogen was not low. However, growth was reduced if two or more of the glutamic-proline-arginine group of amino acids were omitted.

Yokotani et al. (20) fed groups of 10 SD-JCL male rats (98 to 112 g body weight) citric acid (a refined product of yeast fermentation) for 6 weeks at 1.2, 2.4, and 4.8 percent of the diet; measured mean intakes of citric acid were 1.15, 2.26, and 4.67 g per kg body weight per day, respectively. Food intake was depressed as compared to a control group by 0.7, 2.6, and 4 percent, respectively. Growth rate was slightly reduced at all levels of intake. Total plasma protein concentration was significantly less than that of controls only at the 2.4 percent dietary level; slight decreases in blood cell counts and hemoglobin were not statistically significant. At the highest dietary level, plasma cholesterol concentration decreased, serum glutamic oxalacetic transaminase activity increased, the thymus weights were lower, and slight atrophy of the thymus and splenic follicles was found at necropsy.

Daily oral citric acid administration of 600 mg per kg (1.2 percent in the diet) to rats for more than 90 days produced no abnormalities in body weight gain, blood, histopathology of the viscera or reproduction (25). Also, daily oral administration of citric acid to dogs, 1.38 g per kg, for 112 to 120 days was shown to produce no behavioral, biochemical or histopathological abnormalities.

Wehrbein et al. (26) included diammonium citrate for the partial replacement of nonessential amino acid nitrogen in experimental diets fed to replicate groups of three male and three female young Yorkshire-Hampshire pigs. The basal diet fed the control group contained 16 percent crude protein (Nx6.25) at the start of the experiment, and when the pigs reached a weight of about 50.5 kg the protein content was reduced to 14 percent. The experimental diets provided diammonium citrate at an average rate of 230, 465, and 930 mg per kg by replacing 5, 10, and 20 percent of the nitrogen of the basal diet with an equimolar mixture of diammonium citrate and diammonium phosphate. The experiment lasted 81 days. Average daily weight gain and feed intake decreased with increasing levels of added salts. In comparison

to controls, the growth depression was only significant at the 20 percent replacement level. The depressed feed intake and growth were partly reversed by addition of lysine, methionine and tryptophan to the experimental diets. The total blood nitrogen was not affected by feeding the ammonium salts; however, there was a significant depression in blood urea nitrogen levels with increasing levels of the salts.

Rats maintained on a "high protein diet" and given a single oral dose of [N^{15}] ammonium citrate (about 70 mg per kg) excreted the ammonia nitrogen almost quantitatively within 48 hours, while rats on a "low protein diet" incorporated a significant fraction of the ammonia nitrogen into proteins (27). Similar results occurred after administration of diammonium citrate (about 5 mg per kg) to a human subject on a normal diet and to a patient suffering from Addison's disease on a low protein diet. The authors concluded that ammonia is extensively utilized for protein synthesis only when there is a deficiency of dietary amino acids.

Premature infants fed a formula with added citric acid, 680 mg per kg per day, developed metabolic acidosis without clinical signs when a high protein diet (3.19 g protein per 100 ml formula) but not when a lower protein diet (1.62 g protein per 100 ml) was given (28).

Swendseid et al. (29) found that a combination of diammonium citrate and glycine was as effective as a mixture of nonessential amino acids in maintaining nitrogen equilibrium of four young adult subjects fed minimal amounts of essential nitrogen in the form of egg protein in a basal diet. Diammonium citrate (430 to 580 mg per kg) included in the experimental diets as an isonitrogenous mixture with glycine for periods of 6 or 7 days was better utilized as a source of nonessential nitrogen than glycine alone under the experimental conditions.

Scrimshaw et al. (30) replaced isonitrogenously up to 30 percent of the nitrogen contributed by the essential amino acid of whole egg protein with a mixture of glycine and diammonium citrate (each providing equal amounts of nitrogen) without affecting the nutritive value of egg protein in the experimental diets fed to groups of three and eight 17- to 22-year-old male subjects. The eleven subjects received sufficient calorie intake to maintain body weight. In one experiment, three subjects received protein at 0.42 g per kg per day for 13 days, then the protein intake was adjusted by 0.06 g per kg per day for 5-day periods to establish minimum requirements as determined by urinary nitrogen excretion. At this point, the protein of the diet was replaced isonitrogenously by the glycine-diammonium citrate mixture at the rate of 0.06 g per kg for 5-day periods. One 71 kg subject eating a basal diet providing 0.36 g protein per kg was adjudged by the investigators as probably receiving too much of a dilution when 33 percent of the protein nitrogen had been replaced; on this diet the subject was receiving 77 mg diammonium

citrate per kg per day. In a subsequent experiment, the nitrogen contributed by egg protein was increased from 78.6 percent, used in the first experiment, to 90 percent and four of the eight subjects showed no significant difference in urinary nitrogen excretion at a dilution of 40 percent for 8 to 12 days and one 70 kg subject did not increase urinary nitrogen at 50 percent for 4 additional days (124 mg diammonium citrate per kg per day).

Kies et al. (31) measured the nitrogen retention of 10 male subjects fed a basal diet and an isonitrogenous mixture of glycine and diammonium nitrate as a nonspecific nitrogen source. The basal diet provided suboptimal amounts of protein and a daily nitrogen intake of 4.5 g, of which 4 g nitrogen was from dry skim milk solids, white degerminated corn meal, enriched white flour or unenriched polished rice. When added, the salt mixture provided 4 or 8 g of nitrogen daily (215 or 430 mg diammonium citrate per kg body weight), and it was taken in three divided doses in water solution with meals. Nitrogen balance was measured during 5-day periods when the subjects had daily nitrogen intakes of 4.5 g (basal diet alone), 8.5 g or 12.5 g. A negative balance occurred on all diets except one, the basal diet containing milk solids supplemented with 8 g of the glycine and diammonium citrate mixture. However, the degree of nitrogen loss was reduced at each level of total dietary nitrogen increase, and the authors concluded that the nonspecific nitrogen source had a sparing effect on protein requirements.

Long-term studies

Long-term toxicity studies on citric acid have been carried out in rats. Three successive generations of albino Wistar rats were fed citric acid in the diet at 0.15, 0.45, and 1.20 percent, providing an average intake of 100, 300, and 800 mg per kg per day, respectively (32, 33). No effects were noted on growth, reproduction, mortality or blood components after the feeding period of up to 12 months. The teeth were not harmed by the acid diets. Metabolic studies utilized female rats; nitrogen balance, mineral balance, acid-base balance and the gross and microscopic appearance of the tissues were normal. Decrease in ash content of the tibia with an increase in calcium was found, and a slight increase in calcium was observed in muscle. Small changes in tissue composition were not considered evidence of adverse effects; the liver sodium content was decreased and the muscle sodium content was increased; the total muscle phosphorus content was also decreased.

In 1957, Horn et al. (34) reported feeding citric acid for 2 years at 3 and 5 percent of the diet to a group of 20 young male albino Carworth rats (average daily intake was 1.2 and 2.0 g per kg, respectively). Both experimental groups grew more slowly than controls, but survival rates were not decreased. At the time of sacrifice (2 years) there were no differences in organ weights of controls and experimental groups. Results of microscopic

examination of thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small and large intestines, pancreas, bone marrow, and testes were within normal limits.

Special studies

Fatal experimental tuberculosis progressed more rapidly in mice given a diet containing 8 to 10 percent sodium citrate (about 5.5 g per kg body weight) (35). Adding 2 percent sodium citrate to the drinking water (or 1 percent sodium glutarate) also caused accelerated rate of mortality in the animals. The effect on bacterial resistance was not explained but the investigator noted that citrate addition to the diets of control mice markedly reduced the rate of weight gain.

Citric acid and sodium citrate were found to interfere with calcium absorption in vitamin D-deficient rats on a low phosphorus diet (36). Citrates have an antirachitic effect in rats receiving an adequate phosphorus intake (37).

Citric acid was found to decrease the teratogenic effects of insulin and of trypan blue in chicken embryos (38, 39). Incubation of transplantable tumors (Walker carcinoma and Pliss lymphosarcoma) in sodium citrate at concentrations above 30 mg per kg inhibited their growth (40).

A 37-year-old man was reported to be allergic to several organic acids and developed canker sores, headache, general lassitude and irritability from eating foods containing citric acid (41). Direct application of citric acid crystals to the oral mucosa repeatedly produced canker sores but potassium citrate was without effect.

Teratological evaluation of citric acid in pregnant mice ($\leq$241 mg per kg administered on days 6 through 15 of gestation), rats ($\leq$295 mg per kg administered on days 6 through 15 of gestation), hamsters ($\leq$272 mg per kg administered on days 6 through 10 of gestation) and rabbits ($\leq$425 mg per kg administered on days 6 through 18 of gestation), gave no indications of adverse effects on nidation, maternal or fetal survival, and the number of abnormalities seen in either soft or skeletal tissues of the groups did not differ from the number occurring spontaneously in the sham-treated controls (42).

Citric acid was not mutagenic in Salmonella typhimurium strains TA-1530 and G-46 in the host-mediated assay (43). Although it appeared to induce mitotic recombination in Saccharomyces cerevisiae strain D3 in both in vitro and host-mediated assay tests, these tests were repeated at a higher dose level (3.5 g per kg) and all results were negative. Citric acid produced no detectable significant aberration of the bone marrow metaphase chromosomes of rats when given orally up to 3 g per kg per day for 5 days. There

were also no significant chromosomal (anaphase) aberrations in human embryonic lung culture cells (WI-38) when tested up to 600 μg per ml. Citric acid was considered to be nonmutagenic in rats in the dominant lethal assay when tested at levels up to 3 g per kg per day for 5 days.

Tests of citric acid in developing chick embryos showed that at dose levels of 10 mg per kg of egg or above there was higher mortality after air cell treatment at 96 hours and after yolk treatment at 0 and 96 hours. A dose level of 5 mg per kg increased mortality after yolk treatment at 96 hours of embryonic development. No abnormalities were observed in the hatched chicks for the test conditions employed (44).

Neither potassium nor sodium citrate was considered mutagenic when evaluated in microbial assays with and without the addition of mammalian metabolic activation preparations (45, 46). The indicator microorganisms were S. cerevisiae D4 and S. typhimurium, strains TA-1535, 1537, and 1538. Potassium citrate and sodium citrate showed no teratogenicity in the developing chicken embryo (47, 48).

B. Ethyl, isopropyl and stearyl esters of citric acid

Absorption and metabolism

Isopropyl citrate (predominantly the monoisopropyl ester) in a mono- and diglyceride vehicle at levels up to 10 percent of the diet was nearly completely absorbed and did not lower the digestibility of margarine in rats (49). Stearyl citrate, predominantly distearyl citrate, fed at 2.5 to 10 percent of the ration was poorly absorbed by the rat and incomplete digestion of stearyl citrate owing to inefficient hydrolysis of the ester in the gastrointestinal tract was described. The dog was able to digest stearyl citrate more effectively than the rat.

Acute toxicity

The oral LD_{50} for 20 percent stearyl citrate in cottonseed oil in rats was greater than 5.4 g stearyl citrate per kg of body weight (50). Isopropyl citrate (38 percent isopropyl citrate esters in a mono- and diglyceride vehicle) given orally to rats gave an LD_{50} value of greater than 20.7 g per kg for male rats and greater than 18.8 g per kg for female rats. The LD_{50} was 2.8 to 3.7 g per kg body weight when the isopropyl citrate esters were dissolved in 10 percent ethanol; this LD_{50} approximated that expected for the hydrolysis products of this ester. Single doses of 12 g per kg of isopropyl citrate plus vehicle (2.25 g per kg of isopropyl citrate) and 5 g per kg of stearyl citrate were not fatal to dogs.

Finkelstein and Gold (51) included triethyl citrate in a study of the toxicity of several citrate esters administered orally to rats and cats. The single oral LD_{50} for triethyl citrate was about 8 g per kg in rats and 4 g per kg in cats. Lethal doses in cats produced nausea, vomiting, ataxia, weakness, muscle twitching, tremors, reflex hyperexcitability, lowering of body temperature, gasping and shallow respiration, prostration, convulsions, respiratory failure, and death. One cat surviving a dose of 6.2 g per kg body weight was examined at 2-week intervals for 2 months and no toxic effects were shown as judged by weight, blood counts, hemoglobin levels or blood nitrogen and urine analysis.

Short-term studies

Adult rats fed stearyl citrate at various levels up to 10 percent of the diet (over 5 g per kg per day) and rabbits given 2 and 10 percent of the diet (over 4 g per kg per day at the high level) as stearyl citrate for 6 weeks showed no adverse reactions as measured by growth, mortality, or tissue pathology (50). Similarly, rats fed up to 5.3 percent of the diet (over 2 g isopropyl citrate per kg per day) as isopropyl citrate, in a mono- and diglyceride vehicle, and rabbits fed the compound up to 8.5 percent (over 3 g per kg per day) of the diet for 6 weeks showed no signs of toxicity. A group of four dogs was fed a diet containing 0.06 percent isopropyl citrate plus the glyceride vehicle and a similar group was fed a diet with 3 percent stearyl citrate added; no evidence of toxicity was reported.

Young rats were fed triethyl citrate in their diet at an initial rate of 1, 2, and 4 g per kg body weight for 8 weeks (51). Periodic urine examinations and blood counts and growth revealed no toxic effects. At necropsy no gross abnormalities were seen in the thoracic and abdominal organs; histological sections of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen and kidneys were comparable to those from controls. Cats receiving daily doses of triethyl citrate approximating 7 percent of the LD_{50} for an 8-week period did not differ from controls with respect to weight, blood count, hemoglobin, blood sugar and blood nitrogen. However, weakness, ataxia and depression appeared after the fourth or fifth dose and progressed to an advanced degree; the animals appeared normal within 1 to 4 days after treatment was discontinued.

Two groups of four young adult, male and female, beagle dogs were given daily doses of triethyl citrate of 0.05 and 0.25 ml per kg for 6 months (52). The parameters of body and organ weights, blood and urine analyses, and the results of histological examination of tissues revealed no adverse effects. Increasing the daily dose to 2.5 to 3.5 ml per kg for 7 to 12 weeks resulted in a characteristic liver pathology in three treated dogs. A fourth dog that had reacted adversely to a dose of 2 ml per kg showed none of the histological changes after receiving 1.5 ml per kg daily for an additional month.

Long-term studies

Stearyl citrate and isopropyl citrate have been evaluated in a 2-year feeding study and in a multigeneration feeding study in rats (50). The rats were fed stearyl citrate at levels up to 10 percent of the diet in the 2-year study (about 5 g per kg for an adult rat) and either 1.9 or 9.5 percent stearyl citrate in a four-generation study with no adverse effects on growth, mortality, fertility, gestation or lactation, and histopathology.

Isopropyl citrate was fed to weanling rats at a level up to 1.06 percent of the diet (about 1 g per kg) in a 2-year study and in a 5-generation study with no adverse effects (50). The liver, kidney, heart, brain, lung, spleen, stomach, small intestine, large intestine, pancreas, adrenal, and testicle or ovary were examined for histopathological changes at necropsy. Metastatic calcification and tumor formation were noted in the tissues of both test and control rats and were not attributable to the ingestion of isopropyl citrate.

Three groups of 15 male and 15 female weanling Sprague-Dawley rats were fed diets containing 0.33, 1.0, and 3.0 percent triethyl citrate in a lifetime feeding study (2 yr) (53). The dose of triethyl citrate initially ranged from about 0.2 to 2 g per kg body weight. Weight gain and food intake were reduced below that of the control groups when the level of the ester in the diet was increased. Blood and urine studies, survival, and gross and histopathology examinations showed no adverse effects attributable to triethyl citrate ingestion.

Special studies

The intraperitoneal administration of doses in excess of 400 mg per kg of triethyl citrate produced a loss of righting reflex in Swiss albino mice, an effect reversible within 15 minutes (54). Signs of stimulation and a more rapidly reversible loss of righting reflex were observed in Wistar rats dosed at 400 mg per kg. Intravenous administration of a 100 mg per kg dose of the compound to rabbits produced marked increases in motor activity and respiration. A group of 20 mice given intraperitoneal doses of 350 mg of triethyl citrate per kg daily for 14 days had a slightly lower growth rate than controls but no differences were seen in red and white blood cell count, clotting time and hemoglobin levels. Examination of liver, lung, and kidney tissues of two animals at necropsy revealed no pathological cellular changes. Triethyl citrate had a local anesthetic effect and blocked neural transmission when placed in direct contact with a nerve trunk.

Triethyl citrate displayed no teratogenicity to the developing chick embryo when tested in ethanol (as solvent) via the air cell and yolk at preincubation (up to 10 mg per egg) and at 96 hours (up to 0.4 mg per egg) of

incubation (55). After yolk administration, the percent mortality was significantly different from solvent control ($p \leq 0.05$); however, the mortality was not dose dependent from 0.5 to 10 mg per egg preincubation or from 0.02 to 0.40 mg per egg at 96 hours. Verrett concluded that triethyl citrate showed very little toxicity under the four test conditions.

It was not mutagenic in plate and suspension tests using Salmonella typhimurium TA 1535, TA 1537, and TA 1538 and Saccharomyces cerevisiae D4 with and without tissue homogenates (56).

V. OPINION

The citrate ion is widely distributed in plants and animals and is a naturally occurring component of the diet. It is a common metabolite in oxidative metabolism and an important component of bone. Exogenous citrate administered to infants and adults as a component of commonly consumed diets is considered completely metabolizable. The addition of citric acid to foods is considered equivalent to adding citrate salts except in foods of very high acidity. The amount of citrate added to foods by food processors is about 500 mg per person per day. This amount occurs naturally in 2 ounces of orange juice and does not constitute a significant addition to the total body load. Although data on acute and chronic effects of orally administered sodium citrate, calcium citrate and potassium citrate are limited, no biological effects of the citrate-containing substances evaluated in this report cause concern about the safety of these GRAS substances used in reasonable amounts and in accordance with prescribed tolerances and limitations.

In light of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when used at levels that are now current or that might reasonably be expected in the future.

VI. REFERENCES CITED

1. Food and Drug Research Laboratories, Inc. 1973. Monograph on the citrates. Submitted under DHEW contract no. FDA 72-103. Maspeth, N.Y. 100 pp.

2. Tracor Jitco, Inc. 1974. Monograph on citric acid. Submitted under DHEW contract no. FDA 72-100. Rockville, Md. 134 pp.

3. Office of the Federal Register, General Services Administration. 1977. Code of Federal regulations. Title 21. Food and drugs, parts 100 to 199 rev. U.S. Government Printing Office, Washington, D.C.

4. Clements, R.L. 1964. Organic acids in citrus fruits. I. Varietal differences. J. Food Sci. 29:276-280.

5. Johnston, F.B., and M.M. Hammill. 1968. The non-volatile organic acids of some fresh fruits and vegetables. Can. Inst. Food Technol. J. 1:3-5.

6. Thunberg, T. 1953. Occurrence and significance of citric acid in the animal organism. Physiol. Rev. 33:1-12.

7. Office of the Federal Register, General Services Administration. 1976. Part 318 in Code of Federal regulations. Title 9. Animals and animal products, rev. U.S. Government Printing Office, Washington, D.C.

8. National Research Council. 1972. Calcium citrate, page 126; citric acid, page 204; potassium citrate, page 647; sodium citrate, page 636; and triethyl citrate, page 837 in Food chemicals codex, 2nd ed. National Academy of Sciences, Washington, D.C.

9. National Research Council. 1975. Page 22 in Food chemicals codex, 2nd ed., second supplement. National Academy of Sciences, Washington, D.C.

10. Subcommittee on Review of the GRAS List (Phase II). 1972. A comprehensive survey of industry on the use of food chemicals generally recognized as safe (GRAS). Prepared under DHEW contract no. FDA 70-22 by the Committee on Food Protection, Division of Biology and Agriculture, National Research Council. National Academy of Sciences, Washington, D.C.

11. Neurath, G., and W. Lüttich. 1968. Über das natürliche Vorkommen von Äthylestern der Citronensäure. Z. Lebensm. Unters. Forsch. 136:284-289. (Translation supplied with reference no. 1.)

12. Flavor and Extract Manufacturers' Association of the United States. 1975. Page 56 in Results of second FEMA survey of flavoring ingredients: average maximum use levels. Washington, D.C.

13. Pintauro, N.D. 1974. Page 162 in Food additives to extend shelf life. Food Technol. Rev. No. 17. Noyes Data Corporation, Park Ridge, N.J.

14. Letter dated October 5, 1977, from E.F. Bouchard, Pfizer Central Research, New York, to C.I. Miles, Food and Drug Administration, Washington, D.C.

15. Select Committee on GRAS Substances. 1974. Evaluation of the health aspects of ammonium salts as food ingredients (SCOGS-34). Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.

16. Sjöström, P. 1937. Der Citratgehalt im Blutserum als Diagnosticum bei Krankheiten der Leber und der Gallenwege. Acta Chir Scand. Suppl. (49):229.

17. Ostberg, O. 1931. Studien über die Zitronensäureausscheidung der Menschenniere in normalen und pathologischen Zuständen. Skand. Arch. Physiol. 62:81-222.

18. Smith, A.H., D.J. Barnes, C.E. Meyer, and M. Kaucher. 1940. The metabolism of citric acid by infants. J. Nutr. 20:255-262.

19. Tischler, V., P. Beňo, O. Pavkovčekova, I. Lahita, and J. Jacina. 1968. Obličková clearance citrátu v dojčenskom veku. Cesk. Pediatr. 23:492-496. (Translation supplied with reference no. 2.)

20. Yokotani, H., T. Usui, T. Nakaguchi, T. Kanabayashi, M. Tanda, and Y. Aramaki. 1971. Acute and subacute toxicological studies of TAKEDA-citric acid in mice and rats. J. Takeda Res. Lab. 30(1):25-31.

21. Oelkers, H.-A. 1965. Beitrag zur Pharmakologie des Piperazins. Theor. Med. 19:625-630. (Translation supplied with reference no. 1.)

22. Nazario, G. 1952. Agentes acidulantes utilizados em alimentos. Rev. Inst. Adolfo Lutz 2:141-158. (Translation supplied with reference no. 2.)

23. de Albuquerque, A., and M.A. Henriques. 1970. Ensaios sobre a toxicidade do acido ascórbico. Rev. Port. Farm. 20:41-46. (Translation supplied with reference no. 2.)

24. Rogers, Q.R., D.M.-Y. Chen, and A.E. Harper. 1970. The importance of dispensable amino acids for maximal growth in the rat. Proc. Soc. Exp. Biol. Med. 134:517-522.

25. Krop, S., and H. Gold. 1945. On the toxicity of hydroxyacetic acid after prolonged administration: comparison with its sodium salt and citric and tartaric acids. J. Am. Pharm. Assoc. Sci. Ed. 34:86-89.

26. Wehrbein, G.F., P.E. Vipperman, Jr., E.R. Peo, Jr., and P.J. Cunningham. 1970. Diammonium citrate and diammonium phosphate as sources of dietary nitrogen for growing-finishing swine. J. Anim. Sci. 31:327-332.

27. Sprinson, D.B., and D. Rittenberg. 1949. The rate of utilization of ammonia for protein synthesis. J. Biol. Chem. 180:707-714.

28. Ballabriga, A., C. Conde, and A. Gallart-Catala. 1970. Metabolic response of prematures to milk formulas with different lactic acid isomers or citric acid. Helv. Paediatr. Acta 25:25-34.

29. Swendseid, M.E., C.L. Harris, and S.G. Tuttle. 1960. The effect of sources of nonessential nitrogen on nitrogen balance in young adults. J. Nutr. 71:105-108.

30. Scrimshaw, N.S., V.R. Young, R. Schwartz, M.L. Piche, and J.B. Das. 1966. Minimum dietary essential amino acid-to-total nitrogen ratio for whole egg protein fed to young men. J. Nutr. 89:9-18.

31. Kies, C., H.M. Fox, and S.C.-S. Chen. 1972. Effect of quantitative variation in nonspecific nitrogen supplementation of corn, wheat, rice, and milk diets for adult men. Cereal Chem. 49:26-33.

32. Bonting, S.L. 1952. The effect of a prolonged intake of phosphoric and citric acids in rats. Thesis, University of Amsterdam. 97 pp.

33. Bonting, S.L., and B.C.P. Jansen. 1956. The effect of a prolonged intake of phosphoric acid and citric acid in rats. Voeding 17:137-148.

34. Horn, H.J., E.G. Holland, and L.W. Hazleton. 1957. Safety of adipic acid as compared with citric and tartaric acid. J. Agric. Food Chem. 5:759-761.

35. Dubos, R.J. 1955. Effect of metabolic factors on the susceptibility of albino mice to experimental tuberculosis. J. Exp. Med. 101: 59-84.

36. Cramer, J.W., E.I. Porrata-Doria, and H. Steenbock. 1956. A rachitogenic and growth-promoting effect of citrate. Arch. Biochem. Biophys. 60:58-63.

37. Pileggi, V.J., H.F. DeLuca, J.W. Cramer, and H. Steenbock. 1956. Citrate in the prevention of rickets in rats. Arch. Biochem. Biophys. 60:52-57.

38. Landauer, W., and M.B. Rhodes. 1952. Further observations on the teratogenic nature of insulin and its modification by supplementary treatment. J. Exp. Zool. 119:221-261.

39. Beaudoin, A.R. 1968. The effect of citric acid on the teratogenic action of Trypan Blue. Life Sci. 7:635-640.

40. Prizhivoit, G.N. 1969. O vliyanii limonnokislogo natriya na rost perevivaemykh opukholei. Vopr. Onkol. 15:77-83. (Translation supplied with reference no. 1.)

41. Tuft, L., and L.N. Ettelson. 1956. Canker sores from allergy to weak organic acids (citric and acetic). J. Allergy 27:536-543.

42. Food and Drug Research Laboratories, Inc. 1973. Teratologic evaluation of FDA 71-54 (citric acid) in mice, rats, hamsters, and rabbits. Report prepared under DHEW contract no. FDA 71-260. Maspeth, N.Y. [56 pp.]

43. Litton Bionetics, Inc. 1975. Summary of mutagenicity screening studies: host-mediated assay, cytogenetics, dominant lethal assay, compound FDA 71-54, citric acid. Prepared for Food and Drug Administration under contract no. FDA 71-268. Kensington, Md. [137 pp.]

44. Anonymous. Investigation of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: citric acid. [Report supplied by Mississippi State University to Food and Drug Administration, Washington, D.C., under contract no. FDA 72-342. 7 pp.]

45. Litton Bionetics, Inc. 1975. Mutagenic evaluation of compound FDA 75-4 (006100-05-6) potassium citrate, NF, FCC granular. Prepared for Food and Drug Administration under DHEW contract no. 223-74-2104. Kensington, Md. [42 pp.]

46. Litton Bionetics, Inc. 1975. Mutagenic evaluation of compound FDA 75-12 (006132-04-3) sodium citrate, USP, FCC hydrous, granular. Prepared for Food and Drug Administration under DHEW contract no. 223-74-2104. Kensington, Md. [41 pp.]

47. Verrett, M.J. 1976. Investigations of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: potassium citrate. Food and Drug Administration, Washington, D.C. [7 pp.]

48. Verrett, M.J. 1976. Investigations of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: sodium citrate. Food and Drug Administration, Washington, D.C. [7 pp.]

49. Calbert, C.E., S.M. Greenberg, G. Kryder, and H.J. Deuel, Jr. 1951. The digestibility of stearyl alcohol, isopropyl citrates, and stearyl citrates, and the effect of these materials on the rate and degree of absorption of margarine fat. Food Res. 16:294-305.

50. Deuel, H.J., Jr., S.M. Greenberg, C.E. Calbert, R. Baker, and H.R. Fisher. 1951. Toxicological studies on isopropyl and stearyl citrates. Food Res. 16:258-280.

51. Finkelstein, M., and H. Gold. 1959. Toxicology of the citric acid esters: tributyl citrate, acetyl tributyl citrate, triethyl citrate, and acetyl triethyl citrate. Toxicol. Appl. Pharmacol. 1:283-298.

52. Hodge, H.C. 1954. Chronic oral toxicity studies of triethyl citrate in dogs. Unpublished report submitted February 17, 1977 by the Fleischmann Laboratories, Standard Brands, Inc. Stamford, Conn., to the Federation of American Societies for Experimental Biology, Bethesda, Md.

53. LaWall and Harrisson, Consultants, Philadelphia, Pa. 1954. Triethyl citrate lifetime study on rats. Unpublished report prepared for Fleischmann Laboratories, Standard Brands, Inc., Stamford, Conn. Submitted February 17, 1977 to the Federation of American Societies for Experimental Biology, Bethesda, Md.

54. Meyers, D.B., J. Autian, and W.L. Guess. 1964. Toxicity of plastics used in medical practice. II. Toxicity of citric acid esters used as plasticizers. J. Pharm. Sci. 53:774-777.

55. Verrett, M.J. 1976. Investigations of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: triethyl citrate. Food and Drug Administration, Washington, D.C. [7 pp.]

56. Litton Bionetics, Inc. 1976. Mutagenic evaluation of compound FDA 75-10 (000077-93-0) triethyl citrate, FCC. Prepared for Food and Drug Administration under DHEW contract no. 223-74-2104. Kensington, Md. [38 pp.]

VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Ind.

Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N.J.

Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Michael B. Shimkin, M.D., Professor of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, Calif.

Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph.D., (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.

2. LSRO staff:

Kenneth D. Fisher, Ph. D., Director
Frederic R. Senti, Ph. D., Associate Director
C. Jelleff Carr, Ph. D., Director Emeritus
Richard G. Allison, Ph. D., Staff Scientist
Herman I. Chinn, Ph. D., Senior Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M. D., Senior Medical Consultant
Michael J. Wade, Ph. D., Staff Scientist

The Select Committee expresses its appreciation to the following organizations who contributed information and data:

Fleischmann Laboratories, Standard Brands, Inc., Betts Avenue, Stamford, Conn.

Pfizer Central Research, Pfizer, Inc., 235 East 42 Street, New York, N. Y.

Report submitted by:

April 4, 1978
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances

SCIENTIFIC OPINION

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

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

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

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

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

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

3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

Suggested citation: 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. EFSA Journal 2012; 10(3):2563. [127 pp.]. doi:10.2903/j.efsa.2012.2563. Available online: www.efsa.europa.eu/efsajournal.htm

KEYWORDS

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

KEYWORDS

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

TABLE OF CONTENTS

BACKGROUND

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

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

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

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

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

HISTORY OF THE EVALUATION

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

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

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

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

	2011		
FGE.10Rev3	1 February 2012		63

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

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

TERMS OF REFERENCE

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

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

ASSESSMENT

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

1.1. Description

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

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

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

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

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

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

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

1.2. Stereoisomers

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

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

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

1.3. Natural Occurrence in Food

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

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

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

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

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

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

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

2. Specifications

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

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

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

3. Intake Data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Absorption, Distribution, Metabolism and Elimination

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

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

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

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

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

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

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

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

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

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation. It may be anticipated that glutaraldehyde [FL-no: 05.149] is biotransformed through the common pathways of detoxication of aldehydes to innocuous products.

Among the candidate substances, an alkoxy-alcohol, 2-butoxyethanol [FL-no: 02.242], is mainly metabolised to butoxyacetic acid, which has been identified as the metabolite responsible for the haemolysis of red blood cells induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with a second oxygenated functional group and aliphatic lactones included in the present FGE are generally metabolised to innocuous products (many of which are endogenous in humans), at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethan-1-ol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound.

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

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For 5-pentyl-3H-furan-2-one [FL-no: 10.170] flavour industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], –

which is an alpha,beta-unsaturated alcohol (after hydrolysis of the lactone) allocated FGE.19 subgroup 4.1. This subgroup was evaluated in FGE.217 with the conclusion – additional genotoxicity data required. Therefore, the Panel concluded that [FL-no.10.170] should not be evaluated through the Procedure until these data are available.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], should not be evaluated through the Procedure until new data became available because it was found to be genotoxic *in vitro* in bacterial assays. However, in the first revision of FGE.10 (FGE.10Rev1) the Panel reconsidered this compound and concluded that it is an endogenous metabolite of acetone which is formed from the degradation of body fat/fatty acids and that it would be further metabolised to innocuous compounds, and thus not be of concern at the exposure levels resulting from its use as a flavouring substance (see Section 8.4, conclusion on the genotoxicity). The Panel therefore decided that 1-hydroxypropan-2-one [FL-no: 07.169] could be evaluated along the A side of the Procedure in FGE.10Rev1.

For the safety evaluation of the 62 candidate substances in the present revision of FGE.10 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

Fifty-five of the candidate substances are classified according to the decision tree approach by Cramer *et al.* (1978) into structural class I, six are classified into structural class II [FL-no: 02.242, 06.088, 06.090, 06.095, 06.097 and 06.135] and one into structural class III [FL-no: 06.102].

Step 2

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with some structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Therefore, these substances are evaluated via the B-side of the Procedure. The evaluation of the remaining 59 candidate substances proceeds via the A-side of the Procedure.

Step A3

Step A3 applies to 54 candidate substances from structural class I [FL-no: 02.132, 02.198, 05.149, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.832, 09.833, 09.862, 09.874, 09.916, 09.951, 10.038, 10.039, 10.040, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068 and 10.168], four candidate substances from structural class II [FL-no: 06.088, 06.090, 06.095 and 06.135] and one candidate substance from structural class III [FL-no: 06.102].

The 54 candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The four candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the one candidate substance assigned to structural class III has an estimated European daily *per capita* intake of 0.011 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

Accordingly, these 59 candidate substances do not pose a safety concern when used at estimated levels of intake as flavouring substances, based on the MSDI approach.

Step B3

The MSDIs of the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097] and ethyl 2-acetylbutyrate [FL-no: 09.824], were estimated to be 0.0012 microgram/*capita*/day for each. Thus, the MSDI-values of all three candidate substances are below the threshold of concern for their structural classes of 540 microgram/person/day (class II) for [FL-no: 02.242 and 06.097] and of 1800 microgram/person/day (class I) for [FL-no: 09.824]. Accordingly, the three substances proceed to step B4 of the Procedure.

Step B4

The candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824] is expected to be hydrolysed to the corresponding alpha-ethylated carboxylic acid, 2-acetylbutyric acid and ethanol. No toxicity studies that would permit establishing a No Observed Adverse Effect Level (NOAEL) are available for ethyl 2-acetylbutyrate or its hydrolysis product 2-acetylbutyric acid. 2-Acetylbutyric acid is structurally related to 2-ethylhexanol [FL-no: 02.082] for which the JECFA has established an ADI of 0.5 mg/kg bw/day (JECFA, 1993b). The estimated daily *per capita* intake, based on the MSDI approach and expressed in microgram/kg bw/day for the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate (and 2-acetylbutyric acid) is approximately 25 x 10^6 fold below the acceptable daily intake (ADI) value of the structurally related 2-ethylhexanol. Furthermore, the hydrolysis product, 2-acetylbutyric acid, shows some structural similarities to valproic acid, a known teratogenic compound. If 2-acetylbutyric acid is considered to be as potent as valproic acid (NOAEL = 600 mg/kg bw/day) the margin of safety would be 3 x 10^9, based on the MSDI of 0.0012 microgram/*capita*/day. Accordingly, it is concluded that ethyl 2-acetylbutyrate [FL-no: 09.824] does not pose a safety concern at the estimated level of intake, based on the MSDI approach.

For the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-triethoxypropane [FL no: 06.097], the hydrolysis product of which has some structural similarities to 2-butoxyethan-1-ol, a NOAEL could not be established in sub-chronic/chronic toxicity studies with respect to haemotoxicity. Thus, strictly according to the Procedure additional toxicity data would be needed to finalise the evaluation of these two substances in step B4 of the Procedure. However, reconsidering and updating the previous version of this FGE, the Panel noted that at least for 2-butoxyethan-1-ol [FL-no: 02.242] a wealth of toxicity data is available, so that this substance can be evaluated on a broader basis than only the Procedure for the Evaluation of Flavouring substances, which in principle has been designed for the evaluation of data-poor substances.

Considering the data available, especially those on kinetics and mechanism of action (see US-EPA, 1999 and draft EU-RAR 2007, human health part) it becomes clear that there are major differences in sensitivity between humans and rats regarding the prime toxic effect (haemotoxicity) of this substance, with humans (together with dog, guinea pig, pig, cat and rabbit) being considerably less sensitive than rats (together with mouse, hamster and baboon). For that reason it seems inappropriate to ask for further toxicity data in animals, as the available data already cover the most sensitive species. In this case an alternative approach is needed and possible for this data-rich substance (EPA, 1999; EU-RAR, 2007).

In their evaluation, US-EPA, using a Bench Mark Dose approach, combined with physiologically-based kinetic modelling arrived at an oral Reference dose (RfD) for chronic exposure of 0.5 mg/kg body weight (bw)/day (EPA, 1999).

In the EU-RAR (2007) a Human equivalent Lowest Observed Adverse Effect Level (LOAEL) of 9.5 mg/kg bw/day is used, which was derived from the LOAEL in the rat using the same kinetic models as applied by US-EPA. A Margin of Safety of 3 between the Human equivalent LOAEL and estimates

for chronic exposure of "Consumers" or "Humans, exposed via the Environment" was considered sufficient to reach a conclusion of no concern.

For each of the two candidate flavouring substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-tri-ethoxypropane [FL no: 06.097] an MSDI of 0.0012 microgram/*capita*/day (see Table 6.1) can be calculated. The RfD from US-EPA and the LOAEL from the draft EU-RAR are factors of 2.5×10^7 or 4.75×10^8 above the MSDI, respectively. The Panel concluded that these margins are sufficiently large to decide that based on the MSDI exposure estimates, these substances are of no concern when used as flavouring substances.

In conclusion the Panel considered that all candidate substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The mTAMDI for the 54 candidate substances in structural class I and for which use levels information is available, range from 800 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day.

The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day.

For the two substances from structural class III the mTAMDI is 3800 and 4100 microgram/person/day, which is above the threshold of 90 microgram/person/day.

Thus, for the 58 candidate substances further information is required as the mTAMDIs are above the threshold for the structural class. This would include more reliable intake data and then, if required, additional toxicological data. For two substances [FL-no: 06.135 and 08.113] use levels are required for the food categories as listen in Commission Regulation (EC) No 1565/2000 (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n).

For comparison of the MSDI- and mTAMDI-values see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	0.0061	3900	Class I	1800
02.198	Octane-1,3-diol	0.0012	3900	Class I	1800
05.149	Glutaraldehyde	0.055	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	0.22	1600	Class I	1800
08.053	Malonic acid	0.0012	3200	Class I	1800
08.082	Glutaric acid	0.0012	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	0.0012	3800	Class I	1800
08.103	Nonanedioic acid	0.0012	3200	Class I	1800
08.113	Succinic acid, disodium salt	1500		Class I	1800
09.333	sec-Butyl lactate	3.7	3900	Class I	1800
09.345	Di-isopentyl succinate	0.037	3900	Class I	1800
09.346	Dibutyl malate	0.0012	3900	Class I	1800
09.347	Dibutyl succinate	0.12	3900	Class I	1800
09.348	Diethyl adipate	0.027	3900	Class I	1800
09.349	Diethyl citrate	0.12	3900	Class I	1800
09.350	Diethyl fumarate	0.0012	3900	Class I	1800
09.351	Diethyl maleate	12	3900	Class I	1800
09.352	Diethyl nonanedioate	0.0012	3900	Class I	1800
09.353	Diethyl oxalate	0.0012	3900	Class I	1800
09.354	Diethyl pentanedioate	0.0012	3900	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.360	Ethyl 2-acetoxypropionate	4.9	3900	Class I	1800
09.502	Ethyl butyryl lactate	0.5	3900	Class I	1800
09.558	Dimethyl malonate	0.097	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	0.74	3900	Class I	1800
09.580	Hexyl lactate	0.49	3900	Class I	1800
09.590	Isobutyl lactate	3.7	3900	Class I	1800
09.601	Isopentyl lactate	7.2	5100	Class I	1800
09.626	Methyl 2-oxopropionate	0.024	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	0.0012	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	0.24	3900	Class I	1800
09.634	Methyl acetoacetate	0.012	3900	Class I	1800
09.644	Methyl lactate	0.34	3600	Class I	1800
09.683	Pentyl lactate	0.61	3900	Class I	1800
09.815	Propyl lactate	0.62	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	0.33	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	0.24	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	0.0012	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	0.015	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	0.011	3900	Class I	1800
09.951	Dioctyl adipate	6.1	800	Class I	1800
10.038	Dec-7-eno-1,4-lactone	0.37	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	1.2	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	0.011	3900	Class I	1800
10.045	Heptano-1,5-lactone	0.012	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	0.024	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	0.0061	3900	Class I	1800
10.049	Hexadecano-1,5-lactone	0.024	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	0.61	3900	Class I	1800
10.055	Pentano-1,5-lactone	0.012	3900	Class I	1800
10.058	Tridecano-1,5-lactone	0.61	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	1.9	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	48	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	0.9	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	1.2	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	0.0012	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	0.0061	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	0.012	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	0.012	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane	1.2		Class II	540
02.242	2-Butoxyethan-1-ol	0.0012	3900	Class II	540
06.097	1,1,3-Triethoxypropane	0.0012	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	0.011	4100	Class III	90
10.170	5-Pentyl-3H-furan-2-one	1.2	3800	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

As one of the candidate substances, 5-pentyl-3H-furan-2-one [FL-no: 10.170] show possible genotoxic potential *in vitro*, the substance is not taken through the Procedure. This substance is therefore not included in the calculation of the combined intake of the candidate substances evaluated in FGE.10Rev3.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b; Flavour Industry, 2010n), the combined estimated daily *per capita* intake as flavourings of the 55 candidate flavouring substances assigned to structural class I is 1600 microgram, of the six candidate flavouring substances assigned to structural class II is 1.2 microgram and of the one candidate substance assigned to structural class III, 0.01 microgram. These estimates do not exceed the thresholds of concern for the corresponding structural classes of 1800, 540 and 90 microgram/person/day, respectively.

The candidate lactones are structurally related to 27[8] supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by the JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about 55 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 41 candidate substances are structurally related to 33[9] supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 24000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

8. Toxicity

8.1. Acute Toxicity

Data are available for 16 of the candidate substances (Annex IV, Table IV.1). For the majority of candidate substances, oral LD_{50} values, in mice or rats, varied from 100 mg/kg up to more than 5000 mg/kg body weight (bw). For butane-1,3-diol [FL-no: 02.132] and octane-1,3-diol [FL-no: 02.198] LD_{50} values between 20 g/kg bw and approximately 30 g/kg bw are reported (Annex IV, Table IV.1).

Forty-three supporting substances were tested for acute toxicity in mice and/or rats (Annex IV, Table IV.1). For the majority of the supporting substances, oral LD_{50} values, in mice or rats, varied from 1300 mg/kg up to 18500 mg/kg bw. For diethyl sebacate [FL-no: 09.475] and tributyl acetylcitrate [FL-no: 09.511] LD_{50} values larger than 30 g/kg bw are reported.

The acute toxicity data are summarised in Annex IV, Table IV.1.

[8] European production volumes are only available for 27 of the 29 JECFA evaluated lactones – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.
[9] European production volumes are only available for 33 of the 47 JECFA evaluated alcohols and related substances – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute/subchronic/chronic toxicity data are available for five candidate substances, 2-butoxyethan-1-ol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], glutaraldehyde [FL-no: 05.149], nonanedioic acid [FL-no: 08.103] and for 20 supporting substances of the present Flavouring Group Evaluation (JECFA, 1998a; JECFA, 2000c). Additionally, data are available for two to succinic acid, disodium salt [FL-no: 08.113] structurally related substances, succinate monosodium and disodium hexahydrate.

Available data on repeated dose toxicity show that haemolysis is the primary and critical response elicited in the main animal test models (rats and mice) following oral exposure to 2-butoxyethan-1-ol, in which the haematotoxic action is produced by the metabolite butoxyacetic acid (this effect is also seen following other exposure routes such as inhalation or dermal exposure. These exposure routes are not considered relevant for this evaluation as data from oral exposure are available). Notably, the haematotoxic effect exhibits a pronounced species difference. In sensitive species (rat, mouse, hamster, baboon), 2-butoxyethan-1-ol produces a characteristic toxicity that is revealed clinically by the appearance of haemoglobinuria and pathologically by changes in a variety of blood parameters (EPA, 1999; EU-RAR, 2004a). Slight decrease in body weight gain, haematological and liver effects have been reported for male and female rats, respectively (NTP, 1993a). Human erythrocytes are about 100-times less sensitive than rat erythrocytes as judged by prehaemolytic changes *in vitro* (increase in mean erythrocyte volume, erythrocyte deformability) consistently observed in both species. Studies have also shown that potentially sensitive human sub-populations, including children, the elderly and those with sickle cell anemia, do not show increased sensitivity to the haemolytic action of 2-butoxyethan-1-ol. Furthermore, the *in vivo* blood concentrations producing haemolysis in the animal experiments are considered unlikely to occur under normal conditions of human exposure to 2-butoxyethan-1-ol (EU-RAR, 2004a).

Carcinogenicity:

In a two year inhalation study, F344/N rats were exposed to 0, 0.031, 0.0625 and 0.125 mg/m^3 and B6C3F$_1$ mice were exposed to 0, 0.0625, 0.125 and 0.250 mg/m^3 2-butoxyethan-1-ol (NTP, 2000b). The exposure caused a low incidence of haemangiosarcoma in male mice at the highest exposure concentration; haemangiosarcoma did not occur in female mice or in rats. In female mice, 2-butoxyethan-1-ol caused an increased incidence of forestomach tumours. It was not carcinogenic in rats. The occurrence of haemangiosarcoma in male mice only at highest exposure concentration is suggestive of a threshold phenomenon, related to the induction of haemolysis in rodent species. With regard to human relevance, the mechanism proposed for the induction of haemangiosarcomas strongly supports the conclusion that 2-butoxyethan-1-ol is unlikely to be a carcinogenic hazard at the estimated level of intake as flavouring substance, because human erythrocytes are demonstrably more resistant to haemolysis than are rodent erythrocytes.

Glutaraldehyde[10] [FL-no: 05.149] (50, 250, 1000 mg/l in drinking water, resulting in doses of 2.9-6.9, 14.5-31.8 and 54.7-104.6 mg/kg/day, respectively) was not tumorigenic in a two year carcinogenicity

[10] Glutaraldehyde is also used in food contact material (FCM). It was evaluated by the former Scientific Committee on Food (SCF List 7, http://europa.eu.int/comm/food/fs/sc/scf/out50_en.pdf), however, this is not a final evaluation. According to German recommendations, glutardialdehyde (synonym: glutaraldehyde) may be used for the production of artificial sausage skin (maximum use level 0.1 %). The maximum residual amount of glutardialdehyde is 50 mg per kg artificial sausage skin (ready for use). Furthermore, glutardialdehyde may be used as anti slime agent for the production of paper as FCM (maximum use level 2.5 % based on dry fibre material). The maximum residual amount of glutardialdehyde is 2 mg per kg paper (ready for use). The Panel noted that maximum residual amounts of glutaraldehyde in food contact material (as set e.g. in German recommendations) could apparently conflict with reported use levels of glutaraldehyde as flavouring. However, in the German recommendations, the maximum residual amounts were set considering the technologically needed use levels (limited data submitted) rather than on toxicological data, and the Panel therefore did not find the low maximum residual amounts for glutaraldehyde as such in conflict with higher use levels for glutaraldehyde as flavouring, which could therefore go through the Procedure.

study on male and female rats (Van Miller et al., 2002). Furthermore, malonic acid [FL-no: 08.053] was negative in a liver foci tumour promotion assay.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental toxicity and reproductive toxicity are available for the following five candidate substances: 2-butoxyethan-1-ol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], glutaric acid [FL-no: 08.082], glutaraldehyde [FL-no: 05.149] and nonanedioic acid [FL-no: 08.103]. Studies for supporting substances comprise butyro-1,4-lactone [FL-no: 10.006] and adipic acid [FL-no: 08.026] (JECFA, 1998a; JECFA, 2000c) and one structurally related substance, succinate disodium hexahydrate (Annex IV, Table IV.3).

For 2-butoxyethan-1-ol [FL-no: 02.242] no effects on fertility were observed in female and male mice given 2-butoxyethan-1-ol in the drinking water in a continuous breeding study in which a NOAEL of 720 mg/kg was derived (EU-RAR, 2004a). As to developmental toxicity, studies performed on animals via various administration routes did not demonstrate any teratogenic potential, and foetotoxicity and embryotoxicity (lethality and resorptions) were only observed in the presence of maternal toxicity (regenerative haemolytic anaemia). Other effects seen on foetuses were an increase in the incidence of skeletal variations, which are generally described as ossification delays. The effects seen in developmental toxicity studies with 2-butoxyethan-1-ol are considered to result from haemolysis and subsequent maternal anemia (EU-RAR, 2004a). Overall, 2-butoxyethan-1-ol is not considered to pose a safety concern with respect to reproduction and development at the estimated level of intake as flavouring substance.

No information is available on ethyl 2-acetyl butyrate [FL-no: 09.824], the hydrolysis product of which, 2-acetyl butyric acid, has some structural similarities to valproic acid, which, together with a number of its derivatives, has been recognised as teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Offspring of mothers using > 1000 mg/kg bw/day valproic acid per day were at a significantly increased risk of major congenital malformations especially neural tube defects, compared to offspring exposed < or 600 mg valproic acid/day (RR 6.8; 95 % CI: 1.4 - 32.7). No difference in risk of major congenital malformations was found between the offspring exposed to 601 - 1000 mg/day and < or = 600 mg/kg bw/day. Thus, 600 mg/day is considered as NOAEL for the teratogenic effects of valproic acid in humans.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 12 of the candidate substances. These 12 substances are pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168], glutaraldehyde [FL-no: 05.149], 1-hydroxypropan-2-one [FL-no: 07.169], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], diethyl maleate [FL-no: 09.351], diethyl adipate [FL-no: 09.348], methyl acetoacetate [FL-no: 09.634], 2-butoxyethan-1-ol [FL-no: 02.242], glutaric acid [FL-no: 08.082] and succinic acid, disodium salt [FL-no: 08.113]. There were genotoxicity data on 22 supporting substances and for one structurally related substance (Annex IV, Table IV.4 and IV.5).

For 5-pentyl-3H-furan-2-one [FL-no: 10.170] flavour industry informs that the commercial product is a mixture of two structural isomers – 2/3 is the named compound (5-pentyl-3H-furan-2-one) and 1/3 is the structural isomer - 5-pentyl-5H-furan-2-one. This latter isomer is identical to [FL-no: 10.054], which is an alpha,beta-unsaturated alcohol (after hydrolysis of the lactone) allocated to FGE.19 subgroup 4.1. This subgroup was evaluated in FGE.217 with the conclusion that additional

genotoxicity data required. Therefore, the Panel concluded that [FL-no: 10.170] should not be evaluated through the Procedure until these data are available.

In vitro

Pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168] methyl acetoacetate [FL-no: 09.634] and succinic acid [FL-no: 08.113] were reported to be negative in microbial mutagenicity assays.

1-Hydroxypropan-2-one [FL-no: 07.169] was positive in Ames tests using strains TA 100 and TA 104 in the presence and absence of S-9 metabolic activation (Garst et al., 1983; Marnett et al., 1985a; Yamaguchi, 1982; Yamaguchi and Nakagawa, 1983);. These results are consistent across the four reported studies which, despite limitations in study design and reporting, suggest that 1-hydroxypropan-2-one should be considered an *in vitro* mutagen in bacteria. There are no data provided on either *in vitro* endpoints nor on *in vivo* studies.

Diethyl maleate [FL-no: 09.351] was reported to produce mutations in the TK +/- locus of L5178Y mouse lymphoma cells. However, the concentration required for a two-fold increase of mutations results in 70 % growth reduction (Wangenheim and Bolcsfoldi, 1988), rendering this effect questionable. Diethyl maleate was positive in an aneuploidy test using V79 Chinese hamster lung cells at 8.7 $x10^{-6}$ M but not at 5.2 $x10^{-6}$ M (Önfelt, 1987); generally aneuploidy is considered as a threshold phenomenon.

In vitro and/or in vivo

Glutaric acid [FL-no: 08.082] was reported to be negative in the Ames and Rec test as well as in an *in vivo* test for rat bone marrow aberrations.

2-Butoxyethan-1-ol [FL no: 02.242] was negative in the Ames test and in *in vitro* tests in mammalian cells for induction of forward mutations, chromosomal aberrations and sister chromatid exchanges (SCE). Positive results were only reported in one study in V79 cells (for induction of forward mutations, SCE and micronuclei) at doses above the maximum level recommended by current OECD Guidelines. Equivocal positive results were reported in an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes. *In vivo*, negative results were obtained in an adequate micronucleus tests in rats and mice following oral or intraperitoneal administration. No evidence of DNA binding or alteration of DNA methylation was obtained in a study in rats and mice. The overall experimental evidence indicated that 2-butoxyethan-1-ol is not genotoxic (see Table IV.5).

Glutaraldehyde [FL-no: 05.149] exhibits genotoxic effects in *in vitro* tests, most consistently in the bacterial mutagenicity assays. Forward gene mutation tests *in vitro* in mammalian cells have given variable results depending on the locus: negative with HGPRT and positive with TK. Also, SCE, chromosome aberration and UDS tests have shown no effect to a weakly positive effect, depending on the laboratory, protocol, dosages and sampling times. However, that any *in vitro* potential for genotoxic effects will not be expressed *in vivo* is indicated by the *in vivo* study results, which include chromosomal aberrations, mammalian erythrocyte micronucleus test, UDS and recessive lethal mutations. The only study suggesting an *in vivo* effect was an increase in micronuclei in mouse blood cells up to 15 mg/kg bw. However, the data are insufficiently reported. The negative results from the well-conducted *in vivo* studies may be related to the rapid metabolism and protein binding characteristics of glutaraldehyde, and the related observation that although 14C-labelled glutaraldehyde may be detected in cell cytoplasm there is no nuclear fraction radioactivity (Vergnes and Ballantyne, 2002).

Butane-1,3-diol [FL-no: 02.132] was reported as not inducing chromosomal aberration in bone marrow and was negative in a rat dominant lethal assay. Butane-1,3-diol [FL-no: 02.132] was checked for cytogenetic effects over a period of three generations at doses of 5 % (5000 mg/kg/day), 10 % and

24 %. None of the doses produced abnormal rates of bone marrow metaphase cells as compared to controls (Hess et al., 1981).

Malonic acid [FL-no: 08.053] was found negative in a rat liver foci assay, diethyl adipate [FL-no: 09.348] was reported to be negative in a mouse dominant lethal assay.

Genotoxicity tests are available for 22 supporting substances. Some positive test results from *in vitro* studies are reported for 4-hydroxybutyric acid lactone [FL-no: 10.006], which, however, was found negative in a reliable *Drosophila in vivo* sex-linked recessive lethal mutation assay (Table IV 4 and 5). Results of *in vivo* bone marrow micronucleus assays in mice available for 4-hydroxybutyric acid lactone were also negative, however, since the PCE/NCE ratio was not reported it is not clear if the test substance reached the bone marrow (Table IV.5). Positive *in vitro* data that cannot be evaluated are reported for hexano-1,5-lactone [FL-no: 10.010], nonano-1,4-lactone [FL-no: 10.001], undecano-1,4-lactone [FL-no: 10.002], undecano-1,5-lactone [FL-no: 10.011] and ethyl acetoacetate [FL-no: 09.402] (Annex IV, Table IV.4).

Conclusions on genotoxicity

Genotoxicity data are only available on a very limited number of the candidate substances in this Flavouring Group Evaluation and none has a complete package of mutagenicity endpoints.

One of the candidate substances (1-hydroxypropan-2-one [FL-no: 07.169]) induced gene mutations in bacteria but has not been studied *in vivo* or in other *in vitro* assays.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that for the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], it was necessary to request additional *in vitro* data from studies in mammalian cells. However, in the first revision of FGE.10 (FGE.10Rev1) the Panel reconsidered the fact that 1-hydroxypropan-2-one is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore concluded that 1-hydroxypropan-2-one [FL-no: 07.169] would not be of safety concern at the exposure level resulting from its use as a flavouring substance. Consequently, the Panel decided that further studies on the *in vitro* genotoxicity of 1-hydroxypropan-2-one [FL-no: 07.169] would not be required.

Glutaraldehyde was tested *in vitro* and *in vivo*, with positive findings *in vitro*. However, based upon the negative results of *in vivo* genotoxicity assays, along with the lack of tumorigenicity in mice and rats, the *in vitro* genotoxicity data are not considered relevant for the safety evaluation of glutaraldehyde.

Disodium succinate [FL-no: 08.113] did not induce mutations in bacterial reverse mutation assays using *S.typhimurium* strains TA97, TA94, TA98, TA100, TA1535, and TA1537 at 5 mg/plate (with metabolic activation) and in TA97 and TA102 at 15 mg/plate (with or without metabolic activation). A chromosomal test with Chinese hamster lung (CHL) cells revealed equivocal effects on polyploidy at 15 mg/mL (Ishidate et al., 1984; Fujita et al., 1994; OECD, 2003). These results are supported by studies on disodium succinate hexahydrate.

5-pentyl-3H-furan-2-one [FL-no: 10.170] should not be evaluated through the Procedure until the additional gentoxicity data for FL-no: 10.054 are available, as stated in FGE 217.

The available experimental data indicate that 2-butoxyethan-1-ol is not genotoxic.

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

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

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

Thirty-six of the candidate substances possess one or more chiral centres and eight can exist as geometrical isomers due to the presence and the position of a double bond. For four of these eight substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition has not been specified sufficiently. For [FL-no: 10.170] the Industry has informed that the commercial substance is a mixture of two structural isomers. One of these isomers posses a chiral centre for which the configuration has not been specified.

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

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

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

The combined estimated daily *per capita* intake as flavourings of the 55 candidate substances assigned to structural class I is 1600 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. Likewise, the combined estimated daily *per capita* intake as flavouring of the six candidate substances assigned to structural class II is 1.2 microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The candidate lactones are structurally related to 27 supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about 55 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 41 candidate substances are structurally related to 33 supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 24000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

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

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

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern.

It can be anticipated that, at the estimated levels of intake as flavouring substances, the alcohols, aldehydes, acetals, carboxylic acids and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products, many of which are endogenous in humans. The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350]. However, as the estimated levels of intake as flavouring substances are sufficiently low for these two substances, profound GSH depletion is not expected. For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance which has structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Adequate margins of safety could be established for these three substances in step B4 of the Procedure.

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

It was considered that on the basis of the default MSDI approach that the 62 flavouring substances, to which the Procedure have been applied, would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

The mTAMDI for the 54 candidate substances in structural class I, for which use levels information is available, range from 800 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day. For the two substances from structural class III the mTAMDIs are 3800 and 4100, which is above the threshold of 90 microgram/person/day. For two substances [FL-no: 06.135 and 08.113] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000.

Thus, for 60 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. The three candidate substances [FL-no: 05.149, and 07.169 and 09.951] which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

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

In order to determine whether the conclusion for the 62 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 58 flavouring substances. For four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] information on composition of mixture and/or stereoisomerism has not been specified sufficiently. For one substance [FL-no: 10.063] an identity test is missing. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 10.038, 10.040, 10.059 and 10.063], pending further information.

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

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN FGE.10REV3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

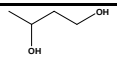
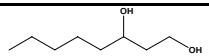
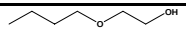
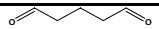
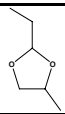
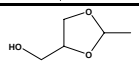
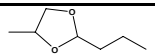
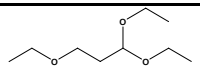
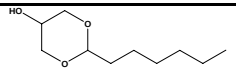
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.132	Butane-1,3-diol		 107-88-0	Liquid $C_4H_{10}O_2$ 90.12	Soluble Freely soluble	102 (13 hPa) MS 95 %	1.436-1.442 0.992-0.998	Racemate.
02.198	Octane-1,3-diol		 23433-05-8	Liquid $C_8H_{18}O_2$ 146.23	Sparingly soluble Freely soluble	82 (7 hPa) MS 95 %	1.452-1.458 0.980-0.986	Racemate.
02.242	2-Butoxyethan-1-ol		 10182 111-76-2	Liquid $C_6H_{14}O_2$ 118.18	Slightly soluble Freely soluble	170 MS 95 %	1.416-1.422 0.899-0.905	
05.149	Glutaraldehyde		 111-30-8	Liquid $C_5H_8O_2$ 100.12	Soluble Freely soluble	188 MS 95 %	1.430-1.436 1.005-1.011	
06.088	2-Ethyl-4-methyl-1,3-dioxolane		 4359-46-0	Liquid $C_6H_{12}O_2$ 116.16	Soluble Freely soluble	116 MS 95 %	1.402-1.408 0.916-0.922	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		 3674-21-3	Liquid $C_5H_{10}O_3$ 118.13	Practically insoluble or insoluble Freely soluble	187 MS 95 %	1.440-1.446 1.120-1.126	Racemate. CASrn in Register to be changed to 3773-93-1 (EFFA, 2006ac). CASrn in Register refers to the (2R, 4S) enantiomer.
06.095	4-Methyl-2-propyl-1,3-dioxolane		 4352-99-2	Liquid $C_7H_{14}O_2$ 130.19	Soluble Freely soluble	143 MS 95 %	1.409-1.415 0.907-0.913	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
06.097	1,1,3-Triethoxypropane		 10075 7789-92-6	Liquid $C_9H_{20}O_3$ 176.26	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.403-1.409 0.890-0.896	
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		 2016 1708-36-7	Solid $C_{10}H_{20}O_3$ 188.22	Practically insoluble or insoluble Freely soluble	255 44 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

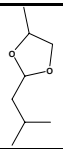
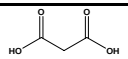
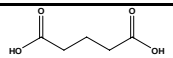
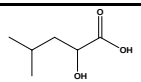
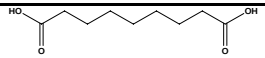
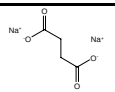
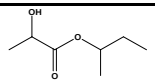
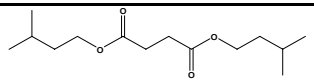
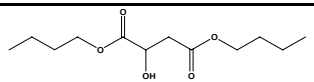
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		4378 18433-93-7	Liquid $C_8H_{16}O_2$ 144.21	Insoluble Soluble	150 MS 96 %	n.a. 0.895	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
07.169	1-Hydroxypropan-2-one		 11101 116-09-6	Liquid $C_3H_6O_2$ 74.08	Soluble Freely soluble	146 MS 95 %	1.420-1.426 1.084-1.090	
08.053	Malonic acid		 2264 141-82-2	Solid $C_3H_4O_4$ 104.16	Soluble Freely soluble	264 135 MS 95 %	n.a. n.a.	
08.082	Glutaric acid		 110-94-1	Solid $C_5H_8O_4$ 132.12	Soluble Freely soluble	303 98 MS 95 %	n.a. n.a.	
08.090	2-Hydroxy-4-methylvaleric acid		 10118 498-36-2	Solid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	249 76 MS 95 %	n.a. n.a.	Racemate.
08.103	Nonanedioic acid		 10079 123-99-9	Solid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	225 (13 hPa) 107 MS 95 %	n.a. n.a.	
08.113	Succinic acid, disodium salt		3277 150-90-3	Solid $C_4H_4Na_2O_4$ 162.05	Soluble Insoluble	426.03 156.43 IR 60	n.a. n.a.	Anhydrous when heated to 120°C. Min.assay: Anhydrous 60 %, hydrate 40 % (Fenaroli, 1995).
09.333	sec-Butyl lactate		 18449-60-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	172 MS 95 %	1.414-1.420 0.970-0.976	Racemate.
09.345	Di-isopentyl succinate		 10555 818-04-2	Liquid $C_{14}H_{26}O_4$ 258.36	Practically insoluble or insoluble Freely soluble	298 MS 95 %	1.431-1.437 0.955-0.961	
09.346	Dibutyl malate		 1587-18-4	Solid $C_{12}H_{22}O_5$ 246.30	Practically insoluble Freely soluble	170 (16 hPa) 82 MS 95 %	n.a. n.a.	CASrn in Register to be changed to 6280-99-5 (racemate).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

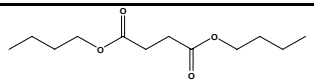
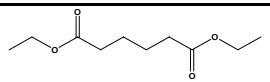
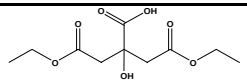
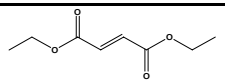
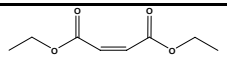
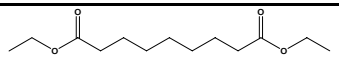
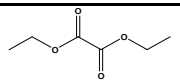
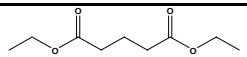
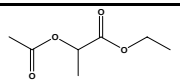
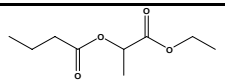
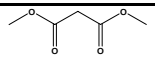
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.347	Dibutyl succinate		 141-03-7	Liquid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	275 MS 95 %	1.426-1.432 0.973-0.979	
09.348	Diethyl adipate		 141-28-6	Liquid $C_{10}H_{18}O_4$ 202.25	Practically insoluble or insoluble Freely soluble	244 MS 95 %	1.425-1.431 1.004-1.010	
09.349	Diethyl citrate		 32074-56-9	Solid $C_{10}H_{16}O_7$ 248.23	Sparingly soluble Freely soluble	354 237 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register refers to incompletely defined substance.
09.350	Diethyl fumarate		 623-91-6	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.444 1.049-1.055	
09.351	Diethyl maleate		 10551 141-05-9	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.445 1.049-1.055	
09.352	Diethyl nonanedioate		 10549 624-17-9	Liquid $C_{13}H_{24}O_4$ 244.33	Practically insoluble or insoluble Freely soluble	290 NMR 95 %	1.432-1.438 0.970-0.976	
09.353	Diethyl oxalate		 95-92-1	Liquid $C_6H_{10}O_4$ 146.14	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.407-1.413 1.076-1.082	
09.354	Diethyl pentanedioate		 818-38-2	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	233 MS 95 %	1.421-1.427 1.019-1.025	
09.360	Ethyl 2-acetoxypropionate		 2985-28-6	Liquid $C_7H_{12}O_4$ 160.17	Practically insoluble or insoluble Freely soluble	76 (13 hPa) MS 95 %	1.405-1.411 1.041-1.047	Racemate.
09.502	Ethyl butyryl lactate		 2242 71662-27-6	Liquid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	208 MS 95 %	1.408-1.414 1.021-1.027	Racemate.
09.558	Dimethyl malonate		 11754 108-59-8	Liquid $C_5H_8O_4$ 132.12	Practically insoluble or insoluble Freely soluble	181 MS	1.411-1.417 1.150-1.156	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

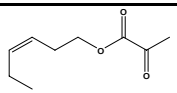
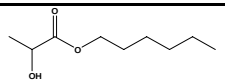
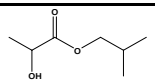
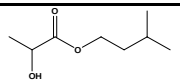
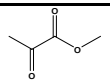
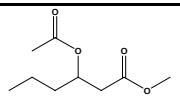
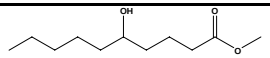
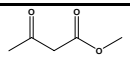
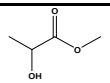
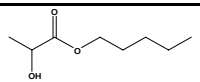
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
09.565 1846	Hex-3-enyl 2-oxopropionate		3934 10684 68133-76-6	Liquid $C_9H_{14}O_3$ 170.21	Practically insoluble or insoluble Freely soluble	76 (0.7 hPa) IR NMR 98 %	1.437-1.445 0.982-0.990	Register name to be changed to Hex-(3Z)-enyl 2-oxopropionate (EFFA, 2010a).
09.580	Hexyl lactate		20279-51-0	Liquid $C_9H_{18}O_3$ 174.24	Slightly soluble Freely soluble	221 MS 95 %	1.426-1.432 0.951-0.957	Racemate.
09.590	Isobutyl lactate		10709 585-24-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	182 MS 95 %	1.415-1.421 0.968-0.974	Racemate.
09.601	Isopentyl lactate		10720 19329-89-6	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	202 MS 97 %	1.421-1.427 0.958-0.974	Racemate.
09.626	Methyl 2-oxopropionate		10848 600-22-6	Liquid $C_4H_6O_3$ 120.09	Sparingly soluble Freely soluble	137 MS 95 %	1.401-1.407 1.145-1.151	
09.629	Methyl 3-acetoxyhexanoate		10755 77118-93-5	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	55 (0.7 hPa) MS 95 %	1.420-1.426 1.013-1.019	Racemate. CASrn in Register to be changed to 21188-60-3. CASrn in Register refers to the (R) enantiomer.
09.633	Methyl 5-hydroxydecanoate		101853-47-8	Solid $C_{11}H_{22}O_3$ 202.29	Practically insoluble or insoluble Freely soluble	278 28 MS 95 %	n.a. n.a.	Racemate.
09.634	Methyl acetoacetate		105-45-3	Liquid $C_5H_8O_3$ 116.12	Sparingly soluble Freely soluble	169 28 MS 95 %	1.415-1.421 1.073-1.079	
09.644	Methyl lactate		27871-49-4	Liquid $C_4H_8O_3$ 104.10	Sparingly soluble Freely soluble	244 MS 95 %	1.408-1.414 1.060-1.066	Register name to be changed to (S)-Methyl lactate.
09.683	Pentyl lactate		6382-06-5	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	206 MS 95 %	1.423-1.429 0.965-0.971	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

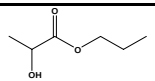
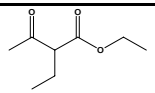
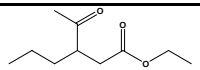
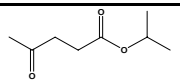
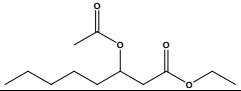
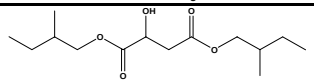
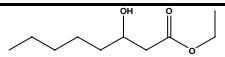
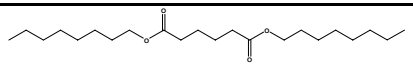
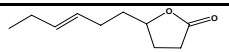
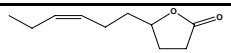
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.815	Propyl lactate		 616-09-1	Liquid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	170 MS 95 %	1.414-1.420 1.000-1.006	Racemate.
09.824	Ethyl 2-acetylbutyrate		 607-97-6	Liquid $C_8H_{14}O_3$ 158.20	Practically insoluble or insoluble Freely soluble	198 MS 95 %	1.417-1.423 0.982-0.988	Racemate.
09.832	Ethyl 3-acetohexanoate		 10566 21188-61-4	Liquid $C_{10}H_{18}O_3$ 186.24	Practically insoluble or insoluble Freely soluble	110 (12 hPa) MS 95 %	1.419-1.425 1.009-1.015	Racemate.
09.833	iso-Propyl 4-oxopentanoate		 21884-26-4	Liquid $C_8H_{14}O_3$ 158.20	Sparingly soluble Freely soluble	209 MS 95 %	1.418-1.424 0.981-0.987	
09.862	Ethyl 3-acetoxy octanoate		 85554-66-1	Solid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	276 21 MS 95 %	n.a. n.a.	Racemate.
09.874	Di(2-methylbutyl) malate			Solid $C_{14}H_{26}O_5$ 274.35	Sparingly soluble Freely soluble	335 74 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register to be introduced 253596-99-5.
09.916	Ethyl 3-hydroxyoctanoate		 10603 7367-90-0	Liquid $C_{10}H_{20}O_3$ 188.27	Practically insoluble or insoluble Freely soluble	118 (12 hPa) MS 95 %	1.421-1.427 0.973-0.979	Racemate (EFFA, 2010a).
09.951 1968	Dioctyl adipate		4476 123-79-5	Liquid $C_{22}H_{42}O_4$ 370.6	Insoluble Soluble	175 (3hPa) -70 MS 99 %	1.443-1.447 0.925	
10.038	Dec-7-eno-1,4-lactone		 67114-38-9	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a). Composition of mixture to be specified.
10.039	cis-Dec-7-eno-1,4-lactone		 63095-33-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

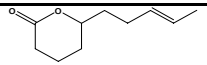
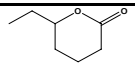
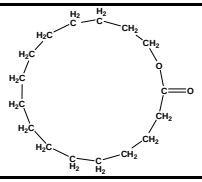
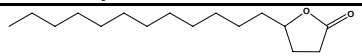
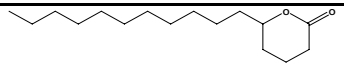
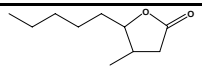
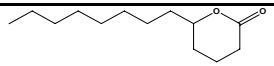
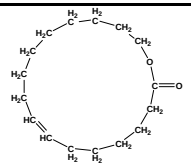
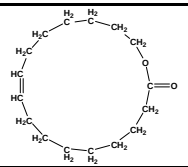
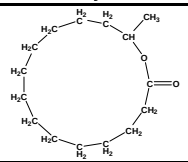
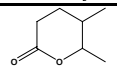
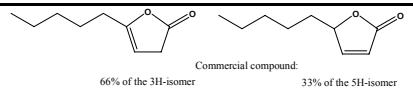
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.040	Dec-8-eno-1,5-lactone		 32764-98-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	157 (15 hPa) MS 95 %	1.462-1.468 0.972-0.978	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a). Composition of mixture to be specified.
10.045	Heptano-1,5-lactone		 10660 3301-90-4	Liquid $C_7H_{12}O_2$ 128.17	Practically insoluble or insoluble Freely soluble	104 (12 hPa) MS 95 %	1.451-1.457 1.031-1.037	Racemate.
10.047	Hexadecano-1,16-lactone		 109-29-5	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	128 (1 hPa) 34 MS 95 %	n.a. n.a.	
10.048	Hexadecano-1,4-lactone		 10673 730-46-1	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	185 (5 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.049	Hexadecano-1,5-lactone		 10674 7370-44-7	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	130 (1 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.052	3-Methylnonano-1,4-lactone		 33673-62-0	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	115 (3 hPa) MS 95 %	1.444-1.450 0.945-0.951	Racemate.
10.055	Pentano-1,5-lactone		 10907 542-28-9	Liquid $C_5H_8O_2$ 100.12	Sparingly soluble Freely soluble	219 MS 95 %	1.451-1.457 1.101-1.107	
10.058	Tridecano-1,5-lactone		 10902 7370-92-5	Liquid $C_{13}H_{24}O_2$ 212.33	Practically insoluble or insoluble Freely soluble	188 (15 hPa) MS 95 %	1.455-1.463 0.939-0.953	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.059	Hexadec-7-en-1,16-lactone 6)		123-69-3	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	188 (20 hPa) MS 95 %	1.482-1.488 0.955-0.961	CASrn in Register refers to the Z-isomer. Stereoisomeric composition to be specified.
10.063	Hexadec-9-en-1,16 lactone 6)		28645-51-4	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	131 (0.9 hPa) 95 %	1.476-1.482 0.953-0.959	ID 7). CASrn in Register does not specify isomeric composition. Stereoisomeric composition to be specified.
10.068	Pentadecano-1,14-lactone		32539-85-8	Liquid $C_{15}H_{28}O_2$ 240.38	Practically insoluble or insoluble Freely soluble	108 (0.1 hPa) MS 95 %	1.466-1.472 0.942-0.948	Racemate.
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		4141 10413-18-0	Liquid $C_7H_{12}O_2$ 128.17	Slightly soluble Freely soluble	60 NMR MS 98 %	1.452-1.458 1.019-1.025	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
10.170	5-Pentyl-3H-furan-2-one 6)		4323 51352-68-2	Liquid $C_9H_{14}O_2$ 154.2	Sparingly soluble Soluble	73 at 1.2 Torr IR NMR MS 95	1.447-1.459 0.970-0.980	Mixture of 3H and 5H isomer (2:1) (Flavour Industry, 2010g). Stereoisomeric composition to be specified.

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.
7) ID: Missing identification test.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

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

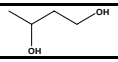
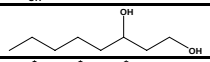
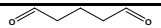
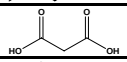
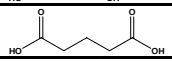
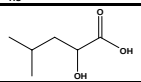
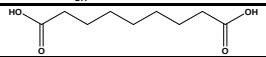
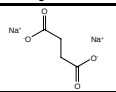
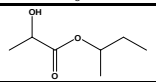
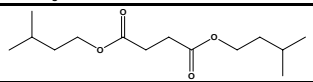
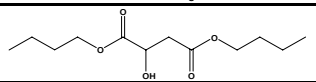
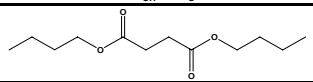
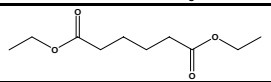
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	
02.198	Octane-1,3-diol		0.0012	Class I A3: Intake below threshold	4)	6)	
05.149	Glutaraldehyde		0.055	Class I A3: Intake below threshold	4)	6)	
07.169	1-Hydroxypropan-2-one		0.22	Class I A3: Intake below threshold	4)	6)	
08.053	Malonic acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.082	Glutaric acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.090	2-Hydroxy-4-methylvaleric acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.103	Nonanedioic acid		0.0012	Class I A3: Intake below threshold	4)	6)	
08.113	Succinic acid, disodium salt		1500	Class I A3: Intake below threshold	4)	6)	
09.333	sec-Butyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	
09.345	Di-isopentyl succinate		0.037	Class I A3: Intake below threshold	4)	6)	
09.346	Dibutyl malate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.347	Dibutyl succinate		0.12	Class I A3: Intake below threshold	4)	6)	
09.348	Diethyl adipate		0.027	Class I A3: Intake below threshold	4)	6)	

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

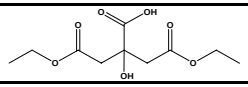
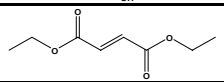
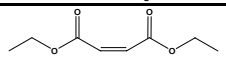
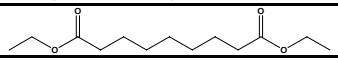
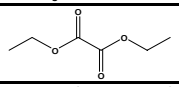
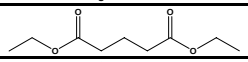
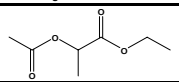
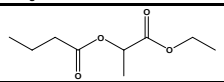
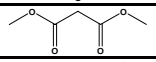
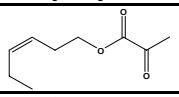
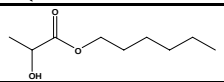
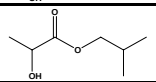
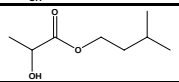
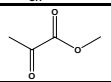
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.349	Diethyl citrate		0.12	Class I A3: Intake below threshold	4)	6)	
09.350	Diethyl fumarate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.351	Diethyl maleate		12	Class I A3: Intake below threshold	4)	6)	
09.352	Diethyl nonanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.353	Diethyl oxalate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.354	Diethyl pentanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.360	Ethyl 2-acetoxypropionate		4.9	Class I A3: Intake below threshold	4)	6)	
09.502	Ethyl butyryl lactate		0.5	Class I A3: Intake below threshold	4)	6)	
09.558	Dimethyl malonate		0.097	Class I A3: Intake below threshold	4)	6)	
09.565 1846	Hex-3-enyl 2-oxopropionate		0.74	Class I A3: Intake below threshold	4)	6)	
09.580	Hexyl lactate		0.49	Class I A3: Intake below threshold	4)	6)	
09.590	Isobutyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	
09.601	Isopentyl lactate		7.2	Class I A3: Intake below threshold	4)	6)	
09.626	Methyl 2-oxopropionate		0.024	Class I A3: Intake below threshold	4)	6)	

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

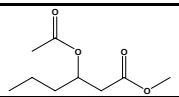
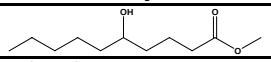
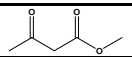
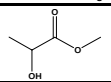
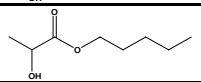
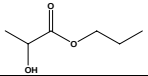
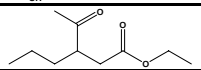
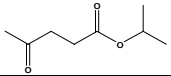
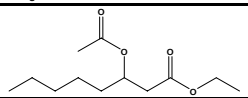
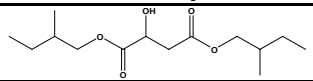
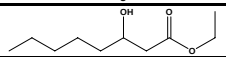
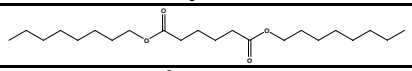
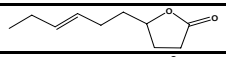
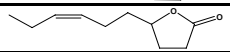
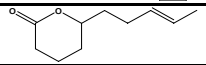
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.633	Methyl 5-hydroxydecanoate		0.24	Class I A3: Intake below threshold	4)	6)	
09.634	Methyl acetoacetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.644	Methyl lactate		0.34	Class I A3: Intake below threshold	4)	6)	
09.683	Pentyl lactate		0.61	Class I A3: Intake below threshold	4)	6)	
09.815	Propyl lactate		0.62	Class I A3: Intake below threshold	4)	6)	
09.832	Ethyl 3-acetohexanoate		0.33	Class I A3: Intake below threshold	4)	6)	
09.833	iso-Propyl 4-oxopentanoate		0.24	Class I A3: Intake below threshold	4)	6)	
09.862	Ethyl 3-acetoxy octanoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.874	Di(2-methylbutyl) malate		0.015	Class I A3: Intake below threshold	4)	6)	
09.916	Ethyl 3-hydroxyoctanoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.951 1968	Dioctyl adipate		6.1	Class I A3: Intake below threshold	4)	6)	
10.038	Dec-7-eno-1,4-lactone		0.37	Class I A3: Intake below threshold	4)	7)	
10.039	cis-Dec-7-eno-1,4-lactone		1.2	Class I A3: Intake below threshold	4)	6)	
10.040	Dec-8-eno-1,5-lactone		0.011	Class I A3: Intake below threshold	4)	7)	

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

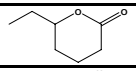
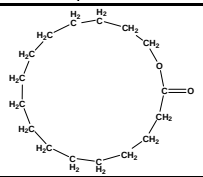
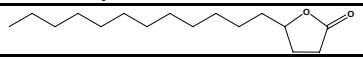
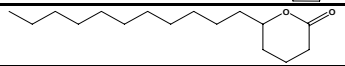
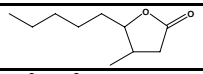
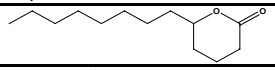
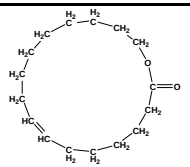
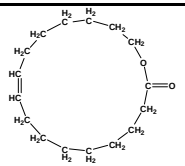
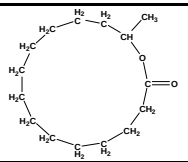
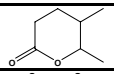
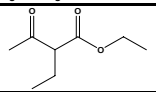
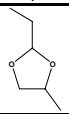
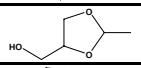
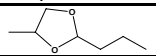
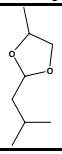
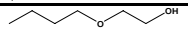
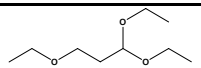
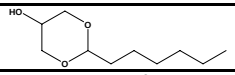
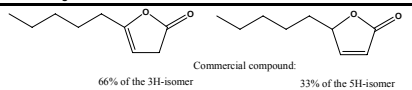
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.045	Heptano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	
10.047	Hexadecano-1,16-lactone		0.024	Class I A3: Intake below threshold	4)	6)	
10.048	Hexadecano-1,4-lactone		0.0061	Class I A3: Intake below threshold	4)	6)	
10.049	Hexadecano-1,5-lactone		0.024	Class I A3: Intake below threshold	4)	6)	
10.052	3-Methylnonano-1,4-lactone		0.61	Class I A3: Intake below threshold	4)	6)	
10.055	Pentano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	
10.058	Tridecano-1,5-lactone		0.61	Class I A3: Intake below threshold	4)	6)	
10.059	Hexadec-7-en-1,16-lactone		1.9	Class I A3: Intake below threshold	4)	7)	
10.063	Hexadec-9-en-1,16 lactone		48	Class I A3: Intake below threshold	4)	7)	
10.068	Pentadecano-1,14-lactone		0.9	Class I A3: Intake below threshold	4)	6)	

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		1.2	Class I A3: Intake below threshold	4)	6)	
09.824	Ethyl 2-acetylbutyrate		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.088	2-Ethyl-4-methyl-1,3-dioxolane		0.0061	Class II A3: Intake below threshold	4)	6)	
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	
06.095	4-Methyl-2-propyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		1.2	Class II A3: Intake below threshold	4)	6)	
02.242	2-Butoxyethan-1-ol		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.097	1,1,3-Triethoxypropane		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		0.011	Class III A3: Intake below threshold	4)	6)	
10.170	5-Pentyl-3H-furan-2-one	 Commercial compound: 66% of the 3H-isomer 33% of the 5H-isomer	1.2	Class III No evaluation			a)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) 1/3 of the named compound correspond to FL-no: 10.054 which is included in FGE.217: additional genotoxicity data required.

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

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

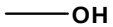
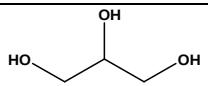
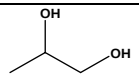
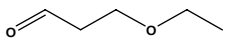
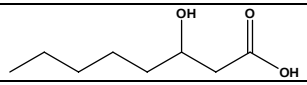
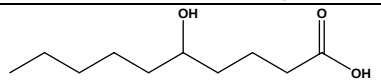
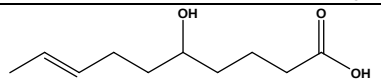
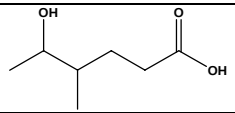
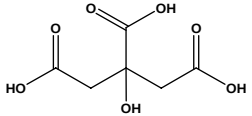
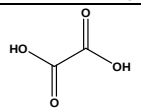
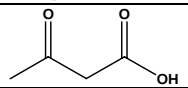
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	3-Ethoxypropan-1-al		Not evaluated as flavouring substance		Not in EU-Register
	3-Hydroxyoctanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxydecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-8-decenoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	Citric acid		Not evaluated as flavouring substance		Not in EU-Register
	Oxalic acid		Not evaluated as flavouring substance		Not in EU-Register
	Acetoacetic acid		Not evaluated as flavouring substance		Not in EU-Register

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

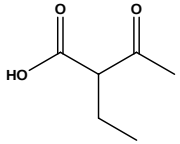
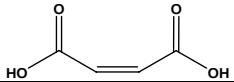
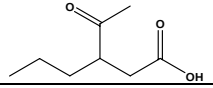
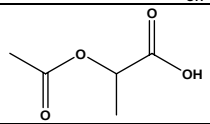
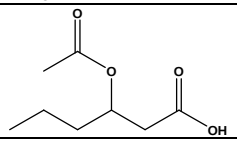
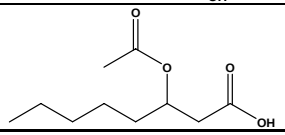
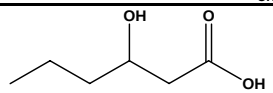
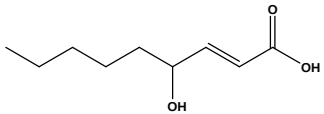
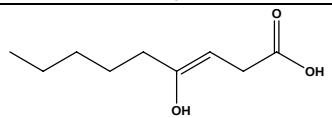
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Acetylbutyric acid		Not evaluated as flavouring substance		Not in EU-Register
	Maleic acid		Not evaluated as flavouring substance		Not in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

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

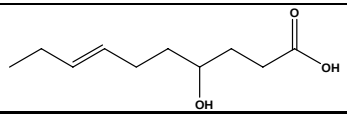
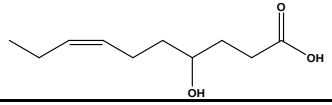
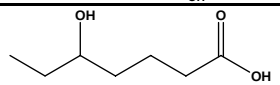
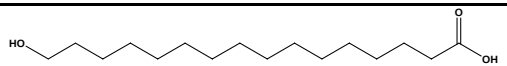
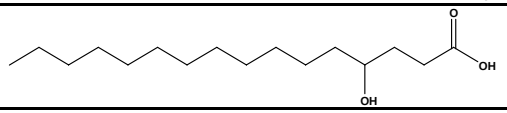
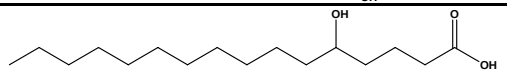
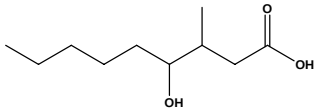
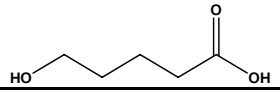
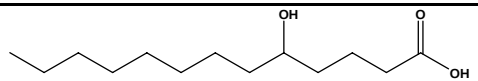
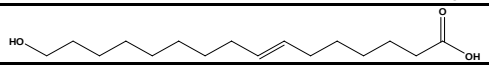
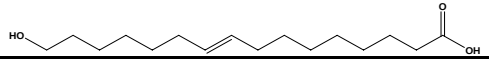
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	(E)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	(Z)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxyheptanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxy-3-methylnonanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxypentanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxytridecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-9-enoic acid		Not evaluated as flavouring substance		Not in EU-Register

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

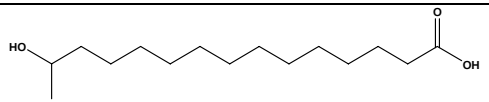
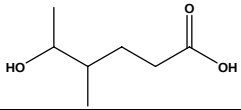
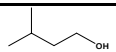
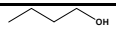
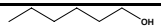
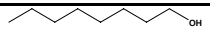
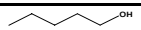
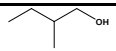
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	14-Hydroxypentadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
02.001	2-Methylpropan-1-ol 251		Category 1 a) Category A b)	Class I A3: Intake above threshold	
02.002	Propan-1-ol 82		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.006	Octan-1-ol 97		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern e) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern d)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are

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

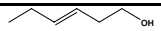
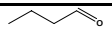
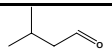
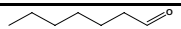
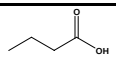
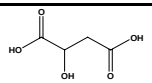
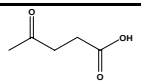
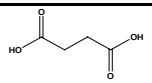
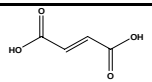
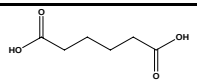
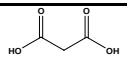
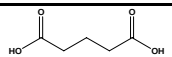
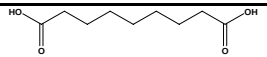
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
					used as flavouring agents.
02.079	Isopropanol 277		Category 1 a) No safety concern f)	Class I A3: Intake above threshold, A4: Endogenous	
02.121	Butan-2-ol		Category 1 a)	No evaluation	
02.159	Hex-3-en-1-ol 315		Category A c)	No evaluation	
05.001	Acetaldehyde 80		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.002	Propanal 83		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.003	Butanal 86		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.006	3-Methylbutanal 258		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.031	Heptanal 95		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern g) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.017	l-Malic acid 619		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.019	Pyruvic acid 936		No safety concern g) Category A c)	Class I A3: Intake below threshold	
08.023	4-Oxovaleric acid 606		No safety concern h) Category A c)	Class I A3: Intake below threshold	
08.024	Succinic acid		Category A c)	No evaluation	
08.025	Fumaric acid 618		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.026	Adipic acid 623		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
08.053	Malonic acid		Category A c) FGE.10	Class I A3: Intake below threshold	
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.103	Nonanedioic acid		FGE.10	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1999b).
c) (CoE, 1992).
d) (JECFA, 1997a).
e) (JECFA, 2004a).
f) (JECFA, 2000a).
g) (JECFA, 2002b).
h) (JECFA, 2000b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

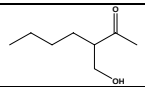
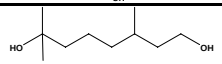
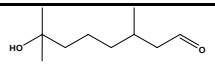
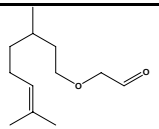
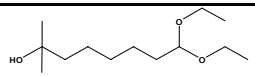
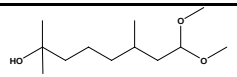
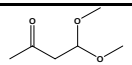
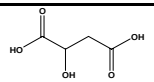
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2003b)	4.6	No safety concern d) Category B	Not in EU-Register.
02.047	3,7-Dimethyloctane-1,7-diol		2586 559 107-74-4	610 JECFA specification (JECFA, 2000d)	9.7	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
05.012	3,7-Dimethyl-7-hydroxyoctanal		2583 100 107-75-5	611 JECFA specification (JECFA, 1999c)	24	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal (CASrn as in Register). CASrn in Register refers to the racemate.
05.079	Citronellyl oxyacetaldehyde		2310 2012 7492-67-3	592 JECFA specification (JECFA, 2003b)	24	No safety concern a) Category B b)	JECFA evaluated citronelloxyacetaldehyde (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.010	1,1-Diethoxy-3,7-dimethyloctan-7-ol		2584 44 7779-94-4	613 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	JECFA evaluated hydroxycitronellal diethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.011	1,1-Dimethoxy-3,7-dimethyloctan-7-ol		2585 45 141-92-4	612 JECFA specification (JECFA, 1999c)	0.037	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal dimethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.038	4,4-Dimethoxybutan-2-one		3381 10029 5436-21-5	593 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	
08.017	l-Malic acid		2655 17 6915-15-7	619 JECFA specification (JECFA, 2000d)	13000	No safety concern a) Category A b)	JECFA evaluated l-malic acid (CASrn 97-67-6). (R)- or (S)- enantiomer not specified by CASrn in Register. GrADI: not specified

Table 3: Supporting Substances Summary

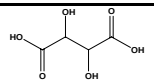
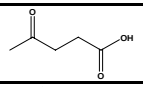
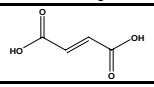
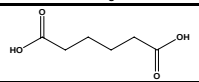
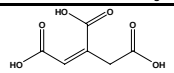
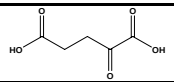
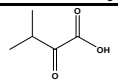
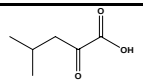
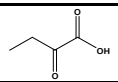
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							(JECFA, 1970a).
08.018	Tartaric acid		3044 18 133-37-9	621 JECFA specification (JECFA, 1999c)	3800	No safety concern a) Category A b)	JECFA evaluated tartaric acid ((+)-, (-)-, (+/-)-, meso-) (CASrn 87-69-4). CASrn in Register refers to (2R,3R)-isomer. No ADI (JECFA, 1978a).
08.023	4-Oxovaleric acid		2627 23 123-76-2	606 JECFA specification (JECFA, 2002d)	190	No safety concern a) Category A b)	
08.025	Fumaric acid		2488 25 110-17-8	618 JECFA specification (JECFA, 2000d)	780	No safety concern a) Category A b)	GrADI not specified (JECFA, 1990a).
08.026	Adipic acid		2011 26 124-04-9	623 JECFA specification (JECFA, 1999c)	11	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1978a).
08.033	Prop-1-ene-1,2,3-tricarboxylic acid		2010 33 499-12-7	627 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category A b)	JECFA evaluated aconitic acid (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
08.037	2-Oxoglutaric acid		3891 653 328-50-7	634 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category A b)	
08.051	3-Methyl-2-oxobutyric acid		3869 2262 759-05-7	631 JECFA specification (JECFA, 1999c)	0.012	No safety concern a) Category B b)	JECFA evaluated 3-methyl-2-oxobutanoic acid (the acid and sodium salt) (CASrn as in Register). CASrn in Register refers to the acid.
08.052	4-Methyl-2-oxovaleric acid		3871 2263 816-66-0	633 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category B b)	JECFA evaluated 4-Methyl-2-oxopentanoic acid and its sodium salt (CASrn 816-66-0 and 4502-00-5).
08.066	2-Oxobutyric acid		3723 600-18-0	589 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	

Table 3: Supporting Substances Summary

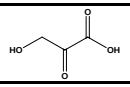
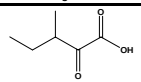
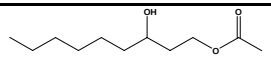
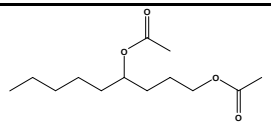
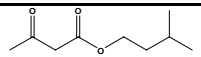
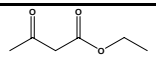
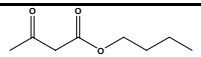
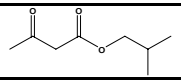
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.086	3-Hydroxy-2-oxopropionic acid		3843 1113-60-6	635 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
08.093	3-Methyl-2-oxovaleric acid		3870 10146 39748-49-7	632 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	JECFA evaluated 3-methyl-2-oxopentanoic acid (the acid and sodium salt) (CASrn 1460-34-0). CASrn 39748-49-7 replaced by CASrn 1460-34-0 in the CASrn system (SciFinder). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.225	1,3-Nonanediol acetate		2783 2075 1322-17-4	605 JECFA specification (JECFA, 2005b)	1.8	No safety concern a) Deleted b)	Reg. CASrn refers to incompletely defined substance (mixed esters). Deleted: Subst. for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available (CoE, 1992).
09.280	Nonane-1,4-diyl diacetate		3579 11927 67715-81-5	609 JECFA specification (JECFA, 2002d)	0.037	No safety concern a)	JECFA evaluated 1,4-nonanediol diacetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.401	Isopentyl acetoacetate		3551 227 2308-18-1	598 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.402	Ethyl acetoacetate		2415 240 141-97-9	595 JECFA specification (JECFA, 1999c)	1200	No safety concern a) Category B b)	
09.403	Butyl acetoacetate		2176 241 591-60-6	596 JECFA specification (JECFA, 2000d)	63	No safety concern a) Category B b)	
09.404	Isobutyl acetoacetate		2177 242 7779-75-1	597 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

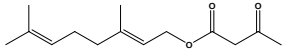
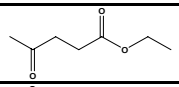
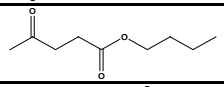
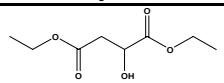
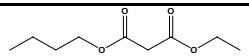
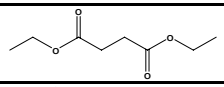
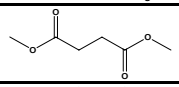
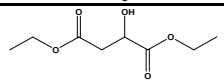
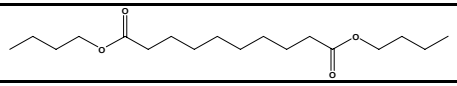
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.405	Geranyl acetoacetate		2510 243 10032-00-5	599 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	
09.435	Ethyl 4-oxovalerate		2442 373 539-88-8	607 JECFA specification (JECFA, 1999c)	470	No safety concern a) Category B b)	
09.436	Butyl 4-oxovalerate		2207 374 2052-15-5	608 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.439	Diethyl malate		2374 382 7554-12-3	620 JECFA specification (JECFA, 2000d)	3.7	No safety concern a) Deleted b)	JECFA evaluated diethyl malate. CASrn in Register refers to the racemate. Deleted: Subst. for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available(CoE, 1992).
09.441	Butyl ethyl malonate		2195 384 17373-84-1	615 Tentative JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	
09.444	Diethyl succinate		2377 438 123-25-1	617 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category B b)	
09.445	Dimethyl succinate		2396 439 106-65-0	616 JECFA specification (JECFA, 2002d)	73	No safety concern a) Category B b)	
09.446	Diethyl tartrate		2378 440 87-91-2	622 JECFA specification (JECFA, 2002d)	15	No safety concern a) Category A b)	JECFA evaluated diethyl tartrate (CASrn as in Register). Register CASrn refers to the (2R,3R)-enantiomer. ADI acceptable (JECFA, 2000b).
09.474	Dibutyl sebacate		2373 622 109-43-3	625 JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

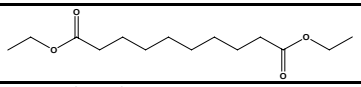
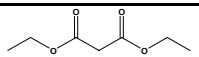
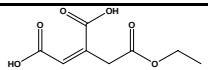
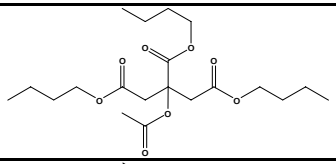
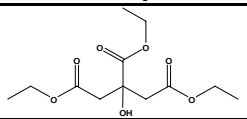
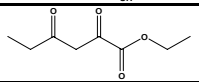
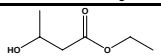
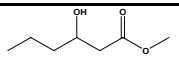
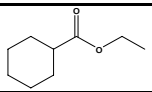
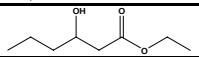
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.475	Diethyl sebacate		2376 623 110-40-7	624 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category A b)	
09.490	Diethyl malonate		2375 2106 105-53-3	614 JECFA specification (JECFA, 2002d)	650	No safety concern a) Category A b)	
09.510	Ethyl aconitate		2417 11845 1321-30-8	628 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	JECFA evaluated ethyl aconitate (mixed esters) (CASrn as in Register). Register CASrn refers to incompletely defined substance.
09.511	Tributyl acetylcitrate		3080 77-90-7	630 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
09.512	Triethyl citrate		3083 11762 77-93-0	629 JECFA specification (JECFA, 2000d)	2900	No safety concern a)	ADI: 0-20 (JECFA, 1984a).
09.514	Ethyl 2,4-dioxohexanoate		3278 11903 13246-52-1	603 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
09.522	Ethyl 3-hydroxybutyrate		3428 10596 5405-41-4	594 JECFA specification (JECFA, 2000d)	7.9	No safety concern a)	JECFA evaluated ethyl 3-hydroxybutyrate (CASrn as in Register). Register CASrn refers to the racemate.
09.532	Methyl 3-hydroxyhexanoate		3508 10812 21188-58-9	600 JECFA specification (JECFA, 2000d)	0.85	No safety concern a)	JECFA evaluated methyl 3-hydroxyhexanoate (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
09.533	Ethyl brassylate		3543 10571 105-95-3	626 JECFA specification (JECFA, 2002d)	3.0	No safety concern a)	
09.535	Ethyl 3-hydroxyhexanoate		3545 11764	601 JECFA specification (JECFA,	60	No safety concern a)	JECFA evaluated ethyl 3-hydroxyhexanoate

Table 3: Supporting Substances Summary

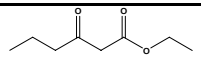
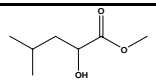
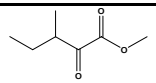
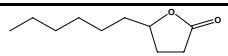
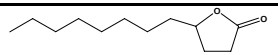
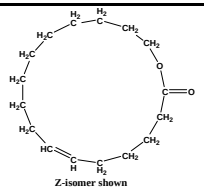
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
			2305-25-1	2002d)			(CASrn as in Register). Register CASrn refers to the racemate.
09.542	Ethyl 3-oxohexanoate		3683 3249-68-1	602 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	
09.548	Methyl 2-hydroxy-4-methylvalerate		3706 40348-72-9	590 JECFA specification (JECFA, 2003b)	0.49	No safety concern a)	JECFA evaluated methyl 2-hydroxy-4-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
09.550	Methyl 2-oxo-3-methylvalerate		3713 3682-42-6	591 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	JECFA evaluated methyl 2-oxo-3-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.001	Nonano-1,4-lactone		2781 178 104-61-0	229 JECFA specification (JECFA, 2000d)	1000	No safety concern c) Category A b)	JECFA evaluated gamma-nonalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn ADI: 0-1.25 (JECFA, 1968).
10.002	Undecano-1,4-lactone		3091 179 104-67-6	233 JECFA specification (JECFA, 1998b)	1200	No safety concern c) Category A b)	JECFA evaluated gamma-undecalactone (CASrn as in Register). Register CASrn refers to the racemate. ADI: 0-1.25 (JECFA, 1968).
10.003	Hexadec-6-eno-1,16-lactone	 Z-isomer shown	2555 180 7779-50-2	240 JECFA specification (JECFA, 2001c)	5.1	No safety concern c) Category B b)	JECFA evaluated omega-6-hexadecenlactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

Table 3: Supporting Substances Summary

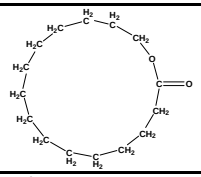
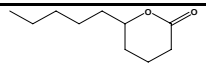
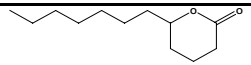
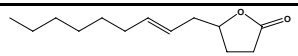
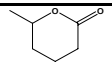
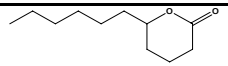
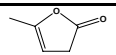
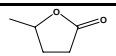
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.004	Pentadecano-1,15-lactone		2840 181 106-02-5	239 JECFA specification (JECFA, 2000d)	73	No safety concern c) Category B b)	
10.006	Butyro-1,4-lactone		3291 615 96-48-0	219 JECFA specification (JECFA, 1998b)	110	No safety concern c) Category A b)	
10.007	Decano-1,5-lactone		2361 621 705-86-2	232 JECFA specification (JECFA, 2000d)	7200	No safety concern c) Category B b)	JECFA evaluated delta-decalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.008	Dodecano-1,5-lactone		2401 624 713-95-1	236 JECFA specification (JECFA, 2000d)	5800	No safety concern c) Category B b)	JECFA evaluated delta-dodecalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.009	Dodec-6-eno-1,4-lactone		3780 625 18679-18-0	249 JECFA specification (JECFA, 2001c)	0.012	No safety concern c) Category A b)	JECFA evaluated 1,4-dodec-6-enolactone (CASrn as in Register). Register CASrn refers to the (Z)-isomer.
10.010	Hexano-1,5-lactone		3167 641 823-22-3	224 JECFA specification (JECFA, 1998b)	320	No safety concern c) Category B b)	JECFA evaluated delta-hexalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.011	Undecano-1,5-lactone		3294 688 710-04-3	234 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	JECFA evaluated 5-hydroxyundecanoic acid delta-lactone (CASrn as in Register). Register CASrn refers to the racemate.
10.012	5-Methylfuran-2(3H)-one		3293 731 591-12-8	221 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	
10.013	Pentano-1,4-lactone		3103 757 108-29-2	220 JECFA specification (JECFA, 1998b)	120	No safety concern c) Category A b)	JECFA evaluted gamma-valerolactone (CASrn as in Register). Register CASrn refers to the racemate.

Table 3: Supporting Substances Summary

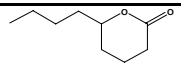
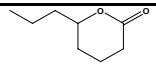
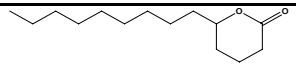
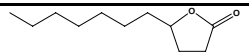
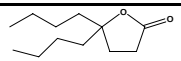
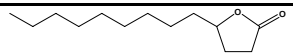
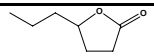
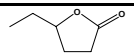
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.014	Nonano-1,5-lactone		3356 2194 3301-94-8	230 JECFA specification (JECFA, 1998b)	130	No safety concern c) Category B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

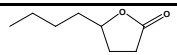
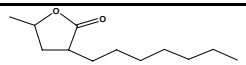
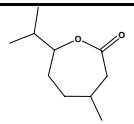
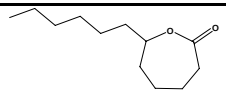
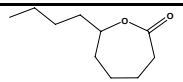
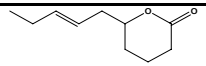
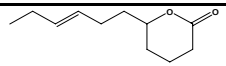
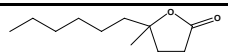
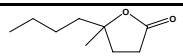
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							the racemate.
10.022	Octano-1,4-lactone		2796 2274 104-50-7	226 JECFA specification (JECFA, 2000d)	430	No safety concern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

1

2

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).

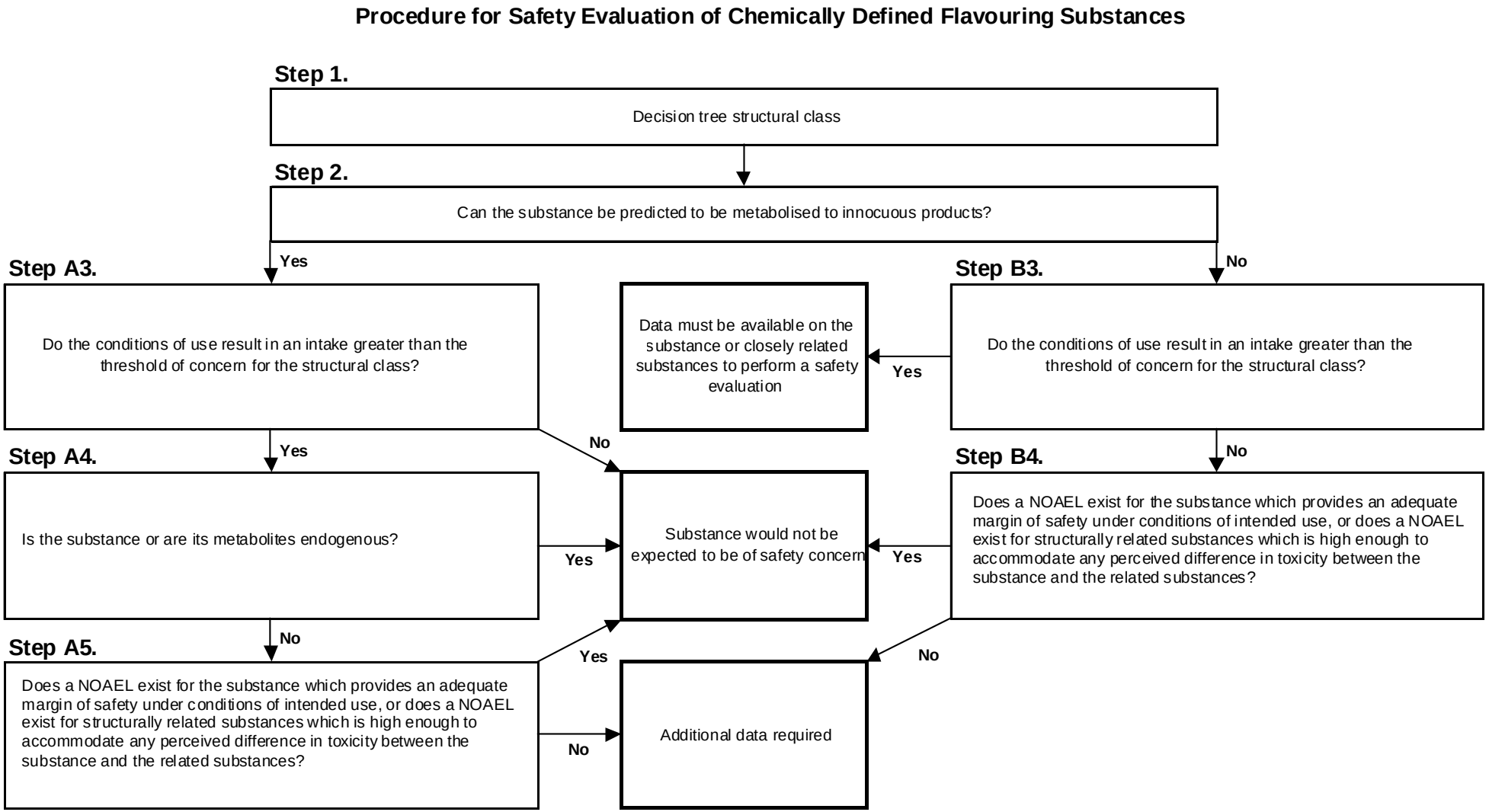


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 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	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10Rev3

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	100	25
06.097	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.102	3	2	3	2	-	10	5	10	2	2	-	-	52	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	5	50	15	50	75	25
07.169	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
08.053	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.082	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.090	3	2	3	2	-	10	5	10	2	-	-	-	5	10	5	10	15	5
	15	10	15	10	-	50	25	50	10	-	-	-	25	50	25	50	75	25
08.103	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
09.333	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.345	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.346	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.347	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.348	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.349	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.350	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.351	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.352	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.353	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.354	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.360	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.502	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.558	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.565	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.580	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	200	10	10	-	-	25	50	25	50	100	25
09.590	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.601	10	5	10	7	-	20	15	15	2	2	-	-	5	10	5	20	20	5
	50	75	50	35	-	100	75	75	10	10	-	-	25	50	50	100	100	25
09.626	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.629	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.633	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.634	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.644	7	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	10	5
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	50	25
09.683	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.815	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.824	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10Rev3

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
09.832	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.833	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.862	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.874	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.916	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.951	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	6
	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	10
10.038	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.039	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.040	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.045	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.047	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.048	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.049	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.055	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.058	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.059	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	30	25	50	100	25
10.063	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.068	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.170	5	2	1	1	1	4	2,2	3	-	-	-	-	101	-	3	2	2	2
	20	10	5	5	5	20	10	15	-	-	-	-	1005	-	10	10	10	10

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories
15.0	Ready-to-eat savouries	Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food

The mTAMDI values (see Table II.2.3) are presented for each of the 61 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2010g; Flavour Industry, 2010n). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	3900	Class I	1800
02.198	Octane-1,3-diol	3900	Class I	1800
05.149	Glutaraldehyde	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	1600	Class I	1800
08.053	Malonic acid	3200	Class I	1800
08.082	Glutaric acid	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	3800	Class I	1800
08.103	Nonanedioic acid	3200	Class I	1800
08.113	Succinic acid, disodium salt		Class I	1800
09.333	sec-Butyl lactate	3900	Class I	1800
09.345	Di-isopentyl succinate	3900	Class I	1800
09.346	Dibutyl malate	3900	Class I	1800
09.347	Dibutyl succinate	3900	Class I	1800
09.348	Diethyl adipate	3900	Class I	1800
09.349	Diethyl citrate	3900	Class I	1800
09.350	Diethyl fumarate	3900	Class I	1800
09.351	Diethyl maleate	3900	Class I	1800
09.352	Diethyl nonanedioate	3900	Class I	1800
09.353	Diethyl oxalate	3900	Class I	1800
09.354	Diethyl pentanedioate	3900	Class I	1800
09.360	Ethyl 2-acetoxypropionate	3900	Class I	1800
09.502	Ethyl butyryl lactate	3900	Class I	1800
09.558	Dimethyl malonate	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	3900	Class I	1800
09.580	Hexyl lactate	3900	Class I	1800
09.590	Isobutyl lactate	3900	Class I	1800
09.601	Isopentyl lactate	5100	Class I	1800
09.626	Methyl 2-oxopropionate	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	3900	Class I	1800
09.634	Methyl acetoacetate	3900	Class I	1800
09.644	Methyl lactate	3600	Class I	1800
09.683	Pentyl lactate	3900	Class I	1800
09.815	Propyl lactate	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	3900	Class I	1800
09.951	Dioctyl adipate	800	Class I	1800
10.038	Dec-7-eno-1,4-lactone	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	3900	Class I	1800
10.045	Heptano-1,5-lactone	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	3900	Class I	1800

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
10.049	Hexadecano-1,5-lactone	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	3900	Class I	1800
10.055	Pentano-1,5-lactone	3900	Class I	1800
10.058	Tridecano-1,5-lactone	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane		Class II	540
02.242	2-Butoxyethan-1-ol	3900	Class II	540
06.097	1,1,3-Triethoxypropane	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	4100	Class III	90
10.170	5-Pentyl-3H-furan-2-one	3800	Class III	90

1

ANNEX III: METABOLISM

III.1. Introduction

III.1.1. Equilibrium Between Aliphatic Lactones and Ring-opened Hydroxycarboxylic Acids: Effect of pH

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured (see Figure III.1). Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclisation are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones, with low molecular weight, being uncharged, may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

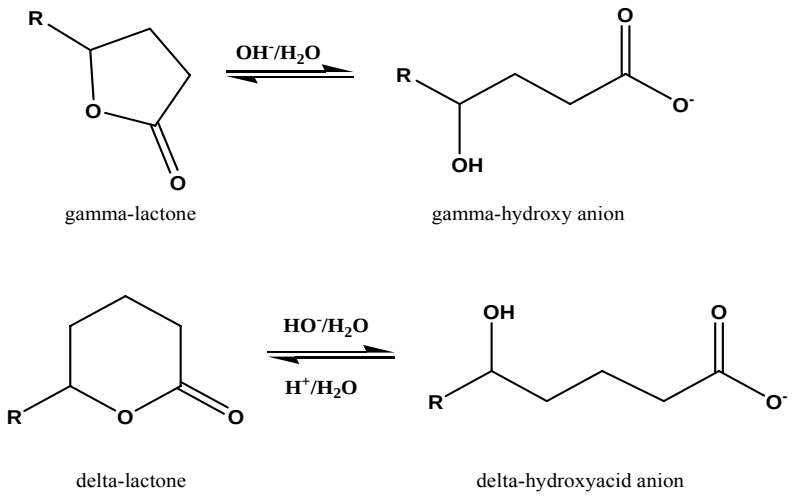


Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion*

III.1.2. Hydrolysis of Aliphatic Lactones

Fifteen candidate substances [FL-no: 10.038, 10.039, 10.040, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068, 10.168 and 10.170] are simple aliphatic lactones that are expected to readily undergo hydrolysis *in vivo*.

Information on the disposition of these substances is mainly derived from studies on a single supporting substance, butyro-1,4-lactone [FL-no: 10.006], which has been extensively studied due to the production of CNS depression, attributed to its hydrolysis product, gamma-hydroxybutyrate. No data on the candidate substances are available.

When 4-hydroxybutanoic acid gamma-lactone (butyro-1,4-lactone) is administered intravenously (Roth and Giarman, 1966), intraperitoneally (i.p.) or orally (Guidotti and Ballotti, 1970) to rats, the open-chain 4-hydroxybutanoate anion is detected in the blood and tissues and the sedative effect produced by 4-hydroxybutanoate was evidenced (Roth and Giarman, 1966; Guidotti and Ballotti, 1970). The half-life for the conversion of the lactone ring to the open-chain anion in the blood is less than one minute. The reaction is catalysed by gamma-lactonase, which shows greater activity in the plasma than in the liver or brain (Fishbein and Bessman, 1966).

Hydrolysis of various aliphatic lactones (1 mM), including those formed from tertiary alcohols, has been described after *in vitro* incubation in basic simulated intestinal fluid and rat liver homogenate, (Morgareidge, 1962a; Morgareidge, 1963a).

Table III.1. Hydrolysis of various aliphatic lactones

Substance	Test System	% Hydrolysis	Time (hr)	Reference
Gamma-Valerolactone	Simulated intestinal fluid	32	4	(Morgareidge, 1962a)
	Rat liver homogenate	93	1	(Morgareidge, 1963a)
Gamma-Nonalactone	Rat liver homogenate (pH= 7.5)	62-94	1	(Morgareidge, 1963a)
	Rat liver homogenate (pH =8)	81-88	1	(Morgareidge, 1963a)
Gamma-Undecalactone	Simulated intestinal fluid	58	1	(Morgareidge, 1962a)
	Rat liver homogenate (pH= 7.5)	26-40	4	(Morgareidge, 1963a)
	Rat liver homogenate (pH= 8)	45-70	1	(Morgareidge, 1963a)
Omega-6-Hexadecenlactone	Simulated intestinal fluid	92	0.25	(Morgareidge, 1962a)
	Simulated intestinal fluid	96	1	(Morgareidge, 1963a)
4,4-Dibutyl-gamma-butyrolactone	Simulated intestinal fluid	92	1	(Morgareidge, 1962a)

As shown in Table III.1, the rate and the extent of hydrolysis differ, depending on the lactone tested. The observation that gamma-lactones, sterically hindered gamma-lactones and omega-lactones are hydrolysed to the ring-opened form under these conditions supports the conclusion that the ring-opened hydroxycarboxylic acid anion exists in body fluids at basic pH. In acidic media, such as the gastric juice and the urine, the lactone form predominates.

Gamma-valerolactone and gamma-hexalactone have been detected in the urine of normal human adults (Zlatkis and Liebich, 1971).

III.1.3.Absorption of Aliphatic Lactones

Aliphatic lactones or the ring-opened hydroxycarboxylic acids are expected to be absorbed from the gastrointestinal tract. In rats, single oral doses >100 mg/kg bw/day of the supporting substance gamma-butyrolactone [FL-no: 10.006] were absorbed rapidly and completely from the intestinal tract (Arena and Fung, 1980; Guidotti and Ballotti, 1970; Lettieri and Fung, 1978). However, the lactone being an uncharged low molecular weight molecule may cross the cell membrane more easily than the ring-opened form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma-, delta- and omega-lactones, lactones fused to alicyclic rings such as 2-(2-hydroxycyclopent-4-enyl)ethanoic

acid gamma-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993).

Incubation of 1 mM of human R-type PON1 with aliphatic lactones gamma-butyrolactone, gamma-valerolactone, gamma-decanolactone and undecano-gamma-lactone resulted in hydrolysis rates of 9.1, 7.0, 19.0 and 13.0 µmol/min/ml substrate, respectively (Billecke et al., 2000). Hydrolysis is slower for the alicyclic fused-ring lactone, 2-(2-hydroxycyclopent-4-enyl)ethanoic acid gamma-lactone, with a hydrolysis rate of less than 3 µmol/min/ml substrate in the Q and R isoenzymes of PON1 (Billecke et al., 2000).

Based on these data, it is concluded that a wide variety of lactones readily hydrolyse in human blood serum support either prior to absorption or upon entering systemic circulation.

III.1.4.Metabolism of Lactones Formed From Linear and Branched-chain Aliphatic Hydroxy-carboxylic Acids

No literature data on the candidate substances are available; however, due to the simple structure of the substances, information on their metabolic fate may be derived from text books.

Linear aliphatic hydroxycarboxylic acids are hydrolysed and rapidly oxidised *via* the fatty acid pathway. Linear saturated 5-hydroxycarboxylic acids formed from delta-lactones are converted, *via* acetyl coenzyme A (CoA), to hydroxythioesters, which then undergo beta-oxidation and cleavage to yield an acetyl CoA fragment and a new beta-hydroxythioester reduced by two carbons. Even numbered-carbon acids continue to be oxidised and cleaved to yield acetyl CoA while odd numbered-carbon acids yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly while propionyl CoA is transformed into succinyl CoA, which then enters the citric acid cycle (Voet and Voet, 1990).

Linear saturated 4- or 6-hydroxycarboxylic acids formed from gamma- or epsilon-lactones participate in the same pathway as linear saturated 5-hydroxycarboxylic acids; however, loss of an acetyl CoA fragment produces an alpha-hydroxythioester, which undergoes oxidation and alpha-decarboxylation to yield a linear carboxylic acid and eventually carbon dioxide (Voet and Voet, 1990). In rats and dogs, the supporting substances, $^{14}CO_1$-gamma-decalactone and $^{14}CO_1$-gamma-dodecalactone, are metabolised in a manner similar to $^{14}CO_1$-lauric acid, with approximately 75 % of the labeled ^{14}CO being eliminated as carbon dioxide within 48 hours (Fassett, 1961).

The metabolic fate of the supporting substance butyro-1,4-lactone [FL-no: 10.006] has been extensively studied in animals and humans. The majority of ^{14}C-labeled 4-hydroxybutanoate administered by intravenous injection to rats was recovered as $^{14}CO_2$ within 2.5 hours (Roth and Giarman, 1965). Oxidation of gamma-butyrolactone to succinate by alcohol dehydrogenase and succinic semialdehyde dehydrogenase occurs primarily in the liver (Jakoby and Scott, 1959); succinate then participates in the citric acid cycle (Doherty and Roth, 1978; Lee, 1977; Möhler et al., 1976; Walkenstein et al., 1964). However, this pathway accounts for only a limited proportion of the metabolised compound. The main biotransformation route through which gamma-butyrolactone is metabolised is beta-oxidation as indicated by the presence of (S)-3,4-dihydroxybutyric acid, glycolic acid and 3-oxobutyric acid in the urine of human volunteers given orally 1.0 g gamma-butyrolactone [FL-no: 10.006] (Lee, 1977); other intermediates derived from beta-oxidation have previously been detected in samples of human urine (Walkenstein et al., 1964).

If the lactone is formed from a linear hydroxycarboxylic acid containing unsaturation, cleavage of acetyl CoA units will continue along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl CoA fragmentation will eventually yield a 3-enoyl CoA, which is converted to the *trans*-Δ_2-enoyl CoA before entering the fatty acid pathway. If unsaturation

begins at an even-numbered carbon, acetyl CoA fragmentation yields a Δ_2-enoyl CoA product, which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, hydration yields (R)-3-hydroxyacyl CoA, which is isomerised to (S)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering into normal fatty acid metabolism (Voet and Voet, 1990).

The principal metabolic pathways utilized for detoxication of branched-chain hydroxycarboxylic acids are influenced by the chain length and the position and size of alkyl substituents. Short-chain (< C6) branched aliphatic hydroxycarboxylic acids may be excreted conjugated mainly with glucuronic acid, or undergo alpha- or beta-oxidation followed by cleavage and complete metabolism to CO_2 (Voet and Voet, 1990; Williams, 1959a) via the fatty acid pathway and the tricarboxylic acid cycle. Alternatively, as chain length, substitution and lipophilicity increase, the hydroxycarboxylic acid may undergo a combination of omega-, omega-1 and beta-oxidation to yield polar hydroxyacid, ketoacid and hydroxydiacid metabolites that may be excreted as the glucuronic acid or sulphate conjugates in the urine and, to a lesser extent, in the faeces. Methyl substituted carboxylic acids are, to some extent, omega-oxidised in animals to form diacids, which can be detected in the urine (Williams, 1959a).

Carboxylic acids with a methyl substituent located at an even-numbered carbon (e.g. 2-methylpentanoic acid or 4-methyldecanoic acid) are metabolised extensively in the fatty acid pathway to CO_2 via beta-oxidation and cleavage of the longer branched-chain. If the methyl group is located at an odd-numbered carbon such as the 3-position, beta-oxidation is inhibited and omega-oxidation predominates, primarily leading to polar, acidic metabolites capable of being excreted in the urine as such or as conjugates (Williams, 1959a). Larger alkyl substituents (> C2) located at the alpha- or beta-position inhibit metabolism to CO_2 (Albro, 1975; Deisinger et al., 1994; Deuel, 1957) in which case there is either direct conjugation of the acid with glucuronic acid or omega-oxidation leading to diacid metabolites, which may be conjugated and excreted.

III.2. Absorption, Metabolism and Elimination of: Esters, Acetals, Aliphatic Primary Alcohols, Aldehydes, and Carboxylic Acids Containing Additional Oxygenated Functional Groups

III.2.1.Mono- and Di-esters

Thirty-two candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874 09.916 and 09.951]. They are expected to undergo hydrolysis in humans to yield their corresponding alcohol (linear or branched-chain aliphatic alcohols) and acid components (i.e. alpha-, beta- or gamma-keto or hydroxy acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised. The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters; therefore the discussion and conclusions presented in previous evaluations (FGE.01 and FGE.02) apply equally well to the candidate esters in the present evaluation.

Hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases or esterases (Heymann, 1980), the most important of which are the B-esterases (Anders, 1989; Heymann, 1980). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals, these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980).

The majority of degradation products yielded from the candicate ester hydrolysis are endogenous in mammals and are known to be completely metabolised, through different reactions, depending on their chain length and degree of branching and functional groups. It is likely that multiple metabolic reactions will occur for some hydrolysis products. The most probable metabolic reactions are the following:

- Oxidation of alcohols to aldehydes and acids.
- Conjugation of alcohols and acids to glucuronides and sulphates.
- Beta-oxidation of carboxylic acids.
- Omega-oxidations of carboxylic acids.

However, the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, has some structural similarities to valproic acid, which together with a number of its derivatives has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Although it can be predicted that 2-acetyl butyric acid is further metabolised through the above mentioned pathways of detoxication for carboxylic acids, the structural similarity with valproic acid does no allow to anticipate that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocuous products.

While no hydrolysis data have been provided for the esters of the present group of flavourings, information on some structurally related esters could be found.

In vitro incubation of the supporting substance methyl 2-oxo-3-methylvalerate [FL-no: 09.550], with a 2 % pancreatin solution (pH = 7.5), resulted in virtually complete hydrolysis (> 98 %) within 80 minutes (Leegwater and VanStraten, 1979). The supporting substance dibutyl sebacate [FL-no: 09.474] in 10 % acacia solution, was hydrolysed *in vitro* in a 10 % crude pancreatic lipase solution (Smith, 1953b).

The supporting substance ^{14}C-tributyl acetylcitrate [FL-no: 09.511], administered to male Sprague-Dawley rats by gavage at a dose level of 70 mg/kg bw, was rapidly absorbed ($t_{½}$ = 1 hour) and partially hydrolysed. More than 87 % of the administered radioactivity was eliminated within 24 hours after dosing. At least nine urinary metabolites (59 - 70 %) were detected. Five metabolites were positively identified as the partially hydrolysed mono-, di- and tri-alkylesters of citric acid. Three metabolites (25 - 26 %) were identified in the faeces; approximately 2 % of the administered dose was eliminated as $^{14}CO_2$ (Hiser et al., 1992).

III.2.2.Acetals

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption.

In vitro experiments using simulated gastric fluid revealed the rates of hydrolysis of acetals to be dependent on the structures of the aldehyde and alcohol moieties. Acetals derived from short (< C8) chain saturated aldehydes were hydrolysed almost instantly (Engel, 2003).

Hydroxycitronellal dimethyl acetal similar to the supporting substance hydroxycitronellal diethyl acetal was > 99 % hydrolysed *in vitro* to the terpenoid hydroxycitronellal and methanol in simulated gastric juice (pH about 2.1) after 1 hour and > 6 % hydrolysed in intestinal fluid (pH = 7.5) after 2 hours (Morgareidge, 1962b).

Once hydrolysed, the component alcohol, aldehydes and acids are expected to be completely metabolised, through the above mentioned common routes of biotransformations and excreted.

III.2.3.Alpha-hydroxy- and Alpha-keto-acids and Their Esters

One candidate substance [FL-no: 08.090] is an alpha-hydroxyacid. In addition alpha-keto- and alpha-hydroxyacids are formed by hydrolysis of candidate esters [FL- No: 09.333, 09.346, 09.353, 09.565, 09.580,

09.590, 09.601, 09.626, 09.644, 09.683, 09.815 and 09.874]. They would be expected to be metabolised like endogenous alpha-ketoacids formed from oxidative deamination of amino acids such as isoleucine, methionine and valine *in vivo*.

The supporting substance, 2-oxobutyric acid [FL-no: 08.066] (i.e. alpha-ketobutyric acid), is endogenous in humans as a product of methionine degradation and undergoes alpha-decarboxylation to yield propionyl CoA. Propionyl CoA ultimately enters the tricarboxylic acid cycle as succinyl CoA (Voet and Voet, 1990).

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

One candidate substance [FL-no: 08.053] is a beta-ketoacid. In addition eight candidate substances [FL-no: 09.346, 09.558, 09.629, 09.634, 09.824, 09.862, 09.874 and 09.916] are precursor of acetoacetic acid or its beta-hydroxy or aldehyde precursor. [FL-no: 09.346, 09.629, 09.862, 09.874 and 09.916] can be oxidised *in vivo* to acetoacetic acid. Acetoacetic acid is endogenous in humans and is formed from the condensation of two acetyl CoA units in the fatty acid pathway. It is released from the liver into the bloodstream and transported to peripheral tissues where it is converted to acetyl CoA and is completely metabolised. At elevated endogenous levels, beta-ketoacids may undergo non-enzymatic decarboxylation, which, for acetoacetic acid, yields acetone and CO_2 (Voet and Voet, 1990).

III.2.5.Gamma-keto- and Gamma-hydroxyacids and Their Esters

Gamma-hydroxy and gamma-keto acids are produced by hydrolysis of two candidate substances [FL-no: 09.832 and 09.833]. They are expected to be completely metabolised to CO_2 at low levels of exposure from use as flavouring substances. At elevated levels of exposure, the ketone function may be reduced to the corresponding secondary alcohol (Bosron and Li, 1980) and excreted as the glucuronic acid conjugate (Williams, 1959a).

Products of partial beta-oxidation or glucuronic acid conjugation have also been identified in the urine. When 1.0 g of the structurally related substance gamma-hydroxybutyrate [FL-no: 10.006] was administered to humans, it was excreted in the urine as *S*-3,4-dihydroxybutyrate, 3-oxobutyric acid and glycolate (Lee, 1977).

III.2.6.Aliphatic Di- and Tricarboxylic Acids and Their Esters

Among candidate substances the aliphatic di- and tri-carboxylic acids and their precursors [FL-no: 05.149, 08.053, 08.082, 08.103, 08.113, 09.345, 09.346, 09.347, 09.348, 09.349, 09.350, 09.351, 09.352, 09.353, 09.354, 09.558, 09.874 and 09.951] either occur endogenously in humans or are structurally related to endogenous substances. Succinic acid (from [FL-no: 09.345 and 09.347]), fumaric acid (from [FL-no: 09.350]), *l*-malic acid (from [FL-no: 09.346 and 09.874]), maleic acid (from [FL-no 09.351]) and citric acid (from [FL-no: 09.349]), are components of the tricarboxylic acid cycle (Voet and Voet, 1990). Fumaric acid is present in the blood, brain, liver, muscle and kidney of normal rats (Marshall et al., 1949). Moreover, the following acids are present in the urine of normal adults, citric, tartaric, malic, aconitic, fumaric and adipic (Hanson, 1943; Osteux and Laturaze, 1954). Alpha-ketoglutaric acid is an intermediate metabolite of citric acid, fumaric acid and succinic acid, and is formed via alpha-oxidation (Krebs et al., 1938; Simola and Krusius, 1938).

Simple aliphatic di- and tricarboxylic acid candidate substances and component acids of the candidate esters are metabolised in the fatty acid beta-oxidation pathway or tricarboxylic acid cycle. When the supporting substance ^{14}C-*l*-malic acid [FL-no: 08.017] was administered to male albino Wistar rats by gavage at a dose level of 2.5 mg/kg bw, 93 % of the radioactivity was recovered in expired air, urine and faeces (Dargel, 1966).

After the administration of the radioactive supporting substance adipic acid [FL-no: 08.026] to rats by stomach tube at a dose level of 200 - 300 mg/kg bw, the compound was extensively metabolised. Labelled products identified in the urine included glutamic acid, lactic acid, beta-ketoadipic acid and citric acid. The presence of the beta-oxidation metabolite, beta-ketoadipic acid, indicates that adipic acid participates in beta-oxidation in the fatty acid pathway (Rusoff et al., 1960).

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids (Bosron and Li, 1980; Feldman and Weiner, 1972; Levi and Hodgson, 1989). The resulting carboxylic acids would be metabolised in the fatty acid pathway and tricarboxylic acid cycle (Voet and Voet, 1990) or conjugated to glucuronides and sulphates and excreted. Branched-chain diols or keto alcohols may undergo oxidation to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted, through the common routes of biotransformation of carboxylic acids.

III.2.7.Aliphatic Alkoxy- alcohol and Diols

Among candidate substances, one is an alkoxy-alcohol [FL-no: 02.242] and two are diols [FL-no: 02.132 and 02.198].

The metabolism and disposition of 2-butoxyethanol [FL-no: 02.242] were extensively studied, and details are reported below. However, it can be anticipated that the major metabolite is butoxyacetic acid, which is primarily responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

1-Hydroxypropan-2-one [FL-no: 07.169] (acetol) is an endogenous metabolite of acetone which is also an endogenous substance formed from the degradation of body fat/fatty acids.

The metabolism in mammals of acetone, which at low concentrations, primarily occurs in the liver, is shown in Figure III.2. At low acetone concentrations in blood, i.e. in healthy humans not exposed to external sources, in amounts of approximately 4 - 12 mg per person corresponding to 0.7 to 2 mg/l blood (Ashley et al., 1994; Dick el al., 1988; Wang et al, 1994c), the major pathway is via the methylglyoxal route. At higher acetone concentrations in the blood, e.g. after acetone exposure, after fasting or in relation to certain deceases the propan-1,2-diol route is the dominating pathway. In the fist step acetone is oxidized to 1-hydroxypropan-2-one via acetone monooxygenase (p-450 IIE1). 1-Hydroxypropan-2-one is oxidised to 2-oxopropanal via acetol monooxygenase (p-450 IIE1), or at higher acetone concentrations to propan-1,2-diol. 2-Oxopropanal is then oxidised to pyruvate leading to glucose formation (Morgott, 1993; WHO, 1998a; NAS/COT, 2005).

The diols are anticipated to be metabolised by the common route of alcohol biotransformation, i.e. direct conjugation or oxidation by alcohol-dehydrogenase to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted.

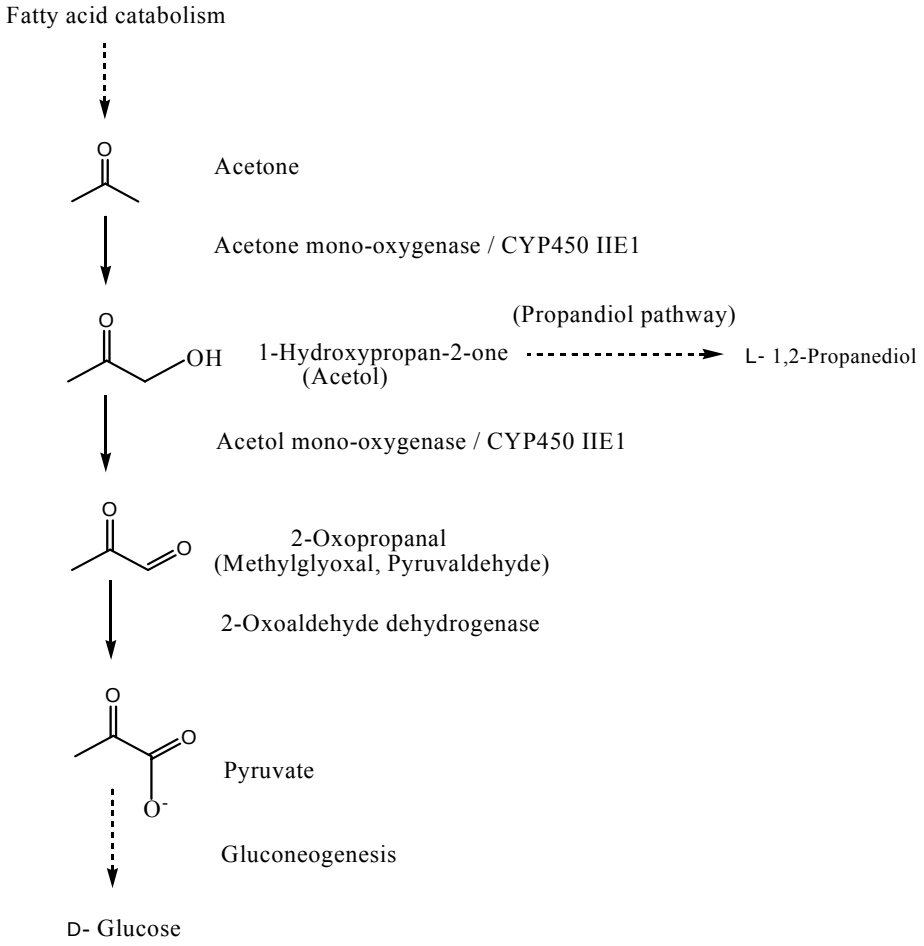


Figure III.2. *Acetone metabolism (methylglyoxal pathway)*

III.3. Studies on Candidate Substances

2-Butoxyethan-1-ol [FL-no: 02.242]

Several experiments by the oral route of administration have been conducted, indicating that 2-butoxyethan-1-ol is rapidly absorbed, metabolised and eliminated. Butoxyacetic acid is its major metabolite, metabolism being mainly catalysed by hepatic alcohol dehydrogenase; most excretion is in the urine (Corley et al., 1994; Ghanayem et al., 1987a; Ghanayem et al., 1987b; Ghanayem et al., 1987c; Medinsky et al., 1990).

The distribution and excretion of ^{14}C-butoxyethanol and its metabolites was evaluated using male F344 rats (9 - 13 weeks old). A single 125 or 500 mg/kg dose of ^{14}C-butoxyethanol was administered to each animal via gavage. Animals were killed 48 hours post-administration and tissues excised. At 48 hours, approximately 18 % and 10 % of the administered dose was exhaled as $^{14}CO_2$ for the 125 and 500 mg/kg doses, respectively; whereas only between 2 and 3 % was excreted in the faeces. The percentage of the 125 mg/kg dose excreted in the urine (70 %) was significantly greater than the percentage excreted after the 500 mg/kg dose (40 %). Butoxyacetic acid was the only urinary metabolite detected for the 125 mg/kg dose; the glucuronide conjugates of butoxyethanol and butoxyacetic acid (23 %) were also detected in the urine of animals dosed with the higher dose. A small portion (8 %) of the 500 mg/kg dose was excreted in the bile 8 hours after dosing. Compared to the 125 mg/kg dose group, tissue concentrations of ^{14}C-butoxyethanol 48

hours after administration were significantly greater in specific organs of rats that received the 500 mg/kg dose. In both dose groups the highest concentration of radioactivity was detected in the forestomach, followed by the liver, kidneys, spleen and the glandular stomach (Ghanayem et al., 1987c).

The metabolism and excretion of 2-butoxyethan-1-ol [FL-no: 02.242] were evaluated using both young (4 to 5 weeks old) and adult (9 to 13 weeks old) male F344 rats with the same experimental design described in Ghanayem *et al*. (1987c), except that ^{14}C-butoxyethanol was administered at a single oral dose (500 mg/kg). There was a significantly higher proportion of the administered dose eliminated as CO_2 in young rats as compared to older rats. Similarly, a significantly higher proportion of the administered dose was excreted in the urine of the young rats. The butoxyacetic acid/butoxyethanol-glucuronide + butoxyethanol-sulphate ratio was significantly greater in older rats (Ghanayem et al., 1987a), which are consistently more susceptible to the toxic action of 2-butoxyethan-1-ol . There was a strong correlation between the amount of butoxyacetic acid in the urine and 2-butoxyethanol-induced haematotoxicity. Moreover, metabolic activation via alcohol and aldehyde dehydrogenases is a prerequisite for the induction of toxic effects, since pre-treatment of rats with pyrazole (alcohol dehydrogenase inhibitor) or cyanamide (aldehyde dehydrogenase inhibitor) protected rats against 2-butoxyethanol-induced haematotoxicity and increased the urinary amount of butoxyethanol-conjugates (glucuronide and sulphate) (Ghanayem et al., 1987b).

2-Butoxyethan-1-ol [FL-no: 02.242] was administered to male F344/N rats (11 to 12 weeks old) at concentrations in drinking water of 290, 860 and 2590 ppm over a 24 hours period. Butoxyethanol was administered as 2-butoxy[U-^{14}C]ethanol, and exhaled air, urine and faeces were collected over a 72 hours period. Most ^{14}C was excreted either in the urine or exhaled as CO_2: 50 - 60 % of the administered dose was eliminated in the urine as butoxyacetic acid and 8 to 10 % as CO_2. Analysis of urine samples collected during the 12 - 24 hours after dosing indicated that the majority of the radioactivity was associated with butoxyacetic acid while 10 % of the administered dose was identified as glycol ether. Minor levels of glucuronide conjugate of butoxyethanol and unmetabolised butoxyethanol were also reported (Medinsky et al., 1990).

Non-oxidative metabolism of 2-butoxyethan-1-ol [FL-no: 02.242] via fatty acid conjugation was also investigated in the liver of F344 male rats following a single oral administration of 500 mg/kg [ethyl-1,2-^{14}C] 2-butoxyethanol. Animals were killed two hours after treatment and samples prepared for analysis. It was demonstrated that 2-butoxyethan-1-ol is metabolised non-oxidatively via conjugation with long-chain fatty acids, and the formation of these esters appears to be catalysed by the enzymes involved in fatty acid conjugation of xenobiotic alcohols. However, the biological significance of 2-butoxyethan-1-ol conjugation with fatty acids remains unclear, although several such lipid conjugates were found to be toxic in laboratory animals and cell lines (Kaphalia et al., 1996).

The elimination kinetics of 2-butoxyethan-1-ol were studied in a once-through isolated perfused rat liver system in the presence and absence of ethanol. Dose-dependent Michaelis-Menten kinetics were observed in the elimination of 2-butoxyethan-1-ol. The apparent K_m ranged from 0.32 to 0.70 mM and the maximum elimination rate ranged from 0.63 to 1.4 micromol/min/g liver in six experiments. The results support the hypothesis that 2-butoxyethan-1-ol is metabolised mainly via oxidation by alcohol dehydrogenase in the rat liver at concentration which can be considered representative of human exposure (Johanson et al., 1986).

Butane-1,3-diol [FL-no: 02.132]

Two groups of 14 rats were administered a control diet (70 % carbohydrate and 30 % fat) or a treatment diet (45 % carbohydrate, 30 % fat and 25 % butane-1,3-diol). Blood acetoacetate and beta-hydroxybutyrate concentrations were increased significantly and blood pyruvate concentration was decreased significantly in rats administered the treatment diet. Addition of butane-1,3-diol to *in vitro* liver tissue slices, as they were metabolising glucose to lactate and pyruvate, greatly decreased pyruvate levels and significantly increased lactate/pyruvate ratios. When butane-1,3-diol and glucose were used as substrates, there was a large increase in acetoacetate and beta-hydroxybutyrate formation in liver tissue slices with butane-1,3-diol. Therefore,

butane-1,3-diol is metabolised in the cytosol and converted by the liver *in vivo* and *in vitro* to ketones prior to its oxidation in the tricarboxylic acid cycle (Mehlman et al., 1971).

Tate *et al.* (1971) found that the conversion of butane-1,3-diol to beta-hydroxybutyrate in rat liver was strongly dependent in NAD^+ and it was inhibited by pyrazole. Since pyrazole is a specific inhibitor of alcohol dehydrogenase (ADH), this inhibition indicated ADH as the catalyst in the catabolism in the cytosol of butane-1,3-diol to an intermediate, aldol. Aldol is then further oxidised to beta-hydroxybutyrate (Tate et al., 1971).

Diethyl maleate [FL-no: 09.351]

Traditionally diethyl maleate [FL-no: 09.351] has been utilised to acutely deplete reduced glutathione (GSH) in the tissues, since it forms GHS-conjugates very rapidly, causing a significant decrease in GSH content (Boyland & Chasseaud, 1970). The liver is the most sensitive organ to diethyl maleate-induced GSH depletion, generally occurring 30 - 90 minutes after intraperitoneal injection of the compound. In the rat, the formed GSH-conjugates are excreted in bile or as mercapturates in urine (Barnhart and Combes, 1978).

The excretion of mercapturic acid was determined in chimpanzees and rats after the administration of diethyl maleate [FL-no: 09.351]. The excretion rate of endogenous thioethers in the urine of untreated chimpanzees and rats was 18.0 and 94.4 micromol/kg bw/24 hours, respectively. The value in man was nearly the same as found in chimpanzees. The administration of diethyl maleate at 30, 75 and 200 mg/kg bw led to a dose-dependent increase in the excretion of urinary mercaptic acids in both species, but the increase in rats was about twice that of chimpanzees. Additional experiments indicate that the observed species differences are due to differences in the glutathione conjugation (Summer et al., 1979a).

Glutaric acid [FL-no: 08.082]

Rat liver mitochondria metabolise glutarate [FL-no: 08.082] at a slow rate as compared with glutaryl CoA. The stimulatory effect of citric acid cycle intermediates, NAD and CoA on glutarate metabolism was interpreted as a manifestation of their involvement in the activation of glutarate by a thiol transferase with succinyl CoA as the coenzyme A donor (Besrat et al., 1969).

Glutaraldehyde [FL-no: 05.149]

Material mass balance and pharmacokinetics studies were conducted with glutaraldehyde [FL-no: 05.149] in groups of F344 rats (four/sex) and New Zealand white rabbits (two/sex) using the intravenous route of exposure at dose volumes of 0.2 ml and 2.5 ml, respectively. Rats and rabbits received intravenous doses of 0.075 and 0.75 % glutaraldehyde in the tail vein or ear vein, respectively. Glutaraldehyde was distributed rapidly and eliminated when administered intravenously to rats and rabbits. When a single infusion of 0.075 % glutaraldehyde was administered, 75 to 80 % of the dose in the rat and 66 to 71 % in the rabbit were recovered as $^{14}CO_2$ during the first 24 hours following administration, with 80 % of the $^{14}CO_2$ being recovered during the first four hours. When a single infusion of 0.75 % glutaraldehyde was administered, the proportion of the dose recovered as $^{14}CO_2$ decreased and the amount of radioactivity recovered in urine, tissues and carcass increased as compared to the 0.075 % glutaraldehyde infusion. Also the average plasma concentration of radioactivity increased 10-fold in rats and rabbits with a 10-fold increase in dose, but the tissue concentration increased by an even greater amount. The results suggest that the mechanisms involved in the disposition of glutaraldehyde were saturated when the higher dose was administered and resulted in a shift in the elimination pathway (McKelvey et al., 1992). Although the metabolism of glutaraldehyde has not been studied in detail, it has been suggested that it is oxidised first to a mono- or dicarboxylic acid by aldehyde dehydrogenase (Weiner, 1980; Hjelle and Peterson, 1983) and then further oxidised through an acidic intermediate to CO_2 (McKelvey et al., 1992).

Nonanedioic acid [FL-no: 08.103]

Following intravenous administration in human volunteers, nonanedioic acid [FL-no: 08.103] and its major catabolite, pimelic acid, are found in serum and urine indicating transformation by mitochondrial beta-oxidative enzymes. Serum levels of nonanedioic acid are short-lived following a single 5 or 10 g intravenous (i.v.) infusion over 1-hour. In the first hour after the cessation of i.v. administration, serum levels of nonanedioic acid decreased to about 25 % of their peak values. Administration of multiple intravenous doses at the same concentrations as the one-hour doses produces sustained higher levels of nonanedioic acid in the serum during the period of administration (Passi et al., 1989).

III.4. Conclusions

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured, while in acidic media, such as gastric juice and urine, the lactone ring is favoured.

Lactones formed from linear saturated and branched-chain aliphatic hydroxycarboxylic acids are hydrolysed to the corresponding hydroxycarboxylic acid that then enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by two-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then completely metabolised in the citric acid cycle.

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (linear or branched-chain aliphatic alcohols) and acid components (i.e. alpha-, beta- or gamma-keto- or hydroxy-acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised and excreted through the common pathways of detoxication of aliphatic alcohols and carboxylic acids). The hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, which shows some structural similarities to valproic acid, which together with a number of its derivatives, has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Therefore, it cannot be anticipated that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocuous products.

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

Beta-keto acids and derivatives like acetoacetic acid undergo decarboxylation. Along with alpha-keto and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways. The gamma-keto-acids and related substances may undergo complete or partial beta-oxidation to yield metabolites that are eliminated in the urine. Omega-substituted derivatives are readily oxidised and/or excreted in the urine. Simple aliphatic di- and tricarboxylic acids participate in the tricarboxylic acid cycle.

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

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their

corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation.

Among candidate substances, an alkoxy-alcohol 2-butoxyethanol [FL-no: 02.242] is mainly metabolised to butoxyacetic acid, which has been identified as the major responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products many of which are endogenous in humans, at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351]; as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to the structurally related ethoxypropanoic acid and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], whose hydrolysis gives rise to 2-acetylbutyric acid, with some structural similarities to valproic acid, a known teratogenic compound.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 16 candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30, for 43 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Methyl 2-hydroxy-4-methylpentanoate [09.548])	Mouse	NR	Oral	4000[1]	(Pellmont, 1978)
(Methyl 2-oxo-3-methylvalerate [09.550])	Rat	M	Gavage	> 5000	(Moreno, 1979b)
(Butyro-1,4-lactone [10.006])	Mouse	NR	Gavage	1245	(Schafer and Bowles, 1985)
(Pentano-1,4-lactone [10.013])	Rat	NR	Oral	> 5000	(Moreno, 1978e)
	Rat	NR	Gavage	8800	(Deichmann et al., 1945)
	Rabbit	NR	Gavage	2480	(Deichmann et al., 1945)
(Hexano-1,4-lactone [10.021])	Rat	NR	Oral	> 5000	(Moreno, 1977f)
(Hexano-1,5-lactone [10.010])	Rat	M	Gavage	13,030	(Smyth et al., 1962)
(Heptano-1,4-lactone [10.020])	Rat	NR	Oral	> 5000	(Moreno, 1977g)
(Octano-1,4-lactone [10.022])	Rat	NR	Oral	> 5000	(Moreno, 1974c)
(Octano-1,5-lactone [10.015])	Rat	NR	Oral	> 5000	(Moreno, 1977h)
(Nonano-1,4-lactone [10.001])	Rat	M, F	Gavage	9780	(Jenner et al., 1964)
	Rat	M	Oral	6600	(Moreno, 1972b)
	Guinea pig	M, F	Gavage	3440	(Jenner et al., 1964)
(Decano-1,4-lactone [10.017])	Rat	NR	Oral	> 5000	(Moreno, 1975h)
(Decano-1,5-lactone [10.007])	Rat	NR	Oral	> 5000	(Levenstein, 1975c)
(Decano-1,6-lactone [10.029])	Mouse	M, F	Gavage	5252	(Moran et al., 1980)
(Undecano-1,4-lactone [10.002])	Rat	M, F	Gavage	18500	(Jenner et al., 1964)
(Undecano-1,5-lactone [10.011])	Rat	NR	Oral	> 5000	(Moreno, 1975i)
(Dodecano-1,4-lactone [10.019])	Rat	NR	Oral	> 5000	(Moreno, 1974d)
(Dodecano-1,5-lactone [10.008])	Rat	NR	Oral	> 5000	(Moreno, 1977e)
(Dodecano-1,6-lactone [10.028])	Mouse	M, F	Gavage	7898	(Moran et al., 1980)
(Pentadecano-1,15-lactone [10.004])	Rat	NR	Oral	> 5000	(Levenstein, 1974c)
(5-Methylfuran-2(3H)-one [10.012])	Mouse	M, F	Gavage	2800	(Moran et al., 1980)
(Dodec-6-eno-1,4-lactone [10.009])	Rat	M, F	Oral	> 5000	(Watanabe and Morimoto, 1990)
(3,7-Dimethyloctano-1,6-lactone [10.027])	Rat	M, F	Gavage	> 5000	(Lewis and Palanker, 1979a)
(5-Hexyl-5-methyldihydrofuran-2(3H)-one [10.051])	Rat	NR	Oral	> 5000	(Moreno, 1976j)
(Citronellyl oxyacetaldehyde [05.079])	Rat	NR	Oral	> 5000	(Moreno, 1973d)
1-Hydroxypropan-2-one [07.169]	Rat	NR	Oral	2200[2]	(Smyth and Carpenter, 1948)
(4,4-Dimethoxybutan-2-one [06.038])	Rat	M	Gavage	6200	(EPA, 1971)
(Ethyl acetoacetate [09.402])	Rat	NR	Oral	3980[3]	(Smyth et al., 1949)
Methyl acetoacetate [09.634]	Rat	NR	Oral	3000	(Smyth and Carpenter, 1948)
	Rat	NR	Oral	2800	(BASF, 1978)
(Butyl acetoacetate [09.403])	Rat	F	Gavage	11260	(Smyth et al., 1954)
(Geranyl acetoacetate [09.405])	Rat	NR	Oral	> 5000	(Moreno, 1976k)
(Ethyl 3-oxohexanoate [09.542])	Mouse	NR	Oral	4000 – 8000	(Pellmont, 1973a)
2-Butoxyethan-1-ol [02.242]	Rat	M	Gavage	1480	(Smyth et al., 1941)
	Rat	NR	Oral	1174	(BASF, 1956)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
	Rat	NR	Oral	620	(Rowe and Wolf, 1982)
	Rat	M, F	Oral	2800	(Carpenter et al., 1956)
	Rat	M	Gavage	2680	(Myers and Homan, 1980)
	Rat	NR	Oral	470	(Wolf, 1959)
	Rat	M	Gavage	1190 – 2800	(Weil and Wright, 1967)
	Rat	M	Gavage	1590	(Moreno, 1976l)
	Rat	M	Gavage	7500	(Moreno, 1976l)
	Rat	NR	Oral	1746	(Eastman Kodak Co., 1989)
	Rat	M	Gavage	7292	(Eastman Kodak Co., 1984)
	Mouse	NR	Oral	1230	(Carpenter et al., 1956)
	Mouse	NR	Oral	1170 – 1700	(Dow Chemical Company, 1982a)
	Mouse	NR	Oral	1519	(Eastman Kodak Co., 1989)
	Mouse	M	Gavage	2406	(Eastman Kodak Co., 1984)
	Rabbit	M	Oral	320 – 370	(Carpenter et al., 1956)
	Guinea pig	M, F	Oral	1200	(Carpenter et al., 1956)
	Guinea pig	M, F	Gavage	1200	(Smyth et al., 1941)
Butane-1,3-diol [02.132]	Rat	F	Gavage	> 5000	(CTFA, 1978)
	Rat	M	Gavage	18610	(Smyth et al., 1941)
	Rat	M	Gavage	22800	(Smyth et al., 1951a)
	Rat	NR5	Oral	29590	(Bornmann, 1954)
	Mouse	NR5	Oral	23440	(Bornmann, 1954)
	Mouse	NR	Oral	23310	(Kopf et al., 1950; Loeser, 1949)
	Mouse	NR	Oral	12980	(Wenzel and Koff, 1956)
	Guinea pig	M, F	Gavage	11460	(Smyth et al., 1941)
(4-Oxovaleric acid [08.023])	Rat	NR	Oral	1850	(Moreno, 1977j)
(Ethyl 4-oxovalerate [09.435])	Rat	NR	Oral	> 5000	(Moreno, 1978f)
Octane-1,3-diol [02.198]	Rat	NR	Oral	> 20000	(Frankenfeld et al., 1975)
(3,7-Dimethyloctane-1,7-diol [02.047])	Rat	M, F	Gavage	> 5000	(Levenstein, 1973b)
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Rat	NR	Oral	> 5000	(Shelanski and Moldovan, 1973b)
1,1,3-Triethoxypropane [06.097]	Rat	M	Gavage	1600	(Smyth et al., 1951a)
Diethyl oxalate [09.353]	Rat	NR	Oral	400 – 1600	(Patty, 1963)
Malonic acid [08.053]	Rat	NR	Oral	1310	(Bio-Fax, 1971)
Dimethyl malonate [09.558]	Rat	NR	Oral	4620	(Levenstein, 1976b)
	Rat	NR	Oral	5331	(Merck Index, 1992)
(Diethyl malonate [09.490])	Rat	NR	Oral	14900	(Smyth et al., 1969a)
	Mouse	NR	Gavage	5400	(Wolven and Levenstein, 1969)
(Diethyl succinate [09.444])	Rat	NR	Oral	8530[3]	(Smyth et al., 1951a)
(Fumaric acid [08.025])	Rat	M, F	Oral	M: 10700; F: 9300	(Vernot et al., 1977)
Diethyl fumarate [09.350]	Rat	NR	Oral	1500	(Hood, 1951)
(l-Malic acid [08.017])	Rat	NR	Oral	3500	(Morgareidge, 1973a)
	Mouse	NR	Oral	2660	(Morgareidge, 1973b)
	Rabbit	NR	Oral	3000	(Morgareidge, 1973c)
Diethyl maleate [09.351]	Rat	M	Gavage	3200	(Smyth et al., 1949)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Rat	NR	Oral	7500[6]	(Foulger, 1947)
Glutaric acid [08.082]	Mouse	NR	Oral	6000	(Boyland, 1940)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
Glutaraldehyde [05.149]	Rat	NR	Gavage	252	(Stonehill et al., 1963)
	Rat	M	Gavage	733[7]	(Ballantyne and Myers, 2001)
	Rat	M	Gavage	2380[8]	(Smyth et al., 1962)
	Rat	M	Gavage	540[9]	(Striegel and Carpenter, 1964)
	Rat	M, F	Oral	M: 134; F: 165	(Ikeda, 1980)
	Rat	M	Gavage	1300[7]	(Myers et al., 1977b)
	Rat	M	Gavage	1870[8]	(Myers et al., 1977c)
	Mouse	NR	Gavage	352	(Stonehill et al., 1963)
	Mouse	M, F	Oral	M: 100; F: 110	(Ikeda, 1980)
	Mouse	M, F	Gavage	M: 152[7] ; F: 113[7]	(Ballantyne and Myers, 2001)
	Mouse	M, F	Gavage	M: 151[8] ; F: 115[8]	(Union Carbide Corp., 1992)
(Adipic acid [08.026])	Mouse	M	Oral	1900[10]	(Horn et al., 1957)
Diethyl adipate [09.348]	Rat	NR	Oral	> 1600	(Patty, 1963)
Nonanedioic acid [08.103]	Rat	M, F	Gavage	> 4000	(Mingrone et al., 1983)
	Rabbit	M, F	Gavage	> 4000	(Mingrone et al., 1983)
(Diethyl sebacate [09.475])	Rat	M, F	Gavage	14470	(Jenner et al., 1964)
	Rat	M	Oral	32000[11]	(Smith, 1953b)
	Mouse	NR	Gavage	> 32000	(Lawrence et al., 1974)
(Triethyl citrate [09.512])	Rat	NR	Gavage	7000[4]	(Finkelstein and Gold, 1959)
(Tributyl acetylcitrate [09.511])	Rat	NR	Gavage	> 30000[12]	(Finkelstein and Gold, 1959)
(3-Hydroxy-2-oxopropionic acid [08.086])	Rat	NR	Oral	2000	(Hoechst, 1995)
Succinic acid, disodium salt [08.113]	Rat	NR	Oral	>1200	MHLW Japan 2002 in: (OECD, 2003)

M = Male; F = Female
NR: Not reported.
[1] Dosed in 5 % gum arabic.
[2] Data derived from a range-finding study.
[3] Actual LD_{50} not reported.Study conducted as a dose range-finder (DRF).
[4] Actual LD_{50} not reported.Value reported as approximate LD_{50}.
[5] Data point not verified.
[6] Actual LD_{50} not reported.Value reported as MFD (assumed to be Median Fatal Dose).
[7] Glutaraldehyde dosed as a 50 % (w/w) solution.The LD_{50} is expressed as mg of actual active ingredients.
[8] Test substance administered as a 25 % solution. The LD_{50} is expressed as mg of actual active ingredients.
[9] Test substance administered as a 45 % aqueous solution.The LD_{50} is expressed as mg of actual active ingredients.
[10] Dosed as a 6 % suspension in 0.5 % methyl cellulose.
[11] Actual LD_{50} not reported.Value represents lowest dose level tested causing mortality. Animals dosed at 16,000 mg/kg had 100 % survival rate, while animals dosed at 32,000 mg/kg had 100 % fatality. Acute lethal dose for dibutyl sebacate is between 16,000 and 32,000 mg/kg.
[12] Value represents the maximum dose level tested. Animals dosed at 30,000 mg/kg had 100 % survival rate.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 and for 20 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for two structurally related substances. The supporting and structurally related substances are listed in brackets.

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

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Mouse; M, F 5/20	Gavage	90	525	(NTP, 1992e)	a)
	Rat; M, F 5/20	Gavage	90	450	(NTP, 1992e)	a)
	Mouse; M, F 2/100	Gavage	2 years	262	(NTP, 1992e)	a)
	Rat; M, F 2/100	Gavage	2 years	112	(NTP, 1992e)	a)
	Rat; M, F 1/7	Diet	4 – 6 months	100[2]	(Fassett, 1961)	
Pentano-1,4-lactone [10.013])	Rat; M, F 1/30	Diet	90	M: 49[2] ; F: 51.1[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/10	Diet	90	500[2]	(Hagan et al., 1967)	a)
(Octano-1,5-lactone [10.015])	Rat; M, F 1/7	Diet	4 - 6 months	32[2]	(Fassett, 1961)	
(Nonano-1,4-lactone [10.001])	Rat; M, F 1/30	Diet	90	M: 62.8[2] ; F: 72.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
(Decano-1,4-lactone [10.017])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
(Decano-1,5-lactone [10.007])	Rat; M, F 1/NR	Diet	49 weeks	150[2]	(Fassett, 1961)	
	Dog; M, F 1/NR	Diet	38 weeks	250[2]	(Fassett, 1961)	
(Undecano-1,4-lactone [10.002])	Rat; M, F 1/30	Diet	90	M: 14.6[2] ; F: 16.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
	Rat; M, F NR[4]	Diet	90	14.1[2, 3]	(Shillinger, 1950)	
(Dodecano-1,4-lactone [10.019])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	
(Dodecano-1,5-lactone [10.008])	Rat; M, F 1/NR	Diet	49 weeks	300[2]	(Fassett, 1961)	
	Dog; M, F	Diet	38 weeks	150[2]	(Fassett, 1961)	

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

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	1/NR					
(5-Methylfuran-2(3H)-one [10.012])	Rat; M, F 1/NR	Diet	90	M: 17.4[2]; F: 17.7[2]	(Shellenberger, 1971c)	a)
(Ethyl acetoacetate [09.402])	Rat; M, F 3/32	Diet	28 - 29	300	(Cook et al., 1992)	a)
2-Butoxyethan-1-ol [02.242]	Rat; M, F 4/20	Diet	91 – 93	40	(Union Carbide Corp., 1963)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	No NOAEL derived [13]	(Union Carbide Corp., 1952)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	76	(Carpenter et al., 1956)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/20	Drinking water	13 weeks	1500 ppm (150 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M 3/10	Gavage	6 weeks	222	(Krasavage, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/10	Drinking water	14	400	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/20	Drinking water	13 weeks	6000 ppm (1200 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/6[4]	Drinking water	21	M: < 2000 ppm (200 mg/kg/day); F: < 1600 ppm (160 mg/kg/day)	(Exon et al., 1991)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/10	Drinking water	14	< 150[5]	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M NR	Oral	5 week	1000	(Bernstein, 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/5	Gavage	5 weeks[6]	< 500	(Nagano et al., 1977)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/NR	Gavage	5 weeks	1000[7]	(Nagano et al., 1979)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M3/NR	Gavage	5 weeks	< 500[8]	(Nagano et al., 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/50	Inhalation	2 years		(NTP, 2000b)	
	Mouse; M, F 4/50	Inhalation	2 years		(NTP, 2000b)	
Butane-1,3-diol [02.132]	Rat; M 15/10	Diet	30 weeks	200000 ppm (10000 mg/kg/day)	(Miller and Dymsza, 1967)	Study aimed at elucidating the usability of butane-1,3-diol as synthetic energy source. It is of limited value for toxicological evaluation.
	Rat; M, F 3/60	Diet	2 years	100000 ppm (5000 mg/kg/day)	(Scala and Paynter, 1967)	Some details of results not reported (e.g. consumption, histopathological evaluation), limited value.
	Dog; M, F 3/8	Diet	2 years	30000 ppm (750 mg/kg/day)	(Scala and Paynter, 1967)	
	Dog; M, F 4/8	Diet	13 weeks	6000	(Reuzel et al., 1978)	Methods, results, discussion comprehensible. Valid study.
(4-Oxovaleric acid [08.023])	Rat: NR	Diet	16	1000[2]	(Tischer et al., 1942)	a)

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

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	2/3					
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Rat; M, F 1/20 1/60	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
Malonic acid [08.053]	Rat; M, F 3/140	Diet	2 years	10[9]	(Hogan and Rinehart, 1979)	
(Diethyl malonate [09.490])	Rat; M, F 2/20	Diet	13 weeks	< 500	(Posternak, 1964a)	a)
	Rat; M, F 1/20-32	Diet	90	40[2]	(Posternak et al., 1969)	a)
(Fumaric acid [08.025])	Rat 2/14 1/20	Diet[10]	2 years	1380[2]	(Levey et al., 1946)	a)
	Guinea pig; M, F 1/NR	Diet	1 year	400[2]	(Levey et al., 1946)	a)
	Rat; M, F Rat; M 4/12 3/12	Diet	2 years	1200	(Fitzhugh and Nelson, 1947)	a)
	Rabbit; NR 3/15	Diet[10]	150	2070[2]	(Packman et al., 1963)	a)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Dog; NR 1/4	Oral	90-114	< 990	(Krop et al., 1945)	a)
	Rat; M, F 4/12	Diet	2 years	1200[2]	(Fitzhugh and Nelson, 1947)	a)
	Rabbit: NR 3/15	Diet[2]	150	2310[2]	(Packman et al., 1963)	a)
Glutaraldehyde [05.149]	Rat; M, F 4/10	Diet	7	1.0	(Union Carbide Corp., 1986)	
	Rat; M, F 3/NR	Drinking water	14	100 ppm (10 mg/kg/day)	(Union Carbide Corp., 1993)	
	Rat; NR 3/3	Drinking water	11 weeks	5000 ppm (500 mg/kg/day)	(Spencer et al., 1978)	
	Mouse; M, F 3/40	Drinking water	90	100 ppm (20 mg/kg/day)	(Bushy Run Research Center, 1989)	
	Rat; M, F 3/NR	Drinking water	13 weeks	50 ppm (5 – 7 mg/kg/day)	(Union Carbide Corp., 1986)	
	Dog;, M, F 3/8	Drinking water	13 weeks	50 ppm (3.2 mg/kg/day)	(Bushy Run Research Center, 1990)	
	Rat; M, F 3/200	Drinking water	2 years	50 ppm (4 mg/kg/day)	(Van Miller et al., 2002)	Large Granular Lymphocytic Leukemia in treated as well as control rats; no clear dose-resposne relationship. Otherwise no significant increase in neoplasia.
(Adipic acid [08.026])	Rat; M, F 4/20-39	Diet	2 years	~ 1500[11]	(Horn et al., 1957)	a)
Nonanedioic acid [08.103]	Rat; M, F 2/30	Diet	90 and 180	280	(Mingrone et al., 1983)	Details of methods not reported, study not performed according to appropiate

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

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
						guidelines. Study of limited value.
	Rabbit; M, F 2/20	Diet	90 and 180	400	(Mingrone et al., 1983)	
	Rat; F 1/10	Diet	3 month[12]	140	(Mingrone et al., 1983)	
	Rabbit; F 1/10	Diet	3 months[12]	200	(Mingrone et al., 1983)	
(Diethyl sebacate [09.475])	Rat; M, F 2/10	Diet	17-18 wks or 27-28 wks	1000[2]	(Hagan et al., 1967)	a)
	Rat; M 4/10	Diet	1 year	1250[2]	(Smith, 1953b)	a)
	Rat; M 5/16	Diet	2 years	6250[2]	(Smith, 1953b)	a)
(Triethyl citrate [09.512])	Rat; M, F 3/7	Diet	2 months	4000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 1/6	Gavage	2 months	< 285	(Finkelstein and Gold, 1959)	a)
(Tributyl acetylcitrate [09.511])	Rat; M, F 2/4	Diet	2 months	5000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 2/4	Gavage	2 months	< 5700	(Finkelstein and Gold, 1959)	a)
(Succinate, monosodium)	Rat; M,F 10/10	Drinking water	13 weeks	1250	(Maekawa et al., 1990) in (OECD, 2003)	
	Rat; M,F 50/50	Drinking water	2 years	2000	(Maekawa et al., 1990) in (OECD, 2003)	Monosodium succinate was given ad libitum in drinking water at levels of 0, 1, or 2 % to F344 rats (50 males, 50 females). No toxic lesion specifically caused by long-term administration of monosodium succinate was detected.
(Succinate, disodium hexahydrate)	Rat; M,F 12 /12	Gavage 0, 100,300, 1000 mg/kg)	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	Males: 100 Females: 300	MHLW, Japan 2002 in (OECD, 2003)	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: males 60 mg/kg; females, 180 mg/kg.

NR: Not reported.

M = Male; F = Female.

a) Study summarised by JECFA at the 49th or 53rd meetings (JECFA, 1998a; JECFA, 2000c).

[1] Number of groups represents the number of treatment groups investigated.Control groups are not reported.

[2] This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.

[3] Article published in Russian. Data point not verified.

[4] Six animals per treatment group. The treatment groups for males were not the same as the females. Males were administered 2000 or 6000 ppm of the test substance, while the corresponding dose levels for the females were 1600 and 4800 ppm, respectively.

[5] Compared to the control group absolute and relative thymus weights were significantly lower in males. These findings were not seen in females receiving up to 650 mg/kg/day.

[6] Animals dosed 5 days a week for five weeks.

[7] Changes in absolute or relative testis weights were not observed.

[8] A decrease in red cell count was noted in the 500 mg/kg dose group and higher dose groups.

[9] No treatment related effects were noted upon mortality, ophthalmology or body weights in the males. Microscopic evaluation noted that the transitional cell carcinomas were found in the urinary bladder. The findings were indicated to be dose related.

[10] Administered as the sodium salt.

[11] Rats fed a maximum dose of ca. 2500 mg/kg/day over a two-year period showed no gross or microscopic changes to their organs. There was no change in the incidence of tumours and mortality was unaffected. There was a slight reduction in body weight in animals dosed at ca. 1500 mg/kg/day and above.

[12] Animals were dosed for 19 gestational days prior to the three month exposure period that is reported.

[13] The value of the study is limited by high mortality in all treatment and control groups.

Developmental and reproductive toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from groups 9, 13 and 30 of the present Flavouring Group Evaluation and for two supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for one structurally related substance. The supporting and structurally related substances are listed in brackets.

Table IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Rat; F	Gavage	5/10	Developmental toxicity: Gestation days 6-15	500	(Kronevi et al., 1988)	
2-Butoxyethan-1-ol [02.242]	Mouse; M, F	Drinking water	5/16	FACB: (Task 1) 2 weeks	0.5 %[2] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	3/40	FACB: (Task 2) 14 weeks[3]	Reproductive: 0.5 %[4] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	1/40	FACB: (Task 3) 14 weeks[3]	M: 1.0 % F: < 1.0 %[5] (2000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Lactation/ Drinking water	1/40	FACB: (Task 4) 32 weeks	0.5 %[6] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; F	Gavage	3/45-47 3/52-59	Developmental toxicity: Gestation days 9 – 11 and 11 - 13	Maternal: 30 Fetal: 100	(Sleet et al., 1989)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	5/6	Developmental toxicity: Gestation days 8 - 14	Maternal: 1000 Fetal: 650	(Wier et al., 1987)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	1/50	Developmental toxicity: Gestation days 6 – 13	Maternal: < 1180[7] Fetal: 1180[7]	(Hardin et al., 1987; Schuler et al., 1984; Smith, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	4/20	During 7 days premating and 98 days cohabitation	Maternal: 720 Fetal: none	(EU_RAR, 2004a)	
Butane-1,3-diol [02.132]	Rat; M, F	Diet	3/50	Five generations ~ 2 years	Reproduction: 5 %[8] (5000 mg/kg/day) Teratogenicity: 5 % (5000 mg/kg/day)	(Hess et al., 1981)	
	Rat; M, F	Gavage	3/10	Developmental toxicity: Gestation days 6 – 15	Maternal: 706; Fetal: 706	(Mankes et al., 1986)	
Glutaric acid [08.082]	Rat; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 1300 Fetal: 1300	(Bradford et al., 1984)	
	Rabbit; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 500 Fetal: 500	(Bradford et al., 1984)	
Glutaraldehyde [05.149]	Rat; M, F	Drinking water	3/56	Reproductive toxicity: 39 weeks[9]	Adult: 50 ppm (5.6 mg/kg/day) Fetal: 250 ppm (24.3 mg/kg/day) Reproductive: > 1000 ppm (84.5mg/kg/day)	(Neeper-Bradley and Ballantyne, 2000)	
	Rat; F	Drinking water	3/25	Developmental toxicity: Gestation days 6 – 16	Maternal: 50 ppm (5 mg/kg/day); Fetal: 750 ppm(68 mg/kg/day)[10]	(Hellwig, 1991a)	
	Rat; F	Gavage	3/21 – 26	Developmental toxicity: Gestation days 6 – 15	Maternal: 50; Fetal: 100	(Ema et al., 1992)	
	Mouse; F	Oral	3/NR	Developmental toxicity: Gestation days 7 – 12	Embryotoxicity: 30; Fetal: 30, Teratogenicity: 30	(Union Carbide Corp., 1986)	

Table IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
	Rabbit; F	Gavage	3/15	Developmental toxicity: Gestation days 7 – 19	Maternal: 15; Fetal: 15	(Hellwig, 1991b)	
(Adipic acid [08.026])	Rat; F	Gavage	4/24-28	Developmental toxicity: Gestation days 6 – 15	288	(Morgareidge, 1973d)	
	Mouse; F	Gavage	4/20 – 21	Developmental toxicity: Gestation days 6 – 15	263	(Morgareidge, 1973d)	
	Rabbit; F	Gavage	4/10 – 14	Developmental toxicity: Gestation days 6 – 18	250	(Morgareidge, 1974a)	
Nonanedioic acid [08.103]	Rat; F	Diet	1/20	Developmental toxicity: Gestation days 0 - 19	140	(Mingrone et al., 1983)	
	Rabbit; F	Diet	1/30	Developmental toxicity: Gestation days 0 - 19	200	(Mingrone et al., 1983)	
(Succinate, disodium hexahydrate)	Rat; M,F	Gavage (0, 100,300, 1000 mg/kg)	4 per sex/ 12	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	M, F: 1000	MHLW, Japan 2002 in (OECD, 2003)	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: m, 600 mg/kg.

M = Male; F = Female.

NR = Not Reported.

FACB = Fertility Assessment by Continuous Breeding.

[1] Number of groups represents the number of treatment groups investigated. Control groups are not reported.

[2] Dose range-finding phase: Based on the results of this dose range-finding study the highest concentration investigated further was 2 % in the drinking water.

[3] Mice were exposed to the test article for a seven day premating period, followed by a 14 week cohabitation/breeding period.

[4] Continuous breeding phase: All breeding pairs in the 0.5 % treatment group were fertile (delivered at least one litter). The fertility of the 1.0 and 2.0 % treatment groups was significantly affected.

[5] Crossover mating trial: Reproductive capacity of female mice is relatively more susceptible than males under the same exposure conditions.

[6] Offspring reproductive performance phase: Reproductive performance was not affected, but the mean liver and kidney weights for females was significantly different from that of the control group when organ weight was adjusted for body weight.

[7] 1180 mg/kg/day was the only dose level tested. Compared to the control group the 1180 mg/kg/day decreased the number of viable litters; therefore increasing the number of failed pregnancies. There were no significant observations noted in the liveborn pups.

[8] Dose related reproductive effects were noted after five successive matings of the F1A generation.

[9] F_0 and F_1 animals dosed for a 10 week pre-breeding period and through mating, and gestation and lactation of offspring.

[10] Glutaraldehyde was evidentially unpalatable, as water consumption was reduced in the mid- and high-dose groups; however, no signs of toxicity were observed at these dose groups.

In vitro mutagenicity/genotoxicity data are available for nine candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for 22 supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for one structurally related substance. Supporting and structurally related substances are listed in brackets.

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	Ames test	*S. typhimurium* TA98, TA100, TA1535	0.1 - 50 µmoles/plate (8.6 - 4305 µg/plate)	Negative[1]	(Loquet et al., 1981)	No control values are given for inactive compounds. Conclusion not comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA102	0.013 - 1.3 mmol (11.2 - 1120 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(NTP, 1992e)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537,	5,000 or 2000 µg/plate	Negative[1]	(MacDonald, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0 - 10000 µg/plate	Negative[1]	(Haworth et al., 1983)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Negative[1]	(Garner et al., 1981)	
	Ames test	*S. typhimurium* TA98,TA100, TA1535, TA1537, TA1538	4 - 2500 µg/plate	Negative[1]	(Trueman, 1981)	
	Ames test	*S. typhimurium* TA92, TA98, TA100, TA1535, TA1537, TA1538	0.2 - 2000 µg/plate	Negative[1]	(Brooks and Dean, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/ml	Negative[1]	(Baker and Bonin, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Rowland and Severn, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Simmon and Shephard, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537	NR	Negative[1]	(Nagao and Takahashi, 1981)	
	Ames test	*S. typhimurium* TA98, TA100,	1000 mg	Negative[1]	(Ichinotsubo et al., 1981b)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 10000 µg/plate	Negative[3]	(Richold and Jones, 1981)	
	Reverse bacterial mutation assay	*E. coli* WP2 (p)	up to 500 µg/plate (high dose studies) up to 100 µg/plate (low dose studies)	Negative[3]	(Venitt and Crofton-Sleigh, 1981)	
	Reverse bacterial mutation assay	*E. coli* SA500	NR	Lethal[4]	(Dambly et al., 1981)	Authors state "toxic, preventing adequate testing".
	Reverse mutation assay	*E. coli* WP2 *uvrA* pKM102	NR	Negative[1]	(Matsushima et al., 1981)	
	Forward mutation assay	*S. typhimurium* TM677	1000 µg/ml	Negative[3]	(Skopeck et al., 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA1535, TA1537	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA100	NR	Negative[3]	(Hubbard et al., 1981)	
(Butyro-1,4-lactone [10.006]) continued	Microtiter fluctuation test	*E. coli* WP2 *uvrA*	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Rec-assay	*Bacillus subtilis* H17, M45	20 µl (20000 µg)	Positive[1]	(Kada, 1981)	Reliable study, conclusion comprehensible.
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA, LexA*	NR	Negative[1]	(Green, 1981)	

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA*, *LexA*	1000 µg/ml	Negative[2]	(Tweats, 1981)	
	Mitotic crossing-over	*S. cerevisiae*	1000 µg/ml	Negative[1]	(Kassinova et al., 1981)	
	Mitotic gene conversion	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	Cell growth inhibition	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	DNA polymerase I inhibition test	*E. coli* W3110 & P3478	10 µl (10000 µg)	Positive[2] Negative[3]	(Rosenkranz et al., 1981)	Reliable study, conclusion comprehensible.
	Forward mutation assay	*S. Pombe*	20 µg/ml[1]	Negative[3]	(Loprieno, 1981)	
	Unscheduled DNA synthesis	Human HeLa S3 cells	0.1 - 100 µg/ml	Negative[1]	(Martin and McDermid, 1981)	
	ADP-ribosyl transferase activity	Human FL cells	10^{-3} to 10^{-7} mol/L (0.0086 – 86 µg/ml)[3]	Negative	(Yingnian et al., 1990)	
	Clastogenic activity	Rat liver cell line RL1	250 µg/ml	Negative	(Dean, 1981)	
	Mammalian cell transformation	BHK-21 hamster kidney cells	250 µg/ml	Positive[1]	(Styles, 1981)	No specific genotoxicity endpoint.
	Degranulation assay	Rat	25 mg/ml (25000 µg/ml)	Positive	(Fey et al., 1981)	No genetic endpoint (displacement of polysomes from ER).
	Sister chromatid exchange	Chinese hamster ovary cells	494 - 4940 µg/ml 494 - 1480 µg/ml 3010 - 4940 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible.
	Chromosomal aberration	Chinese hamster ovary cells	400 - 2580 µg/ml 400 - 1500 µg/ml > 2580 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible. Cells were selected for scoring on the basis of good morphology and completeness of karyotype.
Pentano-1,5-lactone [10.055]	Microbial assay	*E. coli* B/rWP2(*trp*$^-$), WP2(*trp*$^-$), WP2(*uvrA*$^-$)	1 - 3 mg/plate (1000-3000 µg/plate)	Negative[5]	(Kuroda et al., 1986)	Review, data cannot be validated.
(Hexano-1,5-lactone [10.010])	Ames test	*S. typhimurium* TA98, TA100	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Rec-assay	*B. subtilis*	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Sister chromatid exchange	Hamster lung fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Hamster lung fibroblast cells	NR	Positive[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Human embryo fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Heptano-1,4-lactone [10.020])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	100,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Unscheduled DNA synthesis	Rat hepatocytes	3000 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
(Nonano-1,4-lactone [10.001])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	37500 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mammalian	Mouse lymphoma L5178y TK$^{+/-}$	1000 µg/ml	Negative[2]	(Heck et al., 1989)	Abstract only, study cannot be validated.

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
			600 µg/ml	Positive[3]		
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mutation assay	*E.coli* WP2 *uvrA*	0.2 - 1.6 mg/plate (200-1600 µg/plate)	Negative[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
	Rec-assay	*B. subtilis* M45 & H17	20 µl/disk (20000 µg/disk)	Positive[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated
(Undecano-1,4-lactone [10.002])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637	5 mg/plate (5000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102	0.1 mg/disk (100 µg/disk)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[6]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[3] Negative[2]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast	0.5 mg/ml (500 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
(Undecano-1,5-lactone [10.011])	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*	10 µl/plate (10000 µg/plate)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
(Pentadecano-1,15-lactone [10.004])	Ames test	*S. typhimurium* TA98, TA100, TA102	50 µmol (12 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
(5-Methylfuran-2(3H)-one [10.012])	Ames test	*S. typhimurium* TA98, TA100	5 - 50 µg/plate	Negative[1]	(Turek et al., 1997)	
(Dodec-6-eno-1,4-lactone [10.009])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
	Rec-assay	*E. coli* WP2 *uvrA*	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
1-Hydroxypropan-2-one [07.169]	Ames test	*S. typhimurium* TA100	20 - 400 µg/plate	Positive[1]	(Yamaguchi, 1982)	Effect dose-dependent, conclusion comprehensible.
	Ames test	*S. typhimurium* TA104	68 µmoles (5 µg/ml)	Positive[2]	(Marnett et al., 1985a)	Authors state that each compound was tested to its toxic limits, data for maximum non-toxic dose given only.
	Ames test	*S. typhimurium* TA100	500 µg/plate	Positive[1]	(Yamaguchi and Nakagawa, 1983)	Numerical value given was obtained from dose-response curves of five concentration levels.
	Ames test	*S. typhimurium* TA100	NR	Positive[2]	(Garst et al., 1983)	Appropriate controls (idomethan for volatile compounds, sterility of compounds and solvent). Test compound judged positive when dose-related doubling of revertants were found.
(Ethyl 3-hydroxybutyrate [09.522])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535	NR	Negative[4]	(Zeiger and Margolin, 2000)	
(Ethyl acetoacetate [09.402])	Ames test; preincubation protocol	*S. typhimurium* TA92, TA100, TA1535, TA1537, TA94 and TA98	25 mg/plate (25000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test; preincubation protocol	*S. typhimurium* TA97, TA102	0.1 - 10 mg/plate (10 - 10000 µg/plate)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis*; H17, M45	20 µg/disk	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*; H17, M45	20 µl/disk (20000 µg/disk)	Positive	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*E. coli*; WP2 *uvrA*	200 - 1600 µg/plate	Positive[8]	(Yoo, 1986)	Methods in Japanese, tables only in

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						English. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20 µl/ml (10 - 20 µg/ml)	Negative[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20 µl/ml (10 - 20 µg/ml)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast cells	1 mg/ml (2000 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
Methyl acetoacetate [09.634]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *E. coli* WP2 *uvrA*	1 - 5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	Modified Ames, reincubation. Reliable study, conclusion comprehensible.
2-Butoxyethan-1-ol [02.242]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 5000 µg/plate	Negative[1]	(Okamoto and Riccio, 1985)	Study performed in compliance with US-FDA GLP standards. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA*	9.8 - 156.3 µg/plate	Negative[1]	(Henrich and McMahon, 1988)	Test material: mixture of 2-butoxyethanol (2 % w/v) with tricholorbenzene and anionic emulsifiers. Test compound produced no revertants vs solvent control.
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(Zeiger et al., 1992)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, TA1538	5000 - 20000 µg/plate	Negative[1]	(Sippel, 1977)	Negative as defined by less than 2-times of the spontaneous reversion rate. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA100 *E. coli* WP2uvrA	500 - 1000 µg/plate	Negative[1]	(Gollapudi et al., 1996)	Re-examination of EGBE to valdazte report by Hoflack et al (1995) on mutagenicity of the compound in a test with TA97a. reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, TA102	14 mg/plate (14000 µg/plate) conc. range: 0,8 - 115 micromol/plate, positive ab 19 micromol = 2,2mg/plate	Negative with TA98, TA100,TA102, positive with TA97a[1]	(Hoflack et al., 1995)	Positive with TA97a, but not reproduced in study specifically addressing this finding (Gollapudi et al., 1996).
	Mutagenicity Assay	Bacteriophage *T4D E. coli CR63* and *K12*	19.6 - 111.1 µl/ml	Negative[9]	(Kvelland, 1988)	Highly toxic at all concentrations tested, bacteriophage yield less than 1 %.
	Forward mutation assay	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)[3]	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to currunt OECD guidelines.
	Forward mutation assay	Chinese hamster ovary cells V79	1 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study (5 concentrations each test, 1 % without S9 (non-toxic), 0,3 % with S9), conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells AS52	0.38 - 7.6 mM (898 µg/ml)	Negative[1]	(Chiewchanwit and Au, 1995)	Non-cytotoxic concentration range. Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells	0.007 - 0.25 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Positive[2, 10]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to current OECD Guidelines.
	Sister chromatid exchange	Human peripheral lymphocytes	3000 ppm	Positive[1]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						program. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing programme. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	Reliable report with details on purity of test compounds, methods and results. 50 % growth inhibition (at 24 hours) approx. at 90 mM, but value cannot be precisely derived from the graphic presentation.
	Chromosomal aberrations	Human peripheral lymphocytes	3000 ppm	Negative[2]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
2-Butoxyethan-1-ol [02.242] continued	Chromosomal aberrations	Human lymphocytes	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	No information on growth inhibition/ survival of treated human lymphocytes given.
	In vitro micronucleus test	V79 cells	16.92 mM (2000 µg/ml)	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to current OECD Guidelines.
	Unscheduled DNA synthesis	Rat hepatocytes	0.1 - 100 x 10^{-3} %	Positive[1, 11]	(Slesinski and Weil, 1980)	The interpretation of these findings is equivocal due to the methodology applied (liquid scintillation) and the absence of relation with dose.
	Embryo Transformation Assay	Syrian hamster embryo cells	NR	Negative[2]	(Elias et al., 1996)	No specific genotoxic endpoint.
	Embryo Transformation Assay	Syrian hamster embryo cells	500 - 1500 µg/ml	Positive[4]	(Brauninger, 1995)	No specific genotoxic endpoint.
(3,7-Dimethyloctane-1,7-diol [02.047])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Diethyl malonate [09.490])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537.	3 µmol/plate (480 µg/plate)	Negative[1]	(Florin et al., 1980)	
(Dimethyl succinate [09.445])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984a)	
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538	10 mg/plate (10000 µg/plate)	Negative[1]	(Zeiger et al., 1992)	
(Fumaric acid [08.025])	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative[4]	(Rapson et al., 1980)	
	Ames test (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	2000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	10 mg/plate (10000 µg/plate)	Negative	(Ishidate et al., 1984)	
	Chromosomal aberrations	Chinese Hamster fibroblast cells	0.5 mg/ml (500 µg/ml)	Negative	(Ishidate et al., 1984)	
(l-Malic acid [08.017])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA104	2000 µg/plate	Negative[1]	(Al-Ani and Al-Lami, 1988)	

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
Diethyl maleate [09.351]	Forward mutation assay	Mouse lymphocytes L5178Y TK+/-	2.250 - 9.750 x 10^{-4} mol/l (387 - 1679 µg/ml)	Positive[1]	(Wangenheim and Bolcsfoldi, 1988)	No S9 at 2.25 - 9.75 x 10^{-4} mol/L, doubling of the mutation rate at 6 x 10^{-4} mol/L and above, but growth reduction of 70 % or more. Study of insufficient value.
	Aneuploidy test	Chinese hamster lung cells V79	5.2 x 10^{-6} M 8.7 x 10^{-6} M	Negative[4] Positive[4]	(Önfelt, 1987)	Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	REC assay Ames	*B subtilis* M45 & H17 *S. typhimurium* TA98, TA100	NR	Negative[1]	(Sakagami et al., 1989)	Abstract, data cannot be validated.
Glutaraldehyde [05.149]	Ames test	*S. typhimurium* TA104	0.5 µmoles (50.06 µg/ml)	Positive[2]	(Marnett et al., 1985a)	TA104 tested to reassess mutagenic potency of 28 carbonyl compounds. Dose-dependent increase toxic limits of glutaraldehyde. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98	10 mg/plate (10000 µg/plate)	Equivocal[12] Positive[12]	(Haworth et al., 1983)	Part of ring study for re-assessment of 250 chemicals. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA100, TA102, TA104	25 - 300 µg/plate	Positive[1]	(Dillon et al., 1998)	Comparative analysis of TA100, TA102 and TA104 for sensitivity to 13 aldehydes and 4 peroxides. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638, *E. coli* WP2/pKM101, WP2 *uvrA*	20 - 1000 µg/plate	Positive[3, *]	(Watanabe et al., 1998a)	*Cytotoxicity noted in doses as low as 250 µg/plate. Ring study (22 laboratories) for comparative analysis of TA102, TA2638, *E. coli* WP2/pKM101 and WP2 uvrA/pKM101. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, *E. coli* WP2/pKM101, WP2 *uvrA*	5 - 100 µg/plate	Positive[2]	(Wilcox et al., 1990)	Comparative analysis of TA102 and E.coli WP2 strains. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	1000 µg/plate	Positive[13]	(Müller et al., 1993)	Ring study (3 laboratories) to evaluate TA102. Reliable, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638a	76 µg/plate	Positive[3, 14]	(Rydén et al., 2000)	Comparative analysis on the sensitivity of bacterial strains and the possibility of using TA2638a. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	25 µg/plate	Positive[1]	(Levin et al., 1982)	Test of TA102 for detection of oxidative mutagens. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, TA102, TA104	0.1 - 60 µg/plate	Positive[1]	(Noblitt et al., 1992)	Abstract, data cannot be validated.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 *uvrA*	100 - 5000 µg/plate	Negative[1]	(Wagner, 1997)	Study in compliance with inter-national (US-FDA, US-EPA, UK, Japan) GLP Guidelines. Negative result not discussed in view of positive results in other studies. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100,	15.4 µg/plate[2, 15]	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
		TA1537, TA1538, TA98	51.6 µg/plate[3]			due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, A102, TA104	0.050 % in 100 µl/plate (100000 µg/plate)	Positive[14]	(Schweikl et al., 1994)	Study aimed at elucidating the mutagenic potency of 3 different dentin bonding agents, pure glutaraldehyde was tested as one of the ingredients of these materials. Conclusion comprehensible.
Glutaraldehyde [05.149] continued	Ames test	*S. typhimurium* TA100, TA98	20 µg/plate	Negative[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration Ames test less sensitive than REC-assay (see below).
	Ames test	*E. coli* WP2 *uvrA*	20 - 10000 µM (2 - 1001 µg/ml)	Negative[2]	(Hemminki et al., 1980)	Study aimed at comparison of alkylation rate with mutagenicity of directly acting chemicals, glutaraldehyde served as reference compound.
	Rec-assay	*B. subtilis*, M-45 (*Rec*$^-$), H-17 (*Rec*$^+$)	300 µg/ml	Positive[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration REC-assay more sensitive than Ames test (see above).
	L-arabinose resistance forward mutation test	*S. typhimurium:* BA9, BA13	62 - 250 nmoles/ml (6.2 - 25 µg/ml)	Negative[15] Positive[15]	(Ruiz-Rubio et al., 1985)	
	Forward mutation assay	Mouse lymphocytes: L5178Y TK+/-	8 µg/ml	Positive[2]	(McGregor et al., 1988b)	Reliable study, conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells	40.8µM (4.08 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	2.5 µM (.25 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	0.5 - 16 µg/ml	Negative/positive[2] Positive[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol.11-16 µg/ml, with S9 positive (at least with one dose) results in both laboratories. 0,36-16 µg/ml, without S9 results not consistent.
	Chromosomal aberrations	Chinese hamster ovary cells	0.5 - 30 µg/ml	Negative/positive[2] Negative[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol. 1-16 µg/ml, with S9 negative results in both laboratories: 0,3-30 µg/ml, without S9 results not consistent.
	Alkaline elution assay	Human TK6 lymphoblasts	25 µM (0.25 µg/ml)[2]	Positive[2]	(St. Clair et al., 1991)	Linear increase in DNA cross linking between 1-25 µM. At 20 µM 10 % survival only.
	TK6 mutation assay	Human TK6 lymphoblasts	20 µM (2 µg/ml)	Positive	(St. Clair et al., 1991)	Majority of trifluorothymidine resistant colonies displayed normal growth, slow-growing colonies small contribution to overall mutant fraction.
Glutaraldehyde [05.149] continued	Unscheduled DNA synthesis	Primary rat hepatocytes	51 µM (5.1 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.

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

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Unscheduled DNA synthesis	Rat hepatocytes	100 µM (10 µg/ml)	Positive[2]	(St. Clair et al., 1991)	Significant increase over controls at 100 µM, this concentration tolerated without morphological signs of toxicity.
(Adipic acid [08.026])	Ames test	*E. coli* WP2 *uvrA*	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98, *E. coli* WP2 *uvrA*	10 mg/plate (10000 µg/plate)	Negative[1]	(Prival et al., 1991)	
	Ames test (preincubation method)	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
(Dibutyl sebacate [09.474])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Ethyl brassylate [09.533])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Prop-1-ene-1,2,3-tricarboxylic acid [08.033])	Ames test	*S. typhimurium* TA100, TA1535, TA1537, TA98	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984a)	
5,6-Dimethyl-tetrahydro-pyran-2-one [10.168]	Ames test	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5000 microgram/plate	Negative[1]	(Uhde, 2004a)	Test performed both in the incorporation and preincubation assays.
Succinic acid, disodium salt [08.113]	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537	5000 microgram/plate	Negative3	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Ames test	*S.typhimurium* TA97, TA102	10000 microgram /plate	Negative[1]	(Fujita et al., 1994) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	15000 microgram/ml	Equivoval[2]	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 473.
(Disodium succinate hexahydrate)	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537	5000 microgram/plate	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	5000 microgram/ml	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	

NR: Not reported.

[1] With and without S-9 metabolic activation.

[2] Without S-9 metabolic activation.

[3] With S-9 metabolic activation.

[4] Presence or absence of metabolic activation not specified.

[5] Anti-mutagenic effects study.

[6] Presence or absence of metabolic activation not specified.

[7] 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one did not form DNA adducts, but 2,5-DMHF does. Study addresses mechanism of chemical reaction of 2,5-dimethyl-4-hydroxy-3(2H)-furanone with DNA.

[8] The concentrations used were 10-fold higher than that of spontaneous revertants.

[9] The test substance had a severe toxic effect on phage yield.

[10] Weak positive results were detected.

[11] The test substance induced statistically significant levels of unscheduled DNA synthesis in two of the six dose levels tested. Therefore, the test substance is considered a weak mutagen.

[12]This test compared the results at two different laboratories. Results were equivocal at Case Western Reserve University, while they were positive at Microbiological Associates.

[13]Article presents the results from three different laboratories. Results were positive in both water and ethanol; however, it was concluded that TA102 is not sufficiently matured to be employed routinely.

[14]Maximum non-toxic dose.

[15]Results were negative in BA9, not BA13.

In vivo mutagenicity/genotoxicity data are available for six candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for eight supporting substances evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Supporting substances are listed in brackets.

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

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	*In vivo* Bone- marrow micronucleus assay	B6C3F1 mice	Single dose *via* intraperitoneal injection	80 % of LD_{50}	Negative	(Salamone et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo Bone- marrow micronucleus assay	CD-1 mice		0.11-0.44 ml/kg (110 – 440 mg/kg)	Negative	(Tsuchimoto and Matter, 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo micronucleus assay	Mice (B6C3F1/BR hybrid)		80 % of LD_{50}	Negative	(Katz et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo sperm abnormality	Mice (CBA X Balb/c)F1	Daily exposure for five days *via* intraperitoneal injection	0.1-1.0 mg/kg bw/day	Negative	(Topham, 1980)	Sperm head abnormality test does not make use of a genetic endpoint.
	In vivo sex-linked recessive test	*D. melanogaster*	A: *via* diet B: injection	A: 20000 or 28000 ppm B. 15.000 ppm	Negative	(Foureman et al., 1994)	Study in compliance with OECD 477.
(Hexano-1,5-lactone [10.010])	Chromosomal aberration *in vivo*	Rat bone-marrow cell		NR	Negative[1]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Undecano-1,4-lactone [10.002])	*In vivo* mouse micronucleus test	2-6 ddY male mice	*Via* intraperitoneal injection	250-2000 mg/kg	Negative	(Hayashi et al., 1988)	Single application, only one sampling time. Not in compliance with current OECD 474.
2-Butoxyethan-1-ol [02.242]	*In vivo* mouse micronucleus test	Mouse bone marrow	Single dose *via* intraperitoneal injection	1000 mg/kg	Negative	(Elias et al., 1996)	Reliable report, decreased PCE/NCE ratio demonstrates bioavailability of compound at target compartment. Conclusion comprehensible.
	In vivo mouse micronucleus test	Mouse bone marrow	3 doses *via* intraperitoneal injection	450 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo micronucleus test	Rat bone marrow	3 doses *via* intraperitoneal injection	550 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo DNA adducts	Rat brain, kidney, liver, spleen and testes	Single dose *via* oral route	120 mg/kg	Negative	(Keith et al., 1996a)	The method (based on ^{32}P-postlabelling) is aimed at detecting hydrophobic DNA

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

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							adducts resulting from CytP450 induction, not from binding of 2-butoxyethan-1-ol to DNA .
	In vivo DNA methylation	Rat brain, kidney, liver, spleen and testes,	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo DNA adducts	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Detection of hydrophobic DNA adducts such as modified nucleotides with aliphatic side chains.
	In vivo DNA methylation	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo tumour formation	Mouse	Daily dose for two weeks *via* oral route	120 mg/kg/day	Inconclusive	(Keith et al., 1996a)	No difference in tumor incident observed. However no conclusion on the oncogenic potential of 2-butoxyethan-1-ol can be drawn because of the limitations of the experimental protocol (treatment, sample size, duration of the study, reporting, etc.).
Butane-1,3-diol [02.132]	*In vivo* cytogenetic assay	Rat femur bone marrow	*Via* diet[2]	5, 10, 24 %	Negative	(Hess et al., 1981)	F1A, F2A, F3A generations in a multigeneration reproductive toxicity study. PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo dominant lethal assay	Rat	Animals exposed for eight weeks *via* diet	5, 10, 24 %	Negative	(Hess et al., 1981)	F1B generation in a multigeneration reproductive toxicity study.
(3,7-Dimethyloctane-1,7-diol [02.047])	*In vivo* micronucleus test	Mouse		516, 860, 1204 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		10 mM (1743 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	*In vivo* Basc test	*D. melanogaster*		37 mM (6374 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		345, 603, 861 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not

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

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							clear whether the substance had reached the bone marrow.
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	*In vivo* Basc test	*D. melanogaster*		25 mM (5459 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		327, 545, 763 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
Malonic acid [08.053]	*In vivo* mutagenicity assay	Rat hepatocytes	400 mg/kg/day exposure for 6 weeks *via* diet	4000 ppm	Negative	(Ito et al., 1988)	GST-P foci assay following diethyl nitrosamine exposure. Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	*In vivo* bone marrow chromosomal aberrations	Rat bone marrow	Single dose *via* oral gavage	Males: 2750 mg/kg Females: 1375 mg/kg	Negative	(San Sebastian, 1989a)	Reliable study, e.g. cells with gaps excluded. Selected copy of report without data tables.
Glutaraldehyde [05.149]	*In vivo* chromosomal aberration	Rat bone marrow	Single dose *via* oral gavage	Males: 120 mg/kg/bw Females: 80 mg/kg/bw	Negative	(Vergnes and Morabit, 1993a)	Study in compliance with international (FDA, TSCA, OECD) GLP guidelines. Selected copy of report (12 of 100 pages) available.
	In vivo chromosomal aberration	Rat bone marrow	A single dose or daily for five days *via* oral gavage	Single dose: 0.55 ml/kg (males), 0,4 ml/kg (females) of a 6, 12 or 36 % solution. Repeated dose: 0,55 ml/kg (males) of a 5 % solution	Negative	(Putman, 1987)	Time points of investigation: single dose: 8, 12 hours. Repeated dose: 12hours. Well conducted study, conclusion comprehensible. Selected copy of report available.
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* oral gavage	250 mg/kg	Negative	(Vergnes and Morabit, 1993b)	Selected pages of report available (29 of 88 pages).
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* intraperitoneal injection	4, 8, 15 mg/kg/bw	Positive	(Noblitt et al., 1993)	Abstract, study cannot be validated.
	In vivo unscheduled DNA synthesis	Rat	Single dose *via* oral gavage	30, 150, 600 mg/kg	Negative	(Mirsalis et al., 1989)	Reliable part of *In vivo* tumour formation study, conclusion comprehensible.
	In vivo SLRL test	*D. melanogaster*	Three day exposure *via* diet	3500 ppm	Negative	(Zimmering et al., 1989)	Study in compliance with OECD 477.
	In vivo SLRL test	*D. melanogaster*	Single dose *via* intraperitoneal injection three day exposure *via* diet	Injection: 4000 ppm Diet: 10,000 ppm	Negative	(Yoon et al., 1985)	Study in compliance with OECD 477.
(Adipic acid [08.026])	*In vivo* chromosomal nondisjunction	*D. melanogaster*		4000 ppm	Negative	(Ramel and Magnusson, 1979)	
Diethyl adipate [09.348]	*In vivo* dominant lethal assay	Mouse	(Single1460 mg/kg dose *via* intraperitoneal injection)	1.46 ml/kg	Negative	(Singh et al., 1975)	Reliable study, conclusion comprehensible.

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

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Dibutyl sebacate [09.474])	*In vivo* micronucleus test	Mouse		943, 1886, 2829 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		19 mM (4642 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.

NR: Not reported.
[1]Presence or absence of metabolic activation not specified.
[2] Length of exposure not specified in report. Cytogenetic assay conducted on F1A, F2A and F3A generations of a multiple generation study.

ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADI	Acceptable Daily Intake
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CNS	Central Nervous System
CoA	Coenzyme A
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DRF	Dose Range Finder
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	Environmental Protection Agency
ER	**E**ndoplasmic Reticulum
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
I.V.	Intravenous
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
LOAEL	Lowest Observed Adverse Effect Level
MFD	Median Fatal Dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake

mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
RfD	Reference dose
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation

Inhalation Toxicology, 18:685–706, 2006

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370600742680

Taylor & Francis
Taylor & Francis Group

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.

Received 2 January 2006; accepted 31 March 2006.
The authors are grateful to the following staff for their valuable contributions to this work: J. C. Blessing, M. L. Clark, K. M. Gideon, B. K. Hayden, J. D. Penner, J. T. Pierce, B. L. Thomas, and R. L. Thomas.
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.

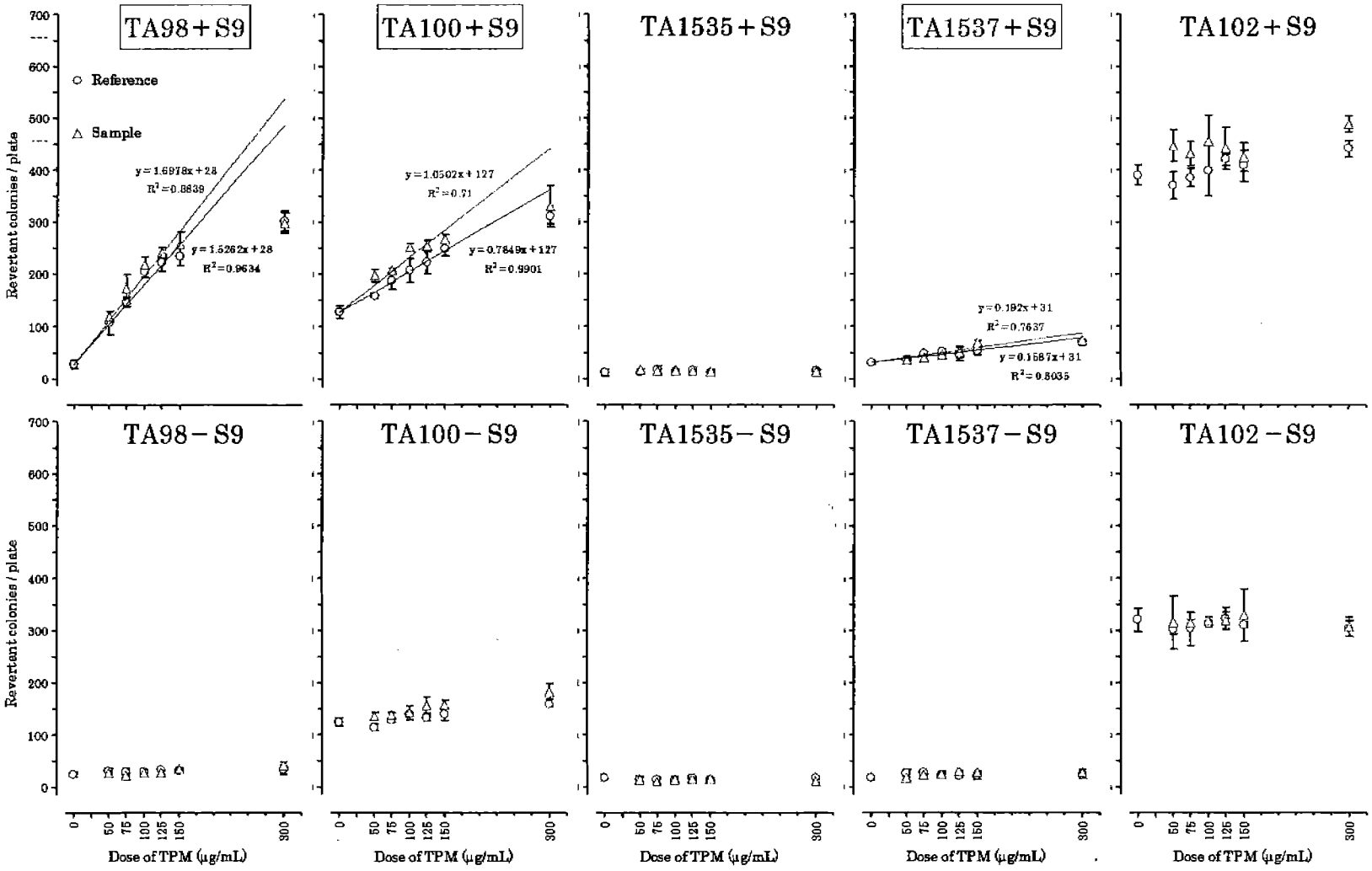
Body Weights and Clinical Observations

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.

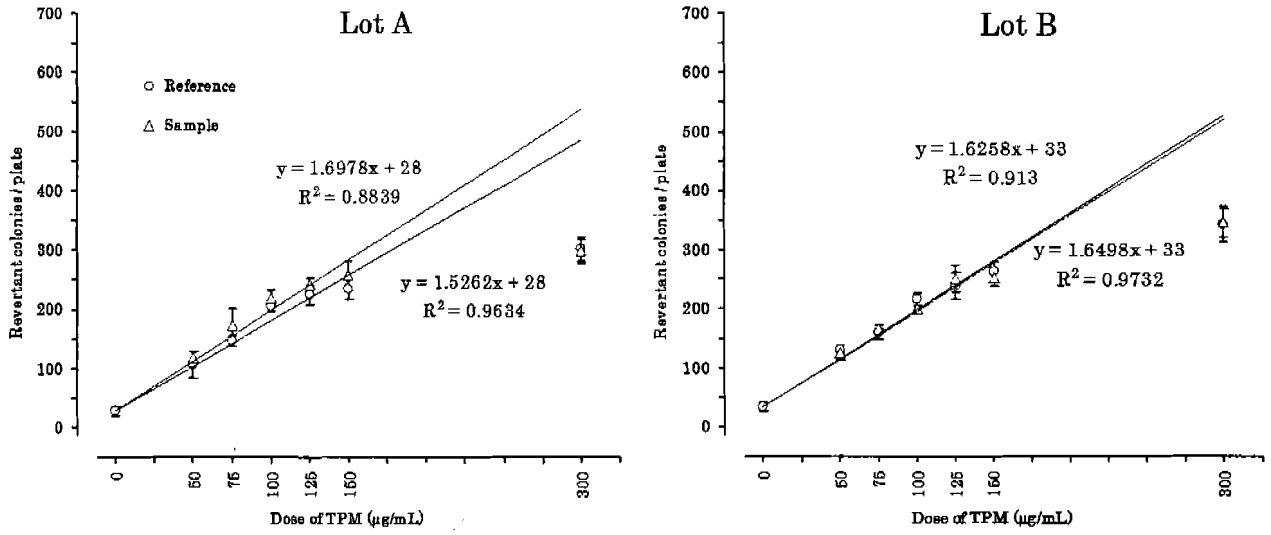
Organ Weights

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

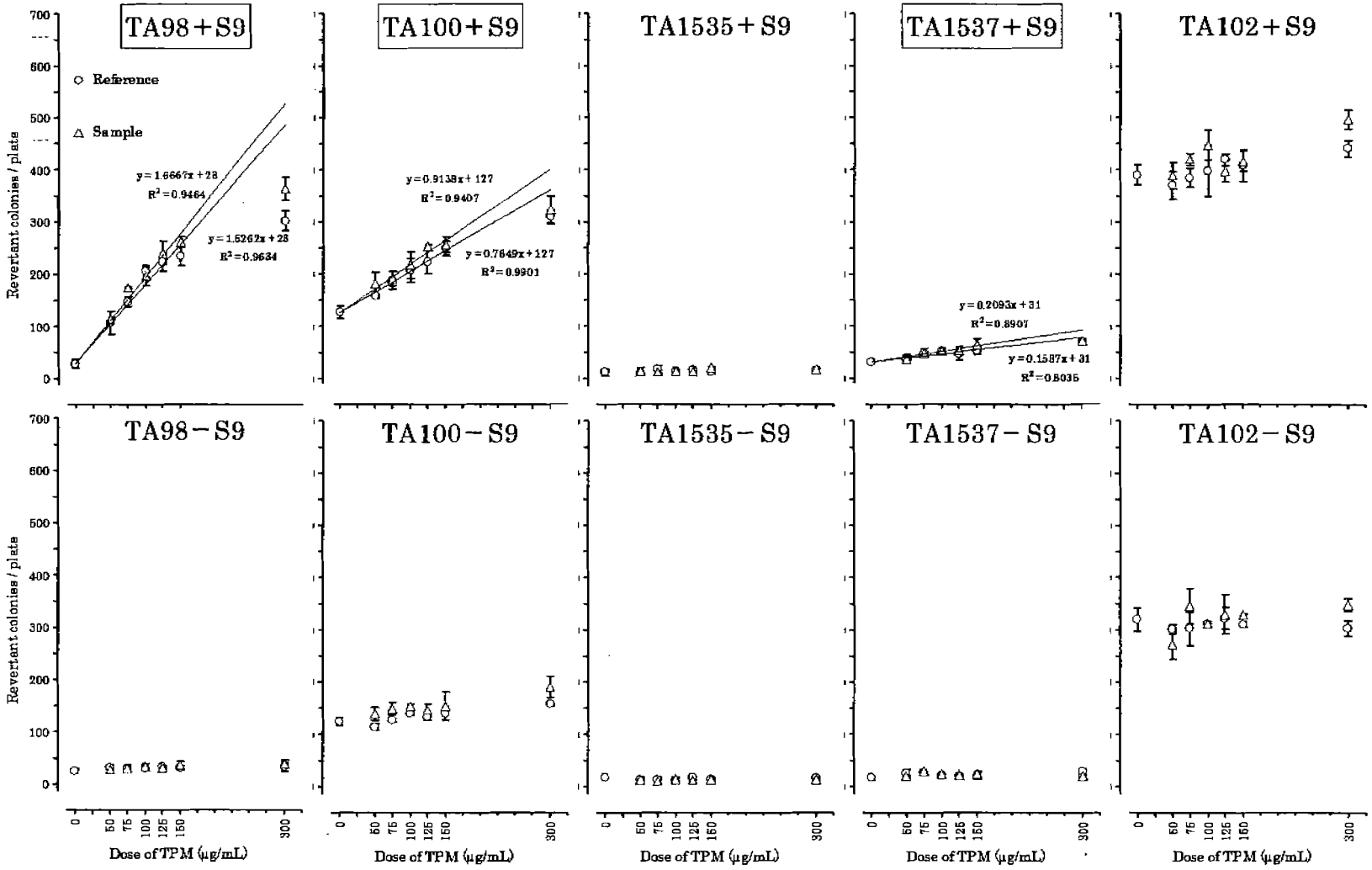
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 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



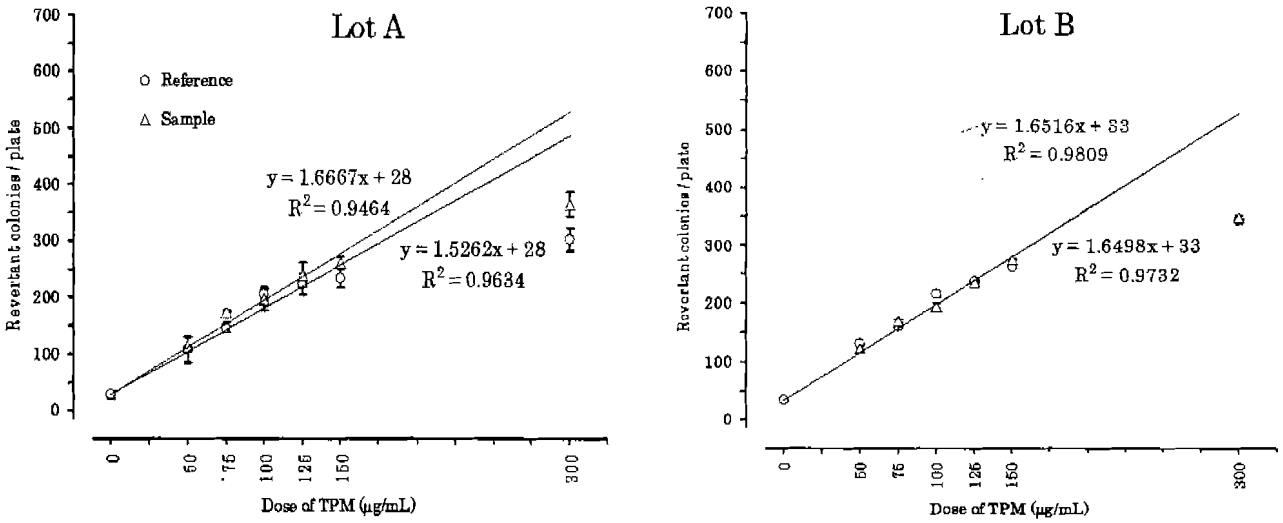
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

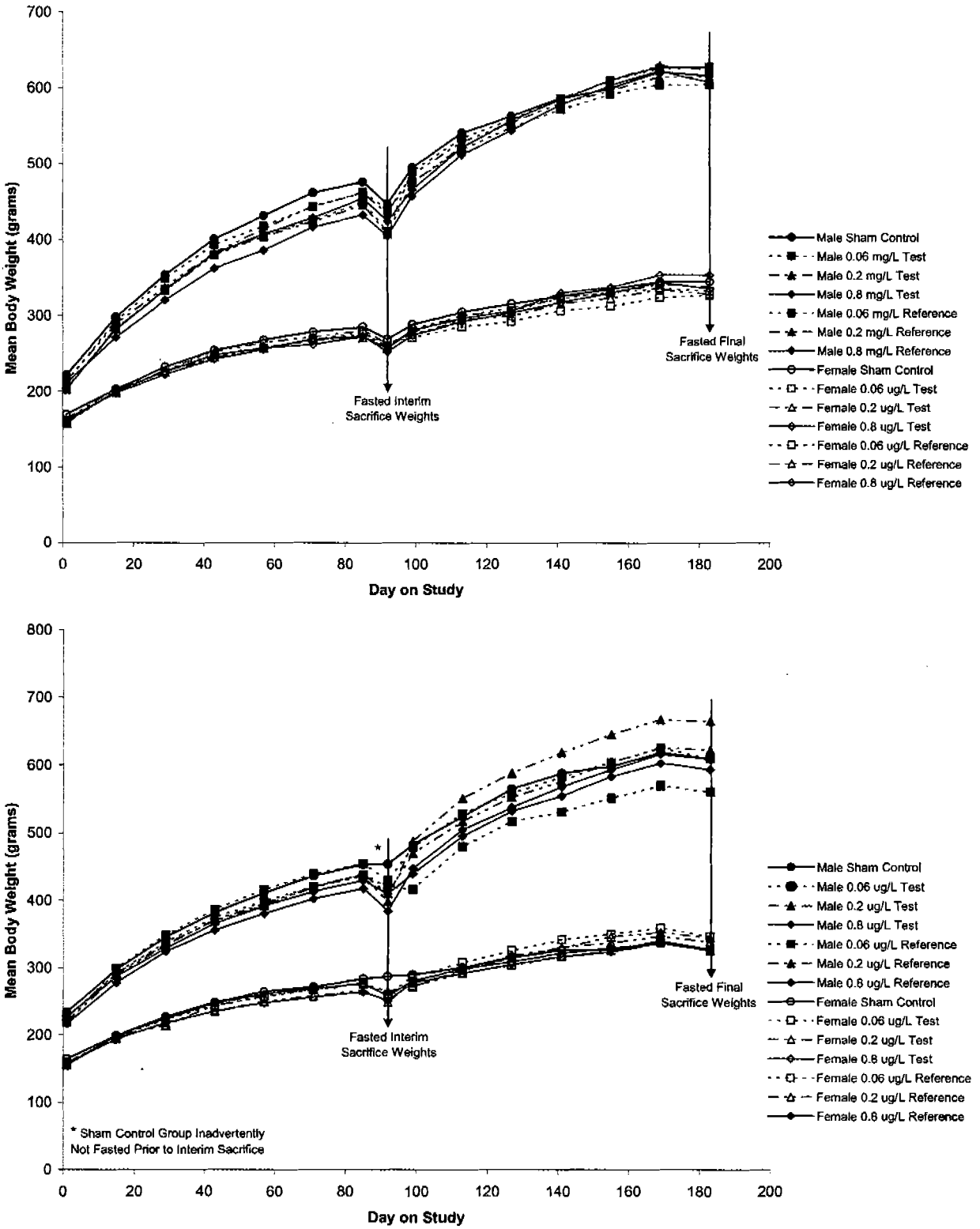


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL HEALTH AND CONSUMER PROTECTION
Directorate B - Scientific Opinions on Health Matters
Unit B2 - Management of Scientific Committees I
Scientific Committee on Toxicity, Ecotoxicity and the Environment

Brussels, 28/9/99
B2/JCD/csteeop/**cit28999**.D(99)

SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on

the toxicological characteristics and risks of certain citrates and adipates used as a substitute for phthalates as plasticisers in certain soft PVC products

Opinion adopted at the 11th CSTEE plenary meeting

on the 28th of September 1999

1. Summary

The CSTEE has evaluated the toxicological characteristics and risks of certain citrates and adipates in order to examine whether such substances may be used as substitutes for phthalate plasticisers in PVC toys. In doing this, the CSTEE has applied the same general risk assessment principles used in its previous opinions on phthalates in PVC products that may be mouthed by children. The documentation made available to the CSTEE and the information found in the open literature on exposure and effects of the specified citrates and adipates, is too limited to determine whether they are safe to use as plasticisers in materials which may be mouthed by children.

2. Background

In its opinion of 24 April 1998 on 'Phthalates in toys', the CSTEE has recommended that *'before introducing other plasticisers into toys which children can put into their mouth, the risk of their use should be assessed by the same process which has been applied to the phthalates discussed above'*.

Recent announcements by toy manufacturers indicate that substitution of phthalates by other plasticisers will take place in the near future. In this contest citrates have been mentioned as possible promising candidates for such a substitution. Citric acid esters have been available since the 1940s for use as plasticisers in polymers such as polyvinyl chloride (PVC) and cellulose acetate. There are currently several manufacturers of citrate esters for use as plasticisers. Information from industry and national laboratories in Member States confirm the existing use of adipates as plasticisers in PVC toys. Also, there are a number of commercially available alternatives to PVC such as thermoplastic elastomers (styrenic block copolymers, polyolefin blends, elastomeric alloys), ethylene vinyl acetate and polyolefins (polyethylene, polypropylene) (CSTEE/98/17 - Add. 35).

The CSTEE has been presented with the following terms of reference on toxicological characteristics and risk to child health of certain citrates, and notably acetyltributyl citrate and diethylhexyl adipate, used as a substitute for phthalates as plasticisers in soft PVC toys and childcare articles:

1. What are the toxicological profiles of the substances under reference? What ranking of these substances can be made on the basis of their toxicological profiles?
2. How do the substances under reference compare with phthalates in terms of their toxicological profiles?
3. Does the CSTEE consider that the toxicological profiles of the substances under reference support their safe use as plasticisers in the products under consideration? Bearing in mind the potential for migration of these substances from the products under consideration, should limits for the migration of these substances from the products under consideration be set, and if so, which limits? In view of both the toxicological profile and the potential for migration of the substances under reference, does the CSTEE consider that the margins of safety for the use of these substances in products under consideration are adequate?
4. What are the issues on which additional information and/or research is required that may help answer the above questions?

The previously formed 'Phthalates Working Group' of the CSTEE has attempted to address these questions. It became readily apparent that there was less sound toxicological and exposure documentation on which to base qualified answers to the questions, this is especially the case for citrates and adipates other than acetyltributyl citrate and diethylhexyl adipate, respectively. Also, most of the information related to the citrates was not available in the open literature and has thus not undergone scientific peer review. In part due to confidentiality issues, the process of making the documentation available to the CSTEE has been slow. A considerable part of the toxicological data generated on the citrates is old and has not been developed applying modern test guidelines. The documentation on adipates has been gathered after searches in available databases and from a comprehensive evaluation report (BUA, 1996).

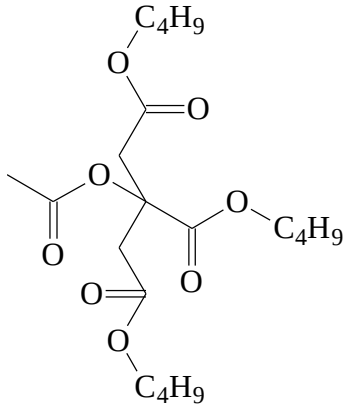
3. Citrates

3.1 O-Acetyltributyl citrate (ATBC)

3.1.1 Physicochemical characteristics

The following properties of ATBC have been identified in the literature (CSTEE/98/17 - Add 1; CSTEE/98/17 – Add.3; CSTEE/97/1-Add.116; CSTEE/97/1-Add.115; CSTEE/98/17 – Add.36):

CAS number: 77-90-7
EINECS number: 201-067-7
Molecular formula: $C_{20}H_{34}O_8$



Molecular weight: 402.5
Vapour pressure: 0.052 mm Hg (20°C)
Melting point: -80°C
Boiling point: 173 °C (1 mm Hg)
200 °C (4 mm Hg)
326 °C (160 mm Hg)
Decomposition temp: >220 °C
Solubility in water: 20 $mg.L^{-1}$
ethanol: Soluble
acetone: Soluble
DMSO: Soluble
toluene: Soluble

3.1.2 Migration from PVC products

Migration of plasticisers from food packaging materials into especially fatty food has been studied a lot. These studies have been performed with static methods (no mechanical treatment) which are known to give much lower results than the *in vivo* studies performed to mimic the mouthing/chewing of a small child.

The migration of ATBC from polyvinylidene chloride film into olive oil have been investigated and 2-30 $mg.dm^{-2}$ was observed. No time for the experiment was given (CSTEE/98/17 - Add. 1).

Medical grade PVC was blended with different plasticisers (about 30%) and moulded to films. These were extracted with different media and the following results were obtained (CSTEE/97/1-Add116):

Plasticiser	**DEHP**	**DEHA**	**ATBC**
Water extraction, %	0.7	1.5	1.2
Soapy water extraction, %	2.7	11.0	9.5
ASTM oil No. 3 extraction, %	11.4	34.7	10.9

The following specific migration (static, one-sided) of ATBC from PVDC film have been reported (CSTEE/98/17 - Add. 36):

Film type	**ATBC (%)**	**Simulant**	**Conditions**	**$mg.dm^{-1}$**
Household cling	4.9	Sunflower oil	10 days 40 °C	4.7
		3% acetic acid		2.8
Industrial cling	4.3	Sunflower oil		3.8
		3% acetic acid		1.5
Industrial non-cling	4.9	Sunflower oil		3.3
		3% acetic acid		2.2
Industrial non-cling	2.6	Olive oil	2 hr 70 °C	4.1
			10 days 40 °C	4.7

One of the producers of plasticisers have performed an extraction study to compare the migrations of ATBC and DINP from PVC (CSTEE/98/17 - Add. 33). The table below shows the loss of plasticiser to a saliva simulant at 60 °C during 24 hours in a static test.

Plasticiser concentration (%) in PVC	**ATBC (% loss)**	**DINP (% loss)**
40	0.8	3.4
65	2.0	6.1

The conditions during this study were rather extreme with 40 mm thick disks (about 50 mm diameter) and a high temperature under static conditions, thereby making it difficult to compare the outcome with results from other studies. The discs were also cleaned with an organic

solvent before the test. It may, however, be possible to compare the extraction efficiencies for the two investigated plasticisers, indicating a faster emission of the DINP compared to ATBC.

There are also several reports on migration of ATBC from plastic films during microwave treatment, but these are less relevant for the exposure of children and will not be reviewed here.

3.1.3 Exposure of children from PVC articles and other products

ATBC is used as a flavouring agent in food. From the used amounts (corrected for underestimation) and under the assumption that the whole amount ends up in the food supply of 10% of the consumers, the daily intake for these has been estimated to 0.02 microg/kg bw (JECFA, 1999).

No information has been found indicating the exposure of children from PVC articles or other products.

3.1.4 Toxicokinetics

ATBC is rapidly absorbed after oral administration in rats with a half-life of 1.0 hr (CSTEE/98/17 - Add. 46). Peak blood concentrations were observed 2-4 hours after administration. At least 67% of the dose is absorbed. The elimination from the blood was biphasic with half-lives of 3.4 hrs and 39 hrs, respectively. The long half-life of the second phase is presumably related to the incorporation of radiolabel into the carbon pool. There are no data on distribution of ATBC. The substance is primarily excreted into the urine (approx. 64%), excretion in faeces amounted to approx. 32% and expired air approx. 2%. ATBC is extensively metabolised, at least 9 metabolites, more polar than ABTC but less polar than citric acid, appear in the urine and at least 3 in faeces. Monobutyl citrate is the major urinary metabolite of ATBC. Theoretically ATBC could be hydrolysed to butanol, however, this has not been documented as a metabolite. There are no structural alerts in the ATBC molecule indicative of chemical reactivity.

3.1.5 Short-term effects

ATBC is virtually non-toxic after single gavage administration to rats and cats since doses of approximately 10 to 30 g/kg did not cause any systemic effects (CSTEE/98/17 - Add. 2).

3.1.6 Irritation

ATBC is not a skin irritant in rabbits, whereas it causes moderate eye irritation in rats (CSTEE/98/17 - Add. 4).

3.1.7 Sensitisation

ATBC did not appear to be a skin sensitiser when tested in the guinea pig maximisation test (CSTEE/98/17 - Adds. 6, 52). In contrast, acetyltriethyl citrate and triethyl citrate appeared to be strong sensitisers in this test. A sensitisation test with ATBC carried out in humans did not show any evidence for sensitising or irritating capacity (CSTEE/98/17 - Adds. 5, 54). Also, acetyltriethyl citrate and triethyl citrate gave a negative response in the human sensitisation test.

3.1.8 Repeated dose toxicity

In a 4-week range-finding feed study in rats, ATBC caused decreased body weights and changes in organ weights from feed concentrations of 2.5% onwards (corresponding to 2700 mg/kg bw/day) (CSTEE/98/17 - Add. 45). No effects were seen at lowest feed concentration of 1% ATBC in the diet (equal to 1000 mg/kg bw/day).

In a 90-day gavage study with male and female Wistar rats (according to OECD Guideline 408) haematological and biochemical changes were noted from 300 mg/kg bw/day onwards (CSTEE/98/17 - Add. 44). At 1000 mg/kg bw/day increased liver weights were observed in both sexes. No histopathological changes were seen. The NOAEL in this study is 100 mg/kg bw/day.

3.1.9 Genotoxicity

ATBC does not induce gene mutations in *Salmonella typhimurium* in the absence or presence of a metabolism system (CSTEE/98/17 - Adds. 10, 47). ATBC does not induce chromosomal aberrations in two studies with rat lymphocytes in the absence or presence of a metabolism system (CSTEE/98/17 - Adds. 48, 50). ATBC increased the mutant frequency of CHO cells (HGPRT-locus) at the highest concentration in the presence of a metabolism system in one experiment, this could not be repeated in a second experiment (CSTEE/98/17 - Add. 49). The compound could not be evaluated without a metabolism system due to severe cytotoxicity. ATBC caused a concentration-dependent increase in the mutant frequency of mouse lymphoma cells (TK-locus) in the presence of a metabolism system in two experiments, in one out of two experiments without a metabolism system increases were seen at the highest and lowest concentration (CSTEE/98/17 - Add. 36). ATBC did not cause unscheduled DNA synthesis (UDS) in rats treated by gavage with a single dose of 800 or 2000 mg/kg bw (CSTEE/98/17 - Add. 61). No other *in vivo* data are available with respect to genotoxicity testing of ATBC. Although there are suggestions of an *in vitro* genotoxic effect of ATBC, the negative UDS study indicates that the *in vivo* genotoxic potential of ATBC is low or absent.

3.1.10 Chronic toxicity/Carcinogenicity

In a two-year feeding carcinogenicity study in the Sherman rat (sex unspecified) (filed with the US FDA in 1950, CSTEE/98/17 - Add. 3), 20 rats per treatment group (40 controls) were given concentrations of 0, 200, 2000 and 20000 ppm ATBC in the diet (the highest dose corresponding to approximately 1000 mg/kg/day). Survival in the highest dose group was more than 50% percent. This study apparently did not reveal any significant toxicological findings related to ATBC exposure. However, the conduct and reporting of this study is not according to modern guidelines. It is not possible to properly evaluate the carcinogenic potential of ATBC from this study. It appears that ATBC is not a potent multi-site carcinogen, but the induction of a low incidence of a site-specific effect cannot be excluded.

3.1.11 Reproductive toxicity

A 2-generation reproduction study has been performed in Sprague-Dawley rats (according to OECD Guideline 416) with ATBC administered in the diet corresponding to doses of 0, 100, 300 and 1000 mg/kg bw/day (CSTEE/98/17 - Add. 36). Decreased body weights were seen from the mid-dose in F_1 male rats and at the high dose in F_0 male rats. No effects were seen in the pups. The NOAEL from this study is 100 mg/kg bw/day

There are no data available with respect to teratogenicity of ATBC.

3.1.12 Data gaps

There is limited knowledge on migration rates of ATBC from PVC products. From a single *in vitro* study it appears that the extraction loss of ATBC from PVC samples by saliva simulant extraction is approximately one third the rate of diisononyl phthalate (DINP). There is no information on exposure of children to ATBC from PVC products or other articles.

There is no evidence that ATBC is a skin sensitiser, although the structurally similar compounds acetyltriethyl citrate and triethyl citrate are strong sensitisers in guinea pigs. The underlying mechanism for these structural differences is not known. Since there was cross reactivity between acetyltriethyl citrate and triethyl citrate, it could be the triethyl tail which renders these citrates to be immunogenic.

There are deficiencies in the database with respect to genotoxicity of ATBC. There are some suggestions of *in vitro* genotoxicity, whereas one *in vivo* UDS study was negative. Preferably, an *in vivo* chromosomal mutation study should be carried out in order to have a more complete database for a conclusive evaluation of the genotoxic potential of ATBC.

A chronic toxicity/carcinogenicity study on ATBC in compliance with modern guidelines is not available. Since a well-conducted 2-generation reproduction study has been performed, this can be used as a substitute for a chronic toxicity study for identifying a No-Observable-Adverse-Effect-Level (NOAEL). Ideally, a chronic toxicity study on ATBC would be needed to substantiate that this is the proper NOAEL value. An in-depth evaluation of the carcinogenic potential of ATBC is not possible based on the data presented to the CSTEE.

Teratogenicity studies on ATBC are lacking, however, this is not seen as a data deficiency in the present exposure situation involving young children.

3.1.13 Critical effect and NOAEL

There are limited data on which to identify the critical effect and NOAEL properly. From the 2-generation reproduction toxicity study decreased body weight was identified as the critical effect giving a NOAEL of 100 mg/kg bw/day. A similar value was established from the 90-day repeated dose study.

3.1.14 Tolerable daily intake (TDI)

The Scientific Committee on Food (SCF) has placed ATBC on their list 7 of 1995, *Substances for which there were insufficient toxicological or technological data to enable the Committee to express an opinion*, and more specifically *Substances for which some toxicological data exist, but for which an ADI or a TDI could not be established* (CSTEE/98/17 - Add. 37). JECFA at its meeting in June 1999 evaluated the use of ATBC as a flavouring agent. According to the Procedure for the Safety Evaluation of Flavouring Agents (based on estimated intake) it was concluded that the intake does not exceed the exposure threshold of concern (1800 microgram/person/day) and there is no safety concern for its use as a flavouring agent (JECFA, 1999).

The CSTEE considers that it is not possible to do a proper risk assessment, especially because of the lack of exposure information. There also are deficiencies with respect to availability of effects information. A complete database is needed in order to evaluate the safety of a phthalate substitute for children's toys. Thus, it is not possible to set a TDI.

3.1.15 Intake doses from PVC articles

It is not possible to estimate intake doses in children mouthing PVC toys containing ATBC from the present database. Assuming, as indicated in section 3.1.2, ATBC is extracted more or less as effectively as the phthalates from PVC and the same concentrations are used in the polymers, a migration of up to 10 µg/10 cm^2/min could be expected from toys when chewed/mouthed by small children. If the released substance is fully hydrolysed this will give a total daily dose of about 200 microgram/kg butanol if a child weighing 5 kg chews the toys during 3 hours. Such a dose is without toxicological concern.

3.1.16 Other exposures

There are no specific data on ATBC exposure of children from other exposures. Except for the possible intake of ATBC as a flavouring agent, there are no specific data on exposure of children to this compound.

3.1.17 Margin of safety (MOS)

It is not possible to estimate the relationship between exposure levels to ATBC from mouthing soft PVC toys and its NOAEL, due to the data gaps.

3.1.18 Comparison with phthalates

The extraction of ATBC from PVC may be comparable to that of phthalate esters. As can be seen in section 3.1.2 there are indications of both somewhat higher and somewhat lower extractability of ATBC as compared to the phthalates, but the results indicate that they are at least of the same order of magnitude.

3.1.19 *Migration limits*

Migration limits for ATBC from PVC cannot be identified from the available data.

3.2 Other citrates

3.2.1 Triethyl citrate

CAS number: 77-93-0.

No information has been made available to the CSTEE on the extractability of triethyl citrate from PVC toys or the exposure of children from such toys.

The oral LD50 value for triethyl citrate in rats is approximately 7 g/kg (CSTEE/98/17 - Add. 2). The substance appears to be a strong sensitiser in the guinea pig maximisation test (CSTEE/98/17 - Add. 6). However, it did not show any evidence of sensitising capacity or skin irritation in humans (CSTEE/98/17 - Adds. 5, 54). Feeding triethyl citrate (highest dose

approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs (CSTEE/98/17 - Add 2).

Data presented to the Scientific Committee for Food in 1990 showed that triethyl citrate is hydrolysed *in vivo* to citric acid and ethanol, compounds with well-defined, low toxic potential (CSTEE/98/17 - Add. 37/b). Triethyl citrate appeared to be hydrolysed at a slower rate with human serum compared to rat serum (CSTEE/98/17 - Add. 37/d).

If, as indicated for ATBC in section 3.1.2, triethylcitrate is extracted more or less as effectively as the phthalates from PVC and the same concentrations are used in the polymers, a migration of up to 10 $\mu g/10cm^2$/min could be expected from toys when chewed/mouthed by small children. If the released substance is fully hydrolysed this will give a total daily dose of about than 120 microgram/kg ethanol if a child weighing 5 kg chews the toys during 3 hours. Such a dose is without toxicological concern.

No other toxicological data on triethyl citrate have been available to the CSTEE, although the Scientific Committee for Food refers to an older, inadequate long-term study in the rat (CSTEE/98/17 - Add. 37/b).

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) established in 1979 a temporary ADI of 10 mg/kg bw. This was changed in 1984 to an ADI of 20 mg/kg bw. The Scientific Committee for Food agreed in 1981 and 1990, respectively, to these values (CSTEE/98/17 - Add. 37/b). The Scientific Committee for Food has placed triethyl citrate on its positive list, List 1 of 1995, *Substances, e.g. food additives, for which an ADI, a temporary ADI (t-ADI), a MTDI, a PMTDI, a PTWI or the classification "acceptable" has been established by this Committee or by JECFA* (CSTEE/98/17 - Add. 37). JECFA at its meeting in June 1999 evaluated the use of triethyl citrate as a flavouring agent according to the Procedure for the Safety Evaluation of Flavouring Agents. Based on estimated intake for Europeans of 3400 microgram/person/day, it was concluded that the intake exceeds the exposure threshold of concern (1800 microgram/person/day), but that there is no safety concern for its use as a flavouring agent (JECFA, 1999).

Triethyl citrate is a strong sensitiser in guinea pigs using the maximisation test in which the compound was injected adjuvant, although no sensitising capacity for humans was apparent from a repeated insult patch test. Further, it failed to induce irritation in human skin. Thus, triethyl citrate will not readily lead to sensitisation when in contact with normal human skin. However, it cannot be ruled out that it will induce sensitisation when in contact with human skin or mucous membranes that is damaged or affected in such a way that inflammatory responses are present.

3.2.2 Acetyltriethyl citrate

CAS number: 77-89-4.

No information has been made available to the CSTEE on the extractability of acetyltriethyl citrate from PVC toys or the exposure of children from such toys.

The oral LD50 value for acetyltriethyl citrate in rats is approximately 7 g/kg (CSTEE/98/17 - Add. 2). The substance causes slight to moderate eye irritation in rabbits (CSTEE/98/17 - Add. 4). Acetyltriethyl citrate appears to be a strong sensitiser in the guinea pig maximisation test (CSTEE/98/17 - Add 6). However, it did not show any evidence of sensitising capacity or skin irritation in humans (CSTEE/98/17 - Adds. 5, 54). Feeding the substance (highest dose approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs.

No other toxicological data on acetyltriethyl citrate have been available to the CSTEE.

Acetyltriethyl citrate is currently on the Scientific Committee for Food List 8 of 1995, *Substances for which there were insufficient toxicological or technological data to enable the Committee to express an opinion*, and more specifically *Substances for which no or only scanty and inadequate data were available* (CSTEE/98/17 - Add. 37).

Acetyltriethyl citrate is a strong sensitiser in guinea pigs using the maximisation test in which the compound is injected in adjuvant, although no sensitising capacity for humans was apparent from a repeated insult patch test. Further, it failed to induce irritation in human skin. Thus, acetyltriethyl citrate will not readily lead to sensitisation when in contact with normal human skin. However, it cannot be ruled out that it will induce sensitisation when in contact with human skin that is damaged and affected in such a way that inflammatory responses are present.

3.2.3 Tributyl citrate

CAS number: 77-94-1.

No information has been made available to the CSTEE on the extractability of tributyl citrate from PVC toys or the exposure of children from such toys.

Tributyl citrate is virtually non-toxic after single gavage administration to rats and cats in that doses of approximately 10 to 30 g/kg did not cause any systemic effects (CSTEE/98/17 - Add. 2). Feeding the substance (highest dose approx. 20 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs.

No other toxicological data on tributyl citrate have been available to the CSTEE.

The Scientific Committee for Food has placed tributyl citrate in its List 6B of 1995, *Substances for which there exist suspicions about their toxicity and for which data are lacking or are insufficient. (The allocation of substances to this list is mainly based upon similarity of structure with that of chemical substances already evaluated or known to have functional groups that indicate carcinogenic or other severe toxic properties)*, and more specifically *Section 6B: Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated* (CSTEE/98/17 - Add. 37).

3.2.4 *Evaluation*

Triethyl citrate is a potential skin sensitiser for humans. There is no relevant exposure information on the substance and the toxicological database is limited. Thus, it is not possible to perform a proper risk assessment of exposure to children of triethyl citrate from PVC toys.

Acetyltriethyl citrate is a potential skin sensitiser for humans. There is no relevant exposure information on the substance and the toxicological database is extremely limited. Thus, it is not possible to perform a proper risk assessment of exposure to children of acetyltriethyl citrate from PVC toys.

Tributyl citrate has an extremely limited toxicological database and there is no relevant exposure information on the substance. Thus, it is not possible to perform a proper risk assessment of exposure to children of tributyl citrate from PVC toys.

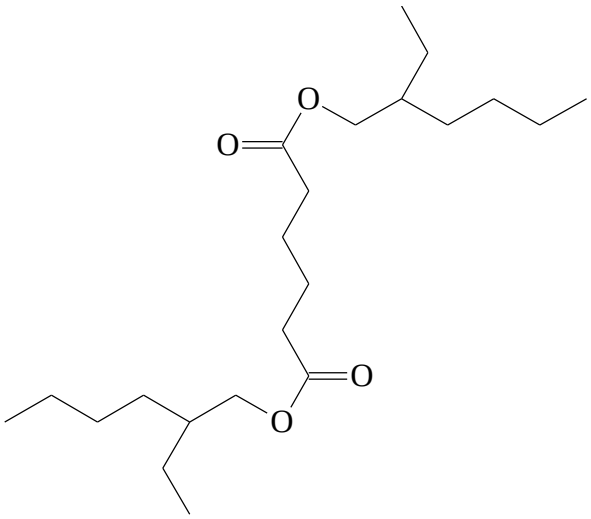
4 Adipates

4.1 Diethylhexyl adipate (DEHA)

4.1.1 Physicochemical characteristics

The following properties of DEHA have been identified in the literature (IUCLID 1996):

CAS number: 103-23-1
EINECS number: 203-090-1
Molecular formula: $C_{22}H_{42}O_4$



Molecular weight: 370.58
Vapour pressure: 0.021 hPa (100 °C)
Melting point: -76 °C
Boiling point: 210-218°C (7 hPa)
$\log P_{ow}$: 8.114
Solubility in water: <100 $mg.L^{-1}$ (20 °C)

4.1.2 Migration from PVC products

The migration of DEHA from PVC film into different foods has been investigated and it is obvious that high lipid content in the food increase the migration (Harrison, 1988). In the same report a maximum dietary intake of DEHA due to this contamination was calculated to 16 $mg.kg^{-1}.day^{-1}$.

In another study the intake of DEHA in the UK was estimated from the concentration of 2-ethylhexanoic acid in urine samples from 112 adults. The results showed a skewed distribution with a median value of 2.7 $mg.day^{-1}$ with a maximum of 8.2 $mg.day^{-1}$ (Loftus et al., 1994).

Urine sampled over 24 hours by approximately 50 male participants from France, Germany and the Netherlands was also analysed for the DEHA metabolite. The median exposure for these three countries was estimated to 1.04, 0.80 and 0.86 mg DEHA/day, respectively (Woollen, 1998).

The content of plasticisers in baby food have been investigated in Denmark (Breidendahl and Petersen, 1998). Of 11 investigated "ready to use" infant formulae DEHA was found in 2 (0.02 and 0.05 mg DEHA/kg), while DEHA was not found in any of the 11 studied baby foods. The content of plasticisers in 21 total diet samples for adults were also measured in this study and the results are shown in the following table:

	Plasticiser amount in total diet (mg/10 mJ)			
	DBP	**BBP**	**DEHA**	**DEHP**
Range	0.13-0.29	0.02-0.03	0.20-0.21	0.19-0.30

In a Danish survey plastic film on the market were tested for DEHA migrations to olive oil (10 days at 40°C). Of the 49 investigated samples 42 exceeded the action limit set at 4 $mg.cm^{-2}$ (Breidendahl and Petersen, 1998).

Models are developed for the prediction of migration of DEHA from plasticised PVC film into different food types and the result is compared with earlier measured data (Mercer et al., 1990). The measured migration varied between 0.6 and 19 $mg.dm^{-2}$ and the result does not seem to change dramatically between 1 and 7 days exposure.

The studies of migration of DEHA into foodstuffs have been published (BUA, 1996) and the maximum value is observed in Brie cheese. After 5 days at 5°C up to 195 $mg.dm^{-1}$ had been transferred from the PVC film containing 17.2% DEHA.

No specific documentation has been found related to the migration of DEHA from PVC toys using salivary simulants. The tests of migration of DEHA into food from packaging materials have been carried out without any mechanical stress (static tests), therefore these results are difficult to extrapolate to the extraction in the mouth of a child.

4.1.3 Exposure of children from PVC articles

No information has been found describing the exposure of children to DEHA from PVC articles.

4.1.4 Toxicokinetics

DEHA is rapidly and completely absorbed from the gastrointestinal tract of experimental animals. In rats, there is evidence for cleavage of the parent compound and subsequent absorption of the monoester and the acid, whereas in cynomolgus monkey also unchanged DEHA is absorbed. DEHA is distributed to a number of tissues with maximum levels reached after 6-12 hours. Liver, fat, kidney and adrenals had relatively high levels of DEHA-associated radiolabel, whereas large amounts of radioactivity were found in the gastrointestinal tract (BUA, 1996).

After oral administration, DEHA is hydrolysed in the gastrointestinal tract to 2-ethylhexanol, mono(2-ethylhexyl)adipate and adipic acid. A half-life of 6 minutes for metabolism of DEHA has been determined in rat small intestine mucus membrane homogenates. The main urinary DEHA metabolite in rats is by far adipic acid (80-90% of administered oral dose). Other major metabolites are 2-ethylhexanoic acid glucuronide and 2-ethyl-1,6-hexanedoic acid. In the monkey the glucuronide of mono(2-ethylhexyl)adipate and traces of unchanged DEHA were found in the urine (BUA, 1996).

In humans given deuterium-labelled DEHA, 2-ethylhexanoic acid was the only metabolite that could be determined in the plasma. It had an elimination half-life of 1.65 hours. In urine, the following metabolites were identified (percentage fraction of administered radioactivity): 2-ethylhexanoic acid (8,6%), 2-ethyl-5-hydroxyhexanoic acid (2.6%), 2-ethyl-1,6-hexanedioic acid (0.7%), 2-ethyl-5-ketohexanoic acid (0.2%), and 2-ethylhexanol (0.1%). The half-life for elimination of all metabolites excreted with the urine averaged 1.5 hours, none of the metabolites could be detected after 36 hours (BUA, 1996).

DEHA is rapidly eliminated, with most of the ^{14}C-radioactivity appearing in the urine after oral administration of rats, mice and cynomolgus monkeys (rats: 34-78% of the dose after 24 hours; mice: 75-92%; monkeys: 47-57%). In rats, the total radioactivity in the body after 96 hours was approx. 0.5%. Some of the biliary (approx. 3% in rats) secreted radioactivity flows into the enterohepatic circulation. Passage of DEHA through the placenta of pregnant mice has been described (BUA, 1996).

4.1.5 Short-term effects

DEHA has very low acute toxicity, the following LD50 values have been reported: Rat (oral) 7,392-45,000 mg/kg bw; mouse (oral) 15,000-24,600 mg/kg bw; rabbit (dermal) 8,410-15,100. The symptoms of intoxication in the rat following oral administration were co-ordination disorders (BUA, 1996).

4.1.6 Irritation

DEHA has been reported to be non-irritating or slightly irritating to the skin of rabbits in some studies. Also, non-irritation or slight eye irritation have been reported in some studies (BUA, 1996; IUCLID, 1999).

4.1.7 Sensitisation

A Draize test failed to produce symptoms of a sensitising potential of DEHA (BUA, 1996).

4.1.8 Repeated dose toxicity

A number of studies have shown DEHA to induce changes indicative of peroxisome proliferation in the liver of rats when the compound is orally administered at dosages generally higher than 1,000 mg/kg bw for 5 to 30 days. Dose dependent changes included increases in relative liver weight, reduction in serum triglyceride and cholesterol levels, increase in hepatic catalase and carnitine acyl transferase activity, as well as biochemical and morphological evidence of peroxisome proliferation. The effects were more pronounced in male rats compared to females. DEHA also acts as a peroxisome proliferator in mice. The peroxisome proliferation appears to be caused by metabolites, rather than the parent compound, with 2-ethylhexanoic acid being the most active metabolite. The peroxisomal effects of DEHA are moderate compared to those of DEHP, which shows a NOAEL for peroxisome proliferation at 5 mg/kg bw/day (RIVM, 1992). There is a marked species difference for the peroxisomal effects. *In vitro* studies with hepatocytes of rats, guinea pigs and marmosets show only in rat hepatocytes a clear effect (BUA, 1996).

There are no adequately performed studies which allow a precise determination of a NOAEL for DEHA from subchronic or chronic studies. An oral rat 90-day study from 1951 quotes a NOAEL of 610 mg/kg bw/day. In one 21-day feeding study in female F344 rats, 122 mg/kg bw/day was cited as the lowest dose which significantly increased peroxisome proliferation. A recent 2-week feeding study in Wistar rats showed a NOAEL of 200 mg/kg bw/day for induction of peroxisomal associated enzymes (BUA, 1996). In a 21-day feeding study in mice, a NOAEL of 325 mg/kg bw/day for peroxisomal proliferation was identified (IUCLID, 1999). The Scientific Committee for Food has assigned a NOAEL for DEHA in the rat, as measured by biochemical parameters and electronmicroscopic analysis of peroxisome proliferation, at around 100 mg/kg bw/day (CSTEE/98/17 - Add. 37/g).

4.1.9 Genotoxicity

DEHA has not induced point mutations in *Salmonella typhimurium* or mouse lymphoma cells, sister chromatide exchanges in primary rat hepatocytes or Chinese hamster ovary cells, nor unscheduled DNA synthesis in primary rat hepatocytes. Further, DEHA did not cause chromosomal aberrations or micronuclei in primary rat hepatocytes. In one test on Chinese hamster ovary cells, an increased rate of chromosomal aberrations was seen in the absence of a metabolic activation system, however, this study did not address cytotoxicity. DEHA has not induced micronuclei in mouse bone marrow cells or sex-linked recessive lethals in *Drosophila melanogaster*. In a dominant-lethal test in mice using intraperitoneal administration, a slight positive effect was seen. At the same time there was a reduction in the fertility index (not seen in oral studies), suggesting cytotoxicity rather than mutagenicity being the underly-

ing cause for the dominant lethality (BUA, 1996). DEHA did not induce cell transformation in Balb-3TR mouse embryo cell cultures (IUCLID, 1999). In an overall assessment of the test results, the CSTEE arrives at the conclusion that DEHA does not have a genotoxic potential.

4.1.10 Carcinogenicity

B6C3F1 mice fed 0, 12000 or 25000 ppm DEHA corresponding to doses of 1,800 and 3,750 mg/kg bw/day (EPA) for 103 weeks showed a dose-dependent incidence of hepatocellular tumours (adenomas and carcinomas combined) in both sexes. The number of females with hepatocellular carcinomas only was also significantly higher in both treatment groups. The male animals of the high dosage group also showed a significantly higher incidence of hepatocellular adenomas only (BUA, 1996).

F344 rats fed 0, 12000 or 25000 ppm DEHA corresponding to doses of 600 and 1,250 mg/kg bw/day (EPA) for 103 weeks did not show evidence of a substance-related carcinogenic effect (BUA, 1996).

In a study designed to explain the underlying species differences in hepatocarcinogenicity of DEHA, the substance showed sustained replicative DNA synthesis at dose levels (2.5% feed concentration) in female mice which were not effective in female rats (4.0% feed concentration). On the other hand, the magnitude of induction of peroxisome proliferation was similar in both species (Lake et al., 1997).

A covalent DNA-binding study in mouse liver and a cell transformation test in BALB/3T3 mouse cells were negative. On the other hand, increased levels of 8-OH-guanine adducts in rat liver DNA have been found after DEHA administration, indicative of the formation of reactive oxygen species (Takagi et al., 1990).

The proposed mechanisms whereby peroxisome proliferators induce liver tumours in rodents include oxidative stress, increased hepatocellular proliferation and/or preferential growth of preneoplastic lesions (IARC, 1995). The available evidence indicates that peroxisome proliferation in mouse and rat liver is mediated by activation of peroxisome proliferator-activated receptors (PPARs), which are members of the steroid hormone receptor superfamily. PPAR expression in human liver is much lower than that observed in mice (Palmer et al., 1998). The CSTEE considers the hepatocarcinogenic response of DEHA in mice to be a dose-thresholded phenomenon. Because of this, and the differences in sensitivity between humans and rodents towards peroxisome proliferators, exposures of children to DEHA orders of magnitude below those doses which induce liver tumours in mice, do not raise any concern.

4.1.11 Reproductive toxicity

In a developmental toxicity study in pregnant Wistar rats fed 0, 300, 1800 or 12000 ppm DEHA, stated by BUA (1996) and IUCLID (1999) to correspond to doses of 0, 28, 170 or 1080, or by the Scientific Committee for Food (CSTEE/98/17 - Add. 37/g) to doses of 0, 30, 110 or 720 mg/kg bw/day (The CSTEE notes that the Scientific Committee for Food may have miscalculated the low dose). The highest dose led to slight reductions in maternal body weight gain and food consumption. In the foetuses at the high dose, reduced ossification and kinked or dilated ureters were found. There was also a slightly significant increase of ureter kinking at the middle dose. The Scientific Committee for Food has in 1994 established a NOAEL for foetotoxicity at 30 mg/kg bw/day (CSTEE/98/17 - Add. 37/g).

In a companion one-generation reproduction toxicity study, Wistar rats were fed with DEHA corresponding to the same doses in the developmental toxicity study. No effects were seen on male or female fertility. The parental generation was fed continuously throughout the study for approx. 18-19 weeks of exposure. At the highest dose of 1080/720 mg/kg bw/day, there was a reduction in the body weight gain of the dams during gestation, an increase in liver weight in both male and female parents, and reductions in offspring weight gain, total litter weight and litter size. From this study a NOAEL of 170 (BUA, 1996) or 110 (SCF: CSTEE/98/17 - Add. 37/g) mg/kg bw/day for both maternal and foetal toxicity can be identified.

A drinking water study where female Long-Evans rats were exposed to di(2-ethylhexyl)-phthalate (DEHP) from day 1 of pregnancy to day 21 after delivery, identified severe histological damage to the testes of the offspring at 32.5 µl DEHP/L (Arcadi et al., 1998). Because of the similarities in chemical structure and metabolism between DEHA and DEHP, DEHA could potentially have a comparable profile to DEHP with respect to testicular toxicity in very young animals (DEHP NOAEL 3.7 mg/kg bw/day; Poon et al., 1997). The CSTEE considers that the one-generation reproduction study may not properly address this issue.

4.1.12 Data gaps

Specific data on the migration of DEHA from PVC products with salivary simulants are lacking. There is limited information on additional exposures of children to DEHA.

Studies to reveal a possible testicular toxic potential of DEHA after foetal and early postnatal exposure are lacking.

4.1.13 Critical effect and NOAEL

DEHA has a toxicological profile similar to DEHP, but is considerably less potent. The most sensitive effect identified so far is foetotoxicity. The lowest NOAEL for this effect is in the order of 30 mg/kg bw/day .

4.1.14 Tolerable daily intake (TDI)

DEHA is on the Scientific Committee for Food List 2 of 1995, *Substances for which the committee was able to express an opinion*, and more specifically *Substances for which a TDI or a t-TDI has been established by this Committee*. Using the NOAEL of 30 mg/kg bw/day for foetotoxicity and an uncertainty factor of 100, the Scientific Committee for Food has established a TDI for DEHA of 0.3 mg/kg bw (CSTEE/98/17 - Add. 37/g).

Given the specific exposure circumstances under consideration and that the structural analogue DEHP has testicular toxicity after pre-/perinatal exposure as its critical effect, the CSTEE considers it premature to establish a TDI for DEHA without a better database to judge any testicular toxic potential of this substance.

4.1.15 Intake doses from PVC articles

It is not possible to assign intake doses of DEHA from children mouthing PVC toys containing this plasticiser.

4.1.16 Other exposures

Mean DEHA exposures to the general population have been measured to be between 0.8 and 2.7 mg/day in 4 EU countries. The main source is assumed to be food packaging materials.

4.1.17 Margin of safety (MOS)

The relationship between exposure levels to DEHA and its NOAEL cannot be estimated because of lack of specific exposure information.

4.1.18 Comparison with phthalates

Three times as much DEHA compared to DEHP is extracted from PVC film into an oil (CSTEE/97/1-Add. 116, see 3.1.2). There are no data on the specific migration of DEHA into salivary simulants which allows a comparison with DEHP. The toxicological profile of DEHA is somewhat similar to, but less potent than DEHP, at least with respect to peroxisome proliferation.

4.1.19 Migration limits

Migration limits for DEHA in soft PVC articles cannot be set.

4.2 Other adipates

The CSTEE has not been supplied with documentation on dicapryl, diisobutyl, diisodecyl or dinonyl adipate and has not found information in the open literature on the migration, exposure and toxicology of these substances.

5 Conclusion

5.1 Terms of reference 1

There are important limitations regarding the toxicological database on O-acetyltributyl citrate (ATBC). The substance has not been studied for chronic toxicity and carcinogenicity according to modern test guidelines. There also are deficiencies in the data base with respect to genotoxicity. Thus at present, the CSTEE cannot evaluate the toxicological profile of this substance on all important endpoints.

Due to its sensitising potential, the CSTEE does not consider triethyl citrate to be a suitable substitute for phthalates as plasticisers in children' toys.

Due to its sensitising potential the CSTEE does not consider acetyltriethyl citrate to be a suitable substitute for phthalates as plasticisers in children' toys. In addition, the database on acetyltriethyl citrate is extremely limited with respect to assessment of additional toxicological endpoints.

The database on tributyl citrate is extremely limited with respect to toxicological endpoints, thus the CSTEE cannot properly evaluate the toxicological profile of this substance.

From the available toxicological data on DEHA, this substance appears to have low toxicity after long-term administration. It induces liver tumours in mice after high doses, but this effect is not considered to be of concern in the present situation given the underlying mechanism of carcinogenicity in mice and the large difference between maximum theoretical exposure doses in children and doses which are carcinogenic in mice. However, a proper assessment of a potential testicular toxic effect of DEHA after foetal/perinatal exposure cannot be performed from the existing data base.

No data have been available to the CSTEE regarding exposure and effects of the other adipates under consideration, therefore a risk assessment is impossible.

5.2 Terms of reference 2

Due to the limitations in the database for ATBC, it is not possible to compare this substance with the phthalates.

DEHA is less potent than DEHP in causing hepatic peroxisome proliferation. However, data are lacking allowing for a comparison of these structural analogues with respect to the critical effect of DEHP, namely testicular toxicity.

5.3 Terms of reference 3

Because of the important data gaps with respect to toxicology, and the dearth of specific migration data, the CSTEE considers that it is at present not possible to support the use of the reference citrates and adipates as plasticisers in the products under consideration. In principle, limits for the migration of these substances from such products could be set given that complete databases were available and that no unacceptable effects were revealed. However, such

limits cannot at present be set. It is not possible to examine the relationship between exposure levels and no effect levels, since data on which to make such comparisons are not sufficient.

5.4 Terms of reference 4

The CSTEE considers that databases on exposure and effects of the citrates and adipates must be comparable in breadth and quality to that of the phthalates, in order to properly evaluate their suitability as substitutes for phthalates as plasticisers in children's toys. Due to the sensitising potential of acetyltriethyl citrate and ethyl citrate, the CSTEE considers that these substances are not candidate alternatives to the phthalates.

5.5 Other considerations

In assessing the toxicological characteristics and risks of certain citrates and adipates which may be used as potential substitutes for phthalates as plasticisers in PVC toys, the CSTEE has applied generally accepted principles of risk assessment. Such assessments are able to assign safe levels of exposure to nongenotoxic chemicals from identification of no-effect levels in toxicological long-term studies and incorporation of appropriate uncertainty factors.

A very important and overall premise for risk assessment of substitution materials, is that the exposure and toxicological databases on the substitutes must be of sufficient quality and cover all the critical endpoints, so that a proper scientific assessment can be carried out. In the case of the citrates and adipates that the CSTEE have considered as substitutes to the phthalates, there are important data gaps with respect to both exposure and toxic effect information.

The CSTEE has given opinions on phthalates, citrates and adipates used as plasticisers in PVC products, since these are, or may be assumed to be, readily extractable from such products when children are mouthing PVC toys. The CSTEE has not evaluated the safety of PVC *per se* in children's toys, since this was not included in the terms of reference to the Committee. However, high-molecular weight polyvinyl chloride is a polymeric material which in itself is not bioavailable when toys are being mouthed by children and thereby non-toxic, unless the PVC product contains additives or residues at levels above those which are estimated to be safe.

The terms of reference given to the CSTEE relate to PVC products, and not to other materials which are or may be used in toys mouthed by children. The CSTEE is aware that there are a number of commercially available alternatives to PVC. Any assessment of the potential risks to children that may result from the use of alternatives to PVC in toys, should follow the same process of risk assessment that the CSTEE has used for plasticisers in PVC. Such a risk assessment must be based on the magnitude, frequency and duration of exposure to those substances which may be extracted from the alternative materials and on data from toxicological tests with such substances on critical endpoints.

6. Statement on the toxicological evaluation

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible.

CSTEE's opinions include evaluations of experiments using laboratory animals; such tests should be conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available should such tests be evaluated and the data accepted, in order to meet the fundamental requirements of protection of consumer health.

7. References

Arcadi FA, Costa C, Imperatore C, Marchese A, Rapisarda A, Salemi M, Trimarchi GR, Costa G. Oral toxicity of bis(2-ethylhexyl)phthalate during pregnancy and suckling in the Long-Evans rat. Fd Chem Toxicol 1998; 36: 963-970.

Breindahl T and Petersen JH. Plasticisers in infant formulae, baby food and total diet samples. Odense Landsdelslaboratorium, Intern rapport IFE 1998:4, 46 p, in Danish.

BUA. German Chemical Society Advisory Committee on Existing Chemicals of Environmental Relevance. Di-(2-ethylhexyl)adipate. BUA Report 196. S. Hirzel Wissenshaftliche Verlagsgesellschaft, Stuttgart, 1996.

Harrison N. Migration of plasticizers from cling-film. Fd Add Contam 1988; 5: 493-499.

IARC. Peroxisome Proliferation and its role in Carcinogenesis. International Agency for Research on Cancer. IARC Technical Report No. 24, Lyon, 1995.

IUCLID. Bis(2-ethylhexyl)adipate IUCLID Data Sheet. European Chemicals Bureau – Existing Chemicals, Ispra, 1999.

JECFA Report of June 1999 meeting, in press

Lake BG, Price RJ, Cunninghame ME, Walters DG. Comparison of the effects of di-(2-ethylhexyl)adipate on hepatic peroxisome proliferation and cell replication in the rat and mouse. Toxicology 1997; 123: 217-226.

Loftus NJ, Woollen BH, Steel GT, Wilks MF, Castle L. An assessment of the dietary uptake of di-2-(ethylhexyl)adipate (DEHA) in a limited population study. Fd Chem Toxicol 1994; 32: 1-5.

Mercer A, Castle L, Comyn J, Gilbert J. Evaluation of a predictive mathematical model of di-(2-ethylhexyl)adipate plasticizer migration from PVC film into foods. Fd Add Contam 1990; 7: 497-507.

Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF. Peroxisome proliferator activated receptor-alpha expression in human liver. Mol Pharmacol 1998; 53: 14-22.

Petersen JH, Breindahl T. Specific migration of di-(2-ethylhexyl)adipate (DEHA) from plasticized PVC film: results from an enforcement campaign. Fd Add Contam 1998; 15: 600-608.

Poon R., Lecavalier P., Mueller R., Valli VE, Procter BG, Chu I (1997) Subchronic oral toxicity of di-n-octyl phthalate and di(2-ethylhexyl)phthalate in the rat. Fd Chem Toxicol. 35, 225-239.

RIVM (1992). Jansen EHJM et al. Toxicological investigation of di(2-diethylhexyl)phthalate in rats. The determination of a no-effect level. Report No. 618902 007. Dated December 1992.

Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa 7: Significant increase of 8-hyddroxydeoxyguanosine in liver DNA of rats following short-term exposure to the peroxisome proliferators di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate. Jpn J Cancer Res 1990; 81: 213-215.

Woollen BH. Survey into the dietary intake of di-2(ethylhexyl)adipate in Member States of the European Union. Report No: CTL/R/1372, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 1998, 32p.

International Journal of Toxicology
2014, Vol. 33(Supplement 2) 16S-46S

DOI: 10.1177/1091581814526891
ijt.sagepub.com
SAGE

Safety Assessment of Citric Acid, Inorganic Citrate Salts, and Alkyl Citrate Esters as Used in Cosmetics

Monice M. Fiume[1], Bart A. Heldreth[2], Wilma F. Bergfeld[3], Donald V. Belsito[3], Ronald A. Hill[3], Curtis D. Klaassen[3], Daniel C. Liebler[3], James G. Marks Jr[3], Ronald C. Shank[3], Thomas J. Slaga[3], Paul W. Snyder[3], and F. Alan Andersen[4]

Abstract

The CIR Expert Panel (Panel) assessed the safety of citric acid, 12 inorganic citrate salts, and 20 alkyl citrate esters as used in cosmetics, concluding that these ingredients are safe in the present practices of use and concentration. Citric acid is reported to function as a pH adjuster, chelating agent, or fragrance ingredient. Some of the salts are also reported to function as chelating agents, and a number of the citrates are reported to function as skin-conditioning agents but other functions are also reported. The Panel reviewed available animal and clinical data, but because citric acid, calcium citrate, ferric citrate, manganese citrate, potassium citrate, sodium citrate, diammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate are generally recognized as safe direct food additives, dermal exposure was the focus for these ingredients in this cosmetic ingredient safety assessment.

Keywords

safety, cosmetics, citric acid, inorganic citrate salts, alkyl citrate esters

Introduction

This assessment reviews the safety of citric acid, an α (and β)-hydroxytricarboxylic acid, as used in cosmetics. The following 12 inorganic citrate salts and 20 alkyl citrate esters are also included in this safety assessment, for a total of 33 ingredients:

Inorganic salts
- aluminum citrate;
- calcium citrate;
- copper citrate;
- diammonium citrate;
- disodium cupric citrate;
- ferric citrate;
- magnesium citrate;
- manganese citrate;
- monosodium citrate;
- potassium citrate;
- sodium citrate;
- zinc citrate;

Alkyl esters
- isodecyl citrate;
- isopropyl citrate;
- stearyl citrate;
- dilauryl citrate;
- distearyl citrate;
- tributyl citrate;
- tri-C12-13 alkyl citrate;
- tri-C14-15 alkyl citrate;
- tricaprylyl citrate;
- triethyl citrate;
- triethylhexyl citrate;
- trihexyldecyl citrate;
- tri-isocetyl citrate;
- tri-isopropyl citrate;
- trilauryl citrate;
- trioctyldodecyl citrate;
- trioleyl citrate;
- triisostearyl citrate;
- tristearyl citrate;
- ethyl citrates;

[1] Cosmetic Ingredient Review Senior Scientific Analyst/Writer, Washington, DC, USA
[2] Cosmetic Ingredient Review Chemist, Washington, DC, USA
[3] Cosmetic Ingredient Review Expert Panel Member, Washington, DC, USA
[4] Former Director, Cosmetic Ingredient Review, Washington, DC, USA

Corresponding Author:
Lillian J. Gill, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.
Email: cirinfo@cir-safety.org

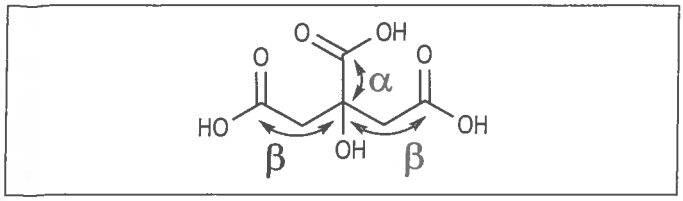


Figure 1. Citric acid.

Citric acid is reported to function in cosmetics as a chelating agent, pH adjuster, or fragrance ingredient. Although some of the inorganic citrate salts are also reported to function as a pH adjuster or chelating agent, there are many other reported functions, including skin-conditioning agent, buffering agent, cosmetic astringent, oral care agent, cosmetic biocide, or pesticide. The alkyl citrate esters are reported to function primarily as skin-conditioning agents but a few have other possible functions reported, including plasticizer, solvent, and fragrance ingredient.

As listed by the Food and Drug Administration (FDA), citric acid, calcium citrate, ferric citrate, manganese citrate, potassium citrate, sodium citrate, diammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate are generally recognized as safe (GRAS) direct food additives. Since these 10 ingredients have been shown to be safe for ingestion, this report will focus on the dermal toxicity of these ingredients. For the other ingredients, all available data will be included.

Structurally, citric acid is an α-hydroxy acid (AHA). The safety of AHAs was previously reviewed by the CIR Expert Panel (Panel).[1] In its *Guidance for Industry: Labeling for Topically Applied Cosmetic Products Containing Alpha Hydroxy Acids as Ingredients*,[2] the FDA specifically mentions citric acid-containing products, for which the following labeling may be warranted:

> Sunburn Alert: This product contains an alpha hydroxy acid (AHA) that may increase your skin's sensitivity to the sun and particularly the possibility of sunburn. Use a sunscreen, wear protective clothing, and limit sun exposure while using this product and for a week afterwards.[2]

Chemistry

Definition, Structure, and Properties

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is a common metabolite of plants and animals and is well known for its part in the Krebs cycle.[3] It precipitates as white, translucent crystals of monoclinic holohedra form. Citric acid is a polyprotic AHA. However, citric acid can also be classified as a β-hydroxy acid as 2 of the carboxylic acid functional groups of citric acid are 2 carbons removed from the hydroxy group (Figure 1).

Citric acid differs structurally from the AHAs reviewed previously[1] (ie, glycolic and lactic acid) having 3 carboxylic acid functional groups instead of just 1. Citric acid is therefore triprotic and thus has 3 different pK_as, making it a prime buffer component. Even for the most acidic of these carboxylates, that is, the center acid functional group, is only a weak acid with a pK_a of 3.1.

Citric acid is soluble in water and in some organic liquids and is very hydrophilic, with an octanol/water partition coefficient around 1. Citric acid and its salts are solids. The citrate alkyl esters, however, vary from oily liquids (for shorter chain analogs like ethyl) to powdery solids (for longer chain analogs like stearyl). Directly dependent on chain length and degree of substitution, these esters are less soluble in water and more soluble in organic liquids and are generally hydrophobic, with octanol/water partition coefficients estimated between 1 and 12.

The definitions and structures of the ingredients included in this review are provided in Table 1. The available physical and chemical property information is found in Table 2. Impurities and composition data are provided in Table 3.

Methods of Manufacture

Industrial, large-scale production of citric acid is accomplished, most commonly, via mycological fermentation of crude sugar stocks (eg, molasses), historically by strains of *Aspergillus niger*.[4] A common problem associated with these fermentation methods is the cosynthesis of isocitric acid (1-hydroxy-1,2,3-propanetricarboxylic acid). However, isocitric acid can be separated using a variety of crystallization techniques. Careful control of the trace element content is very important for high production.[3,5] (While citric acid can also be extracted from citrus fruits, over 99% of the world's citric acid output is produced by microbial fermentation.[5]) The citrate salts are produced by the same fermentation process but are simply crystallized in the presence of appropriate alkaline solutions (eg, citric acid can be crystallized with sodium hydroxide to produce sodium citrate).

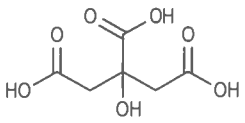
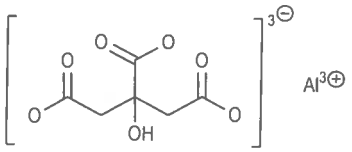
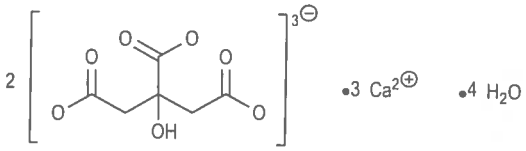
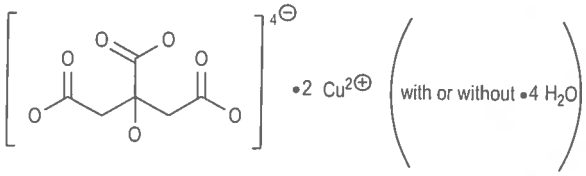
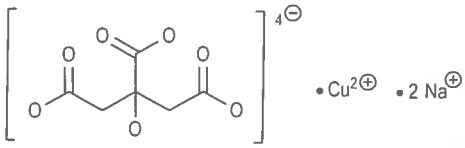
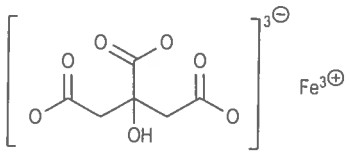
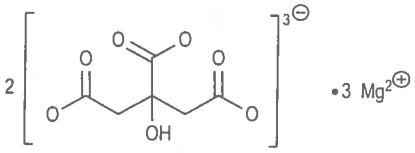
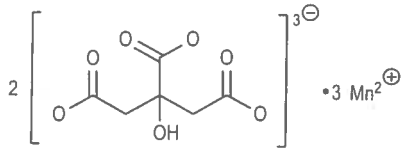
Citrate alkyl esters are typically produced via the condensation of the appropriate alcohol with citric acid (eg, condensing with butyl alcohol to produce tributyl citrate).[6] Some ingredient-specific methods of manufacture are described in Table 4.

Use

Cosmetic

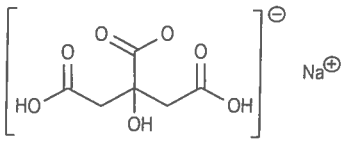
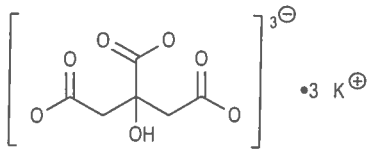
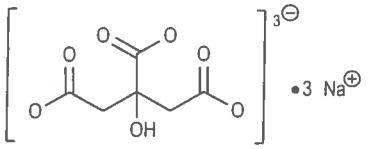
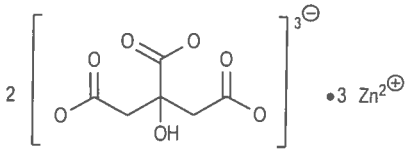
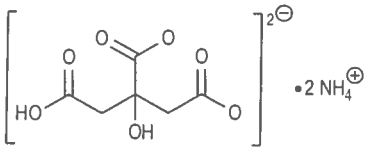
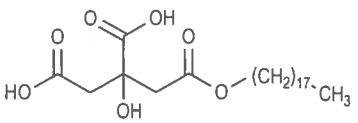
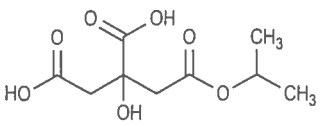
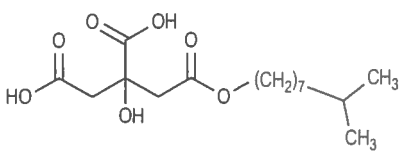
Citric acid is reported to function in cosmetics as a chelating agent, pH adjuster, or fragrance ingredient.[7] Some of the inorganic salts of citric acid are reported to function as a pH adjuster or chelating agent; these salts also have many other reported functions, including skin-conditioning agent, buffering agent, cosmetic astringent, oral care agent, cosmetic biocide, or pesticide. The alkyl esters are reported to function primarily as skin-conditioning agents but a few of these have other reported functions, including plasticizer, solvent, and

Table 1. Definitions and Structures of Citric Acid, Salt and Esters.

Ingredient/CAS No.	Definition	Formula/structure
Citric acid and inorganic salts		
Citric acid/77-92-95949-29-1 [hydrate]	An α-hydroxy tricarboxylic acid	
Aluminum citrate/813-92-331142-56-0	A complex salt of aluminum hydroxide and citric acid[7]	
Calcium citrate/5785-44-4	The calcium salt of citric acid[7]	
Copper citrate/10402-15-0866-82-0 (hemitrihydrate)	The complex copper (II) salt of citric acid. Herein, copper complexes with the carboxylates and the hydroxyl group	
Disodium cupric citrate/ 38218-87-065330-59-8	The disodium salt of the complex formed between copper (II) and citric acid. Herein, copper complexes with the hydroxyl group and one of the carboxylates	
Ferric citrate/2338-05-83522-50-7 [hydrate]28633-45-6	The iron (III) salt of citric acid	
Magnesium citrate/144-23-06150-79-47779-25-1	The magnesium salt of citric acid[7]	
Manganese citrate/10024-66-5	The manganese (II) salt of citric acid[7]	

(continued)

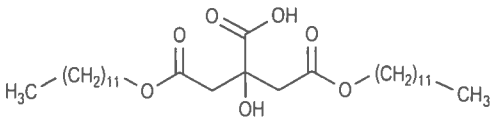
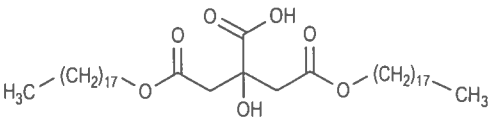
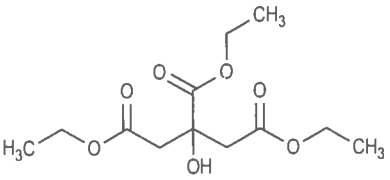
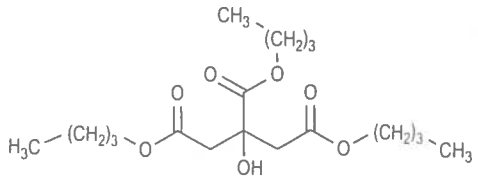
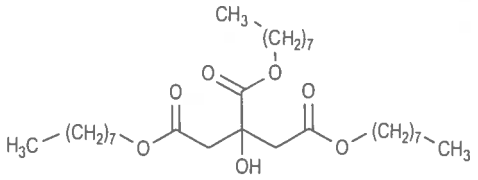
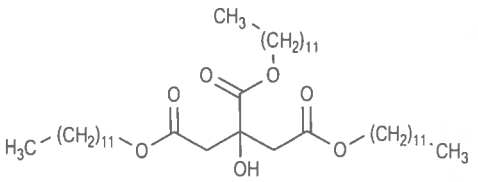
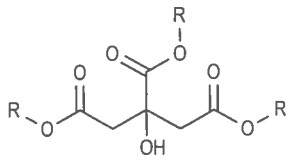
Table 1. (continued)

Ingredient/CAS No.	Definition	Formula/structure
Monosodium citrate/994-36-518996-35-5	The monosodium salt of citric acid	
Potassium citrate/866-84-2	The tripotassium salt of citric acid	
Sodium citrate/68-04-2 (anhydrous)6132-04-3 (dihydrate)	The trisodium salt of citric acid	
Zinc citrate/546-46-3	The zinc (II) salt of citric acid	
Diammonium citrate/3012-65-5	The diammonium salt of citric acid	
Alkyl esters Monoesters Stearyl citrate/1323-66-61337-33-3 [CAS No. is not specific to monoester]	The ester of stearyl alcohol and citric acid[7]	
Isopropyl citrate/39413-05-3 [CAS No. is not specific to monoester]	The ester of isopropanol and citric acid[7]	
Isodecyl citrate/90605-17-7 [CAS No. is not specific to monoester]	The ester of branched chain decyl alcohols and citric acid[7]	

1 example of an "iso"

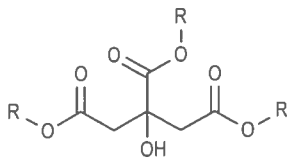
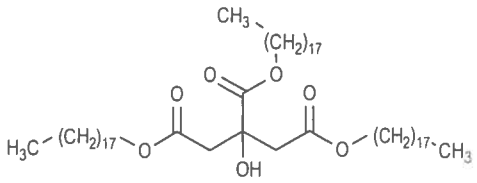
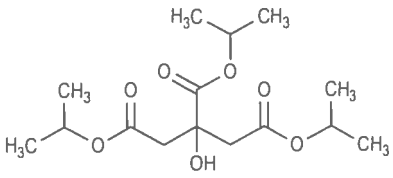
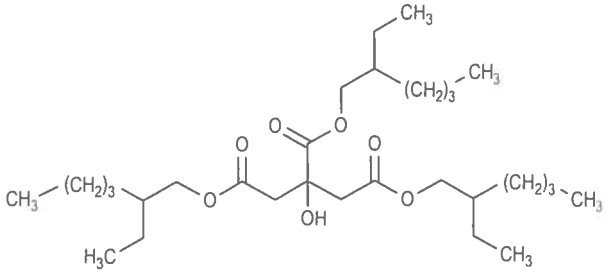
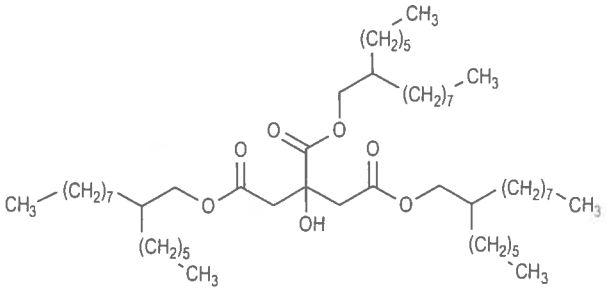
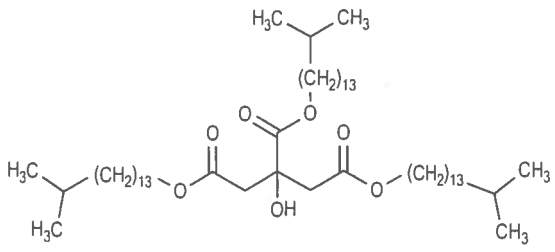
(continued)

Table 1. (continued)

Ingredient/CAS No.	Definition	Formula/structure
Diesters		
Dilauryl citrate/25637-88-1	The diester of lauryl alcohol and citric acid[7]	
Distearyl citrate/29589-99-9	The diester of stearyl alcohol and citric acid[7]	
Triesters		
Triethyl citrate/77-93-0	The triester of ethyl alcohol and citric acid[7]	
Tributyl citrate/77-94-1	The triester of butyl alcohol and citric acid[7]	
Tricaprylyl citrate/76414-35-2	The triester of capryl alcohol and citric acid[7]	
Trilauryl citrate/65277-53-4	The triester of lauryl alcohol and citric acid[7]	
Tri-C12-13 alkyl citrate	The triester of C12-13 alcohols and citric acid[7]	 wherein R is a 12- or 13-carbon chain

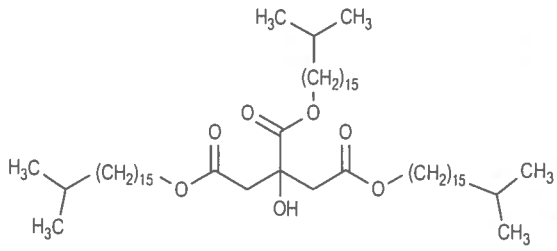
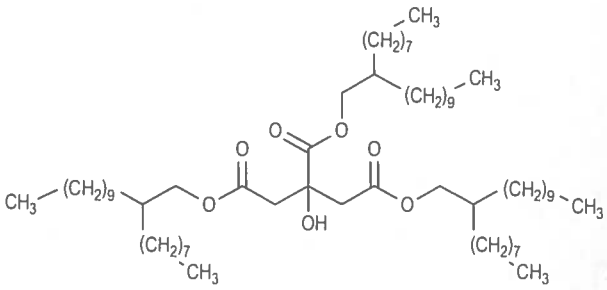
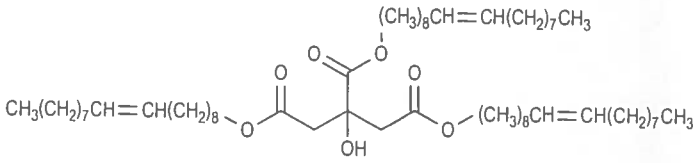
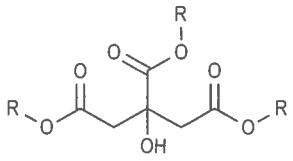
(continued)

Table 1. (continued)

Ingredient/CAS No.	Definition	Formula/structure
Tri-C14-15 alkyl citrate/ 222721-94-0	The triester of C14-15 alcohols and citric acid[7]	 wherein R is a 14 or 15 carbon chain
Tristearyl citrate/7775-50-0	The triester of stearyl alcohol and citric acid[7]	
Triisopropyl citrate/ 74592-76-0	The triester of isopropyl alcohol and citric acid	
Triethylhexyl citrate/ 7147-34-4	The triester of 2-ethylhexanol and citric acid	
Trihexyldecyl citrate	The triester of 2-hexyldecanol and citric acid.	
Triisocetyl citrate/93385-14-9	The triester of isocetyl alcohol and citric acid	 I example of an "iso"

(continued)

Table 1. (continued)

Ingredient/CAS No.	Definition	Formula/structure
Triisostearyl citrate/ 113431-54-2	The triester of isostearyl alcohol and citric acid	 1 example of an "iso"
Trioctyldodecyl citrate/ 126121-35-5	The triester of 2-octyldodecanol and citric acid	
Trioleyl citrate/175831-77-3	The triester of oleyl alcohol and citric acid	
Ethyl citrates/172820-60-9	A mixture of mono-, di-, and triesters of ethanol and citric acid[7]	 wherein R is a hydrogen atom or an ethyl group

fragrance ingredient. The various cosmetic functions of these ingredients are provided in Table 5; some ingredients have more than 1 reported function.

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). The VCRP data obtained from the FDA in 2011[8] and data received in response to a survey of the maximum reported use concentration by category conducted by the Personal Care Products Council (Council)[9] indicate that 22 of the 33 citrates named in this report are currently used in cosmetic formulations. Citric acid is used in almost every category of cosmetic product, with 6795 reported uses[8] at concentrations up to 4% in leave-on formulations, 10% in rinse-off formulations, and 39% in products diluted for (bath) use.[10] Sodium, tributyl, and triethyl citrate are reported to be used in 980, 331, and 244 cosmetic formulations, respectively.[8] All other in-use ingredients have less than 50 uses. The ingredient with the highest concentration of use is tri-isostearyl citrate; it is used at up to 80% in lipstick formulations.[10] Trioctyldodecyl citrate is used at up to 30% in leave-on formulations; it is used at up to 21% in products applied to the eye area and 19% in lipstick formulations. Tricaprylyl citrate is used at up to 27% in leave-on formulations. All other in-use ingredients are used at concentrations of ≤12%.

Frequency and concentration of use data are provided in Table 6. The ingredients not in use, according to the VCRP and Council survey, are listed in Table 7. Products containing citric acid and some of its salts and esters may be applied to baby skin or used near the eye area or mucous membranes. Additionally, citric acid and some of its salts and esters are used in cosmetic sprays, including hair, deodorant, body, and

Table 2. Chemical and Physical Properties.

Property	Description	Reference
Citric acid		
Molecular weight	192.12	4
	monohydrate: 210.14	
Appearance and form	Monoclinic holohedrism crystals	4
	monohydrate: orthorhombic crystals	
	Free-flowing, colorless, translucent crystals, or as a white granular to fine powder	48
Melting point	153°C	4
	Monohydrate: ≈100°C	
Boiling point	Decomposes above 175°C	19
log P	−1.198 ± 0.396 (at 25°C)	49
log Kow	−1.75	50
Vapor pressure	<0.001 mm Hg (20°C)	51
	3.7×10^{-9} mm Hg (25°C)	50
Solubility	Solubility in water increases with temperature (from 54%, w/w at 10°C to 84%)	4
	At 100°C; freely soluble in alcohol; very slightly soluble in ether in water: 162 g/100 mL (at 25°C); in alcohol: 59.1 g/100 mL (at 25°C)	48
	Solubility in water increases with temperature from ~54 wt% at 10°C to ~88 wt% at 100°C	52
Density	1.665monohydrate: 1.542	4
pK_a	pK1 = 3.128; pK2 = 4.761; pK3 = 6.396 (25°C)	4
pH (citric acid–sodium citrate solution)	pH of water solutions with equal percentages of citric acid and sodium citrate ranged from 4.15 (0.25% each chemical) to 3.54 (15% of each chemical)	53
Aluminum citrate		
Density	1.5 g/cm^3	4
Calcium citrate		
Molecular weight	498.43	4
Appearance and form	Fine white, odorless powder	54
Solubility	Soluble in 1050 parts cold water, somewhat soluble in hot water; insoluble in alcohol	4
Copper citrate		
Molecular weight	315.18	4
Appearance and form	Green or bluish-green crystalline powder; odorless	4
Solubility	Slightly soluble in water; soluble in ammonia, diluted acids, and cold alkali citrate solutions; freely soluble in hot alkali citrate solutions	4
Diammonium citrate		
Molecular weight	226.18	4
Appearance and form	Granules or crystals	4
Solubility	Soluble in 1 part water; slightly soluble in alcohol	4
Ferric citrate		
Appearance and form	Garnet-red transparent scales or pale brown powder	4
Solubility	Slowly but completely soluble in cold water; readily soluble in hot water, practically insoluble in alcohol	4
Magnesium citrate		
Molecular weight	Dibasic: 214.41	4
	tribasic: 451.11	
Monosodium citrate		
Molecular weight	214.12	55
Melting point	Decomposes	55
Solubility	570 g/L (at 25°C); insoluble in ethanol and ether	55
Potassium citrate		
Molecular weight	306.39	4
	monohydrate: 324.41	
Appearance and form	Monohydrate: white crystals, granules, or powder; odorless	4
	Monohydrate: white coarse powder	56
Boiling point	211°C (calculated)	50
log Kow (calculated)	−0.28	50
Vapor pressure	2.09×10^{-12} mm Hg (25°C)	50
Solubility	1 g dissolves slowly in 0.65 mL water; practically insoluble in alcohol	4
	Monohydrate: 190 g/100 mL water (at 25°C); insoluble in alcohol and ether	56
Stability	Monohydrate: very hygroscopic; readily deliquesces in moist air	56

(continued)

Table 2. (continued)

Property	Description	Reference
Sodium citrate		
Molecular weight	258.07	4
	dihydrate: 294.10	
Appearance and form	Dihydrate: white crystals, granules, or powder; odorless	4
Melting point	Anhydrous: >300°C	57
	dihydrate: 150°C	58
Density	Monohydrate: 1.814	4
log Kow (calculated)	−0.28	59
Vapor pressure	2.09×10^{-12} mm Hg (25°C)	50
Solubility	Soluble in water, ~425 g/L (25°C)	50
	monohydrate: soluble in 1.3 parts water; insoluble in alcohol	4
Zinc citrate		
Molecular weight	574.43	4
Appearance and form	Powder; odorless	4
Solubility	Slightly soluble in water; soluble in diluted mineral acids and alkali hydroxides	4
Stearyl citrate		
Molecular weight	458.60	60
Distearyl citrate		
Melting point	70-72°C	61
Triethyl citrate		
Molecular weight	276.29	62
Appearance and form	Clear, colorless, oily liquid	30
Melting point	−55°C	63
Boiling point	294°C	63
Vapor pressure	6.4×10^{-3} mm Hg (20°C)	64
Density	1.137 (20°C)	4
Refractive index	1.440-1.442 (25°C/D)	64
Solubility	6.5 g/100 mL water (25°C)	30
	5.5 g/100 mL water (25°C); insoluble in hexane	64
	miscible with alcohol, ether	4
log Kow	1.3 (35°C; measured)	62
	0.33 (calculated)	
Tributyl citrate		
Molecular weight	360.44	4
Appearance and form	Colorless or pale yellow liquid; odorless	4
Melting point	−20°C	4
Boiling point	170°C (1 mm Hg)	30
	233°C (22 mm Hg)	4
Vapor pressure	9.6×10^{-2} mm Hg (20°C)	64
Density	1.045 (20°C)	4
Refractive index	1.443-1.445 (25°C/D)	64
Solubility	Insoluble in water; miscible with most organic liquids	4
log P (predicted)	4.324 ± 0.411 (25°C)	49
pK_a (predicted)	11.3 ± 0.29 (25°C)	49
Tricaprylyl citrate		
Molecular weight	528.76	49
Boiling point	250-255°C (6-7 mm Hg)	65
Density	0.9498 g/cm^3	65
log P (predicted)	10.438 ± 0.411 (25°C)	49
pK_a (predicted)	11.30 ± 0.29	49
Trilauryl citrate		
Molecular weight	697.08	49
Boiling point (predicted)	675.9°C	49
Density (predicted)	0.955 g/cm^3 (20°C)	49
log P (predicted)	16.551 (25°C)	49
pK_a (predicted)	11.29 (25°C)	49
Tristearyl citrate		
Molecular weight	949.56	49
Boiling point (predicted)	840.3°C	49

(continued)

Table 2. (continued)

Property	Description	Reference
Density (predicted)	0.924 g/cm^3 (20°C)	49
log P (predicted)	25.722 (25°C)	49
pK$_a$ (predicted)	11.29 (25°C)	49
Triisopropyl citrate		
Molecular weight	318.36	49
Boiling point (predicted)	331°C	49
Density (predicted)	1.116 g/cm^3 (20°C)	49
log P (predicted)	2.328 (25°C)	49
pK$_a$ (predicted)	11.69 (25°C)	49
Triisostearyl citrate		
Molecular weight	944	66
Appearance	Clear viscous liquid	67
Trioctyldodecyl citrate		
Molecular weight	1032	66
Boiling point (predicted)	883.3°C	49
Density (predicted)	0.917 g/cm^3 (20°C)	49
log P (predicted)	29.634 (25°C)	49
pK$_a$ (predicted)	11.25 (25°C)	49
Trioleyl citrate		
Molecular weight	943.51	49
Boiling point (predicted)	845.8°C	49
Density (predicted)	0.936 g/cm^3 (20°C)	49
log P (predicted)	25.443 (25°C)	49
pK$_a$ (predicted)	11.28 (25°C)	49

Table 3. Impurities and Composition.

Ingredient	Impurities/composition	Reference
Stearyl citrate	10%-15% monostearyl, 70%-80% distearyl, and 10%-15% tristearyl derivatives	18
Isopropyl citrate	65%-80% monoisopropyl, 15%-30% diisopropyl, and 5%-10% triisopropyl citrate	18
Triisostearyl citrate	Supplied as >90% triisostearyl citrate impurities include residual isostearyl alcohol (<10%) and citric acid (<0.5%)	66
Trioctyldodecyl citrate	Supplied as ~100% trioctyldodecyl citrate (according to one supplier) impurities include residual octyldodecyl alcohol (<5%) and citric acid	66

Table 4. Ingredient-Specific Methods of Manufacture.

Ingredient	Method of manufacture	Reference
Calcium citrate	Neutralization of citric acid with calcium hydroxide or calcium carbonate	21CFR184.1195
Copper citrate	Prepared by the interaction of hot aqueous solutions of copper sulfate and sodium citrate	4
Ferric citrate	Prepared from reaction of citric acid with ferric hydroxide	21CFR184.1298
Manganese citrate	Obtained by precipitating manganese carbonate from manganese sulfate and sodium carbonate solutions. The filtered and washed precipitate is digested first with sufficient citric acid solution to form manganous citrate and then with sodium citrate to complete the reaction	21CFR184.1449
Potassium citrate	Crystallizing and drying of a potassium citrate solution that is prepared using a citric acid solution and potassium hydroxide	68
Sodium citrate	Neutralization of citric acid with sodium hydroxide or sodium carbonate	21CFR184.1751
Zinc citrate	Prepared from zinc carbonate and citric acid	4
Diammonium citrate	Partial neutralization of citric acid with ammonia	21CFR184.1140
Isopropyl citrate	Esterification of citric acid with isopropanol	21CFR184.1386
Stearyl citrate	Esterification of citric acid with stearyl alcohol	21CFR184.1851
Triethyl citrate	Esterification of ethyl alcohol with citric acid	21CFR184.1911
Tributyl citrate	Synthesized from n-butyl alcohol and citric acid	4
Triisostearyl citrate	Manufactured from isostearyl alcohol and citric acid in a proprietary esterification process, without the use of heavy metal catalysts	66
Trioctyldodecyl citrate	Manufactured from octyldodecyl alcohol and citric acid in a proprietary esterification process, without the use of heavy metal catalysts	66

Table 5. Reported Cosmetic Functions of Citric Acid and its Salts and Esters.[a]

pH adjuster
Citric acid
Calcium citrate
Monosodium citrate
Potassium citrate
Sodium citrate
Chelating agent
Citric acid
Diammonium citrate
Potassium citrate
Sodium citrate
Fragrance ingredient
Citric acid
Sodium citrate
Triethyl citrate
Buffering agent
Diammonium citrate
Potassium citrate
Sodium citrate
Skin-conditioning agent—emollient
Dilauryl citrate
Distearyl citrate
Isodecyl citrate
Stearyl citrate
Tri-C12-13 alkyl citrate
Tri-C14-15 alkyl citrate
Triethylhexyl citrate
Triisopropyl citrate
Trioleyl citrate
Skin-conditioning agent—occlusive
Tricaprylyl citrate
Trihexyldecyl citrate
Triisocetyl citrate
Triisostearyl citrate
Trilauryl citrate
Trioctyldodecyl citrate
Tristearyl citrate
Triisostearyl citrate
Trilauryl citrate
Skin-conditioning agent—miscellaneous
Ferric citrate
Magnesium citrate
Hair fixative
Ethyl citrates
Plasticizer
Isodecyl citrate
Isopropyl citrate
Tributyl citrate
Triethyl citrate
Triethylhexyl citrate
Cosmetic astringent
Aluminum citrate
Oral care agent
Zinc citrate
Cosmetic biocide
Zinc citrate
Pesticide
Copper citrate
Solvent
Isopropyl citrate
Tributyl citrate
Not reported
Disodium cupric citrate
Manganese citrate

[a]Reference.[7]

other propellant and pump spray products; the maximum reported concentrations of citric acid used in a spray product is 0.7% citric acid, of a salt is 0.2% sodium citrate, of an ester is 4% trioctyldodecyl citrate, and in deodorants is 2% triethyl citrate. These products could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump sprays.[11-14] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[11,13] There is some evidence indicating that, generally, deodorant spray products, compared with hair sprays, can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[11] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

All ingredients included in this review are listed in the European Union inventory of cosmetic ingredients.[15] "Water-soluble zinc compounds" are listed in Annex III of the Cosmetic Directive, with a maximum authorized concentration in the finished cosmetic product of 1% calculated as zinc; therefore, zinc citrate has a maximum authorized concentration of use of 1%, calculated as zinc, in finished cosmetic products in the European Union.[16]

Noncosmetic

The following 10 ingredients are GRAS direct food additives, restricted only by the need to follow good manufacturing practices: citric acid (21CFR184.1033), calcium citrate (21CFR184.1195), ferric citrate (21CFR184.1298), manganese citrate (21CFR184.1449), potassium citrate (21CFR184.1625), sodium citrate (defined as the trisodium salt; 21CFR184.1751), diammonium citrate (21CFR184.1140), isopropyl citrate (21CFR184.1386), stearyl citrate (21CFR184.1851), and triethyl citrate (21CFR184.1911). Additionally, the following are allowed as indirect food additives: citric acid, magnesium citrate, monosodium citrate, potassium citrate, sodium citrate, diammonium citrate, stearyl citrate, isopropyl citrate, distearyl citrate, triethyl citrate, tributyl citrate, and tristearyl citrate.[17] Citrate-containing ingredients are allowed as active ingredients, at a maximum daily dosage of 8 g, in antacid over-the-counter products (21CFR331.11). Examples of other noncosmetic uses of citric acid and some of the citrates are provided in Table 8.

Toxicokinetics

Orally administered citric acid is well absorbed and largely metabolized.[18] Exogenous and endogenous citric acid can be completely metabolized and serve as a source of energy. Citric acid is an intermediate in the Krebs (or tricarboxylic acid)

Table 6. Frequency and Concentration of Use According to Duration and Type of Exposure.

	Citric acid		Aluminum citrate		Diammonium citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	6795	0.0000005-39	4	NR	6	NR
Duration of use						
Leave on	2851	0.0000005-4	3	NR	2	NR
Rinse off	3753	0.000002-10	1	NR	4	NR
Diluted for (bath) use	191	0.3-39	NR	NR	NR	NR
Exposure type						
Eye area	580	0.0000005-2	NR	NR	1	NR
Incidental ingestion	214	0.0006-3	NR	NR	NR	NR
Incidental inhalation—spray	149[b]	0.004-0.7[b]aerosol: 0.05-0.7pump: 0.003-0.1	NR	NR	NR	NR
Incidental inhalation—powder	35	0.0006-0.3	NR	NR	NR	NR
Dermal contact	4055	0.000008-10	4	NR	2	NR
Deodorant (underarm)	22[c]	0.000008-0.2 (not spray)0.002[c]pump: 0.01	NR	NR	NR	NR
Hair—noncoloring	1945	0.0001-5	NR	NR	3	NR
Hair—coloring	210	0.08-10	NR	NR	NR	NR
Nail	290	0.001-45% diluted to 0.025%	NR	NR	NR	NR
Mucous membrane	1875	0.0002-39 (20%-39% is diluted prior to bath use)	1	NR	1	NR
Baby products	112	0.2	NR	NR	NR	NR

	Dilauryl citrate		Ethyl citrates		Ferric citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	1	NR	NR	0.5-1	7	0.5
Duration of use						
Leave on	NR	NR	NR	NR	4	NR
Rinse off	1	NR	NR	0.5-1	3	0.5
Diluted for (bath) use	NR	NR	NR	NR	NR	NR
Exposure type						
Eye area	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR
Incidental inhalation—spray	NR	NR	NR	NR	NR	NR
Incidental inhalation—powder	NR		NR	NR	NR	NR
Dermal contact	1	NR	NR	0.5-1	5	0.5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	NR	NR	2	NR
Hair—coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	NR	1	NR	0.5
Baby products	NR	NR	NR	NR	NR	NR

	Isodecyl citrate		Magnesium citrate		Monosodium citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	4	NR	9	0.01-2	16	0.004-5
Duration of use						
Leave on	4	NR	NR	0.01-2	NR	0.004-5
Rinse off	NR	NR	9	0.5	2	0.8-5
Diluted for (bath) use	NR	NR	NR	NR	14	5
Exposure type						
Eye area	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR
Incidental inhalation—spray	NR	NR	NR	NR	NR	NR
Incidental inhalation—powder	NR	NR	NR	NR	NR	5
Dermal contact	4	NR	NR	0.01-2	16	0.004-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	9	0.5	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	NR	NR	14	5 (diluted prior to use)
Baby products	NR	NR	NR	NR	NR	5

(continued)

Table 6. (continued)

	Potassium citrate		Sodium citrate		Stearyl citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	8	0.002-0.6	980	0.000005-10	23	0.007-12
Duration of use						
Leave on	2	0.002-0.5	587	0.000005-10	1	0.3-12
Rinse off	6	0.002-0.6	386	0.0001-10	22	0.007-2
Diluted for (bath) use	NR	NR	7	0.9	NR	NR
Exposure type						
Eye area	1	NR	47	0.02-2	NR	1-2
Incidental ingestion	1	0.6	7	0.003-0.4	NR	12
Incidental inhalation—spray	NR	0.06-0.07	30[c]	0.000005-0.3; 0.4[b]	NR	1-3[b]
Incidental inhalation—powder	NR	0.02	6	0.03	NR4	NR
Dermal contact	4	0.002-0.5	718	0.0001-10	20	0.007-5
Deodorant (underarm)	NR	NR	1[b]	0.02 (not a spray); 0.1[c]	NR	3[c]
Hair—noncoloring	3	0.002-0.07	206	0.000005-4	3	1
Hair—coloring	NR	NR	5	0.1	NR	NR
Nail	NR	NR	2	0.08-0.5	NR	NR
Mucous membrane	4	0.002	96	0.003-1	7	0.007-12
Baby products	NR	NR	9	NR	NR	NR

	Tributyl citrate		Tri-C12-13 alkyl citrate		Tri-C14-15 alkyl citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	331	0.0005-9	1	NR	19	0.1-5
Duration of use						
Leave on	35	0.0005-9	1	NR	19	0.1-5
Rinse off	267	0.0009-5	NR	NR	NR	NR
Diluted for (bath) use	29	0.0005	NR	NR	NR	NR
Exposure type						
Eye area	NR	NR	1	NR	1	3
Incidental ingestion	NR	NR	NR	NR	NR	NR
Incidental inhalation—spray	6	NR	NR	NR	13[b]	0.1-5[b]
Incidental inhalation—powder	1	NR	NR	NR	NR	NR
Dermal contact	260	0.0005-<0.05	1	NR	19	0.1-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	14	NR	NR	NR	NR	NR
Hair—coloring	55	NR	NR	NR	NR	NR
Nail	2	0.01-9	NR	NR	NR	NR
Mucous membrane	233	0.0005-0.001	NR	NR	NR	NR
Baby products	1	NR	NR	NR	NR	NR

	Tricaprylyl citrate		Triethyl citrate		Triethylhexyl citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	19	0.3-27	244	0.0008-6	1	NR
Duration of use						
Leave on	16	0.3-27	215	0.004-6	1	NR
Rinse off	3	0.5-0.8	29	0.0008-0.2	NR	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	NR
Exposure type						
Eye area	1	3	2	0.2-0.6	NR	NR
Incidental ingestion	3	14-19	11	0.3	NR	NR
Incidental inhalation—spray	NR	NR	96[b]	0.2-2	NR	NR
Incidental inhalation—powder	1	NR	NR	3	NR	NR
Dermal contact	13	0.3-27	127	0.0008-6	1	NR
Deodorant (underarm)	NR	NR	48[c]	2 (aerosol)	NR	NR
Hair—noncoloring	3	0.5-0.8	106	0.1-2	NR	NR
Hair—coloring	NR	NR	NR	0.5	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	3	14-19	18	0.2-0.3	NR	NR
Baby products	NR	NR	NR	0.009	NR	NR

(continued)

Table 6. (continued)

	Triisocetyl citrate		Triisostearyl citrate		Trioctyldodecyl citrate	
	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]	# of uses[8]	Max. conc of use, %[9]
Totals[a]	33	0.6-3	47	0.3-80	56	1-30
Duration of use						
Leave on	33	0.6-3	44	0.3-80	56	1-30
Rinse off	NR	NR	3	NR	NR	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	NR
Exposure type						
Eye area	NR	NR	1	NR	8	5-21
Incidental ingestion	7	NR	39	9-80	37	1-19
Incidental inhalation—spray	NR	NR	NR	NR	1	4
Incidental inhalation—powder	11	2	NR	NR	2	1-3
Dermal contact	26	0.6-3	5	0.3-9	19	1-30
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	3	NR	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	7	NR	39	9-80	37	1-19
Baby products	NR	NR	NR	NR	NR	NR

	Zinc citrate	
	# of uses[8]	Max. conc of use, %[9]
Totals[a]	9	0.05-2
Duration of use		
Leave on	5	0.05
Rinse off	4	0.3-2
Diluted for (bath) use	NR	NR
Exposure type		
Eye area	NR	NR
Incidental ingestion	4	0.3-2
Incidental inhalation—spray	NR	NR
Incidental inhalation—powder	NR	0.05
Dermal contact	5	0.05
Deodorant (underarm)	4[c]	NR
Hair—noncoloring	NR	NR
Hair—coloring	NR	NR
Nail	NR	NR
Mucous membrane	4	0.3-2
Baby products	NR	NR

Abbreviation: NR, no reported uses
[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[b]Includes suntan products, in that it is not known whether or not the reported product is a spray.
[c]It is not known whether or not the product is a spray.

Table 7. Ingredients Not Reported to be Used.

Calcium citrate
Copper citrate
Disodium cupric citrate
Distearyl citrate
Isopropyl citrate
Manganese citrate
Trihexyldecyl citrate
Triisopropyl citrate
Trilauryl citrate
Trioleyl citrate
Tristearyl citrate

cycle.[19] Citric acid completes the breakdown of pyruvate, formed from glucose through glycolysis, and it liberates carbon dioxide. Approximately 2 kg of citric acid are formed and metabolized every day in humans. Citrate is thought to be freely filterable at the glomerulus of the kidney and 65% to 90% of filtered citrate is reabsorbed in humans.[20] Filtered citrate of 10% to 35% is excreted in the urine. The normal blood citrate level in humans is approximately 25 mg/L.[21]

In Vitro

Trihexyl citrate. Although trihexyl citrate is not a cosmetic ingredient, this information is presented because this chemical is structurally similar to cosmetic ingredients included in this review, supporting that citrate esters undergo hydrolysis.

Trihexyl citrate was incubated with rat serum, an intestinal cytosolic fraction, and a liver cytosolic fraction obtained from Sprague-Dawley rats to determine the hydrolysis of trihexyl

Table 8. Examples of Noncosmetic Uses.

Ingredient	Noncosmetic use	Reference
Citric acid	Used in the food, beverage, and pharmaceutical industries; active ingredient in pesticide products; manufacture of ecologically compatible detergents; chemical cleaning; metal cleaning; concrete admixtures; plasticizers; photography	3,5,48,69
Calcium citrate	Calcium fortifier in foods; anticaking agent in dry mixes	4
Copper citrate	As an astringent or antiseptic	4
Diammonium citrate	Determination of phosphate, especially in fertilizers	4
Potassium citrate	As a replacement for sodium citrate in foods; as a buffering agent in foods; as a source of potassium ion in a nutritional supplement; sequestering or emulsifying agent	4,56
Sodium citrate	Anticoagulant; acidulant in beverages, confectionery, effervescent salts, powders, and tablets, pharmaceutical syrups, and elixirs; pH adjuster in food; as an synergistic oxidant; in processing cheese; in the manufacture of alkyd resins; in the manufacture of citric acid salts as a sequestering agent to remove trace metals; in electroplating; in special inks	4
Triethyl citrate	Plasticizer for cellulose derivatives and natural resins; plasticizer in pharmaceutical excipients; solvent in paint removers; emulsifier in food industry; flavor-preserving agent	60,64,70
Tributyl citrate	Plasticizer and solvent for nitrocellulose lacquers; in polishes, inks, and similar preparations; plasticizer in pharmaceutical excipients; as an antifoam agent	4
Zinc citrate	Used in toothpaste and mouthwash	4

citrate in each of these preparations.[22] Dimethyl sulfoxide (DMSO) was used as the vehicle; the volume of DMSO did not exceed 1% of the total volume of the incubation medium. A concentration of 50 nmol/mL was used with all 3 preparations; a concentration of 1000 nmol/mL was also used with rat serum. In rat serum, at the concentrations of 50 and 1000 nmol/mL, the half-life of trihexyl citrate hydrolysis was 4 and 90 minutes, respectively. Hexanol was produced as a product of hydrolysis. Dihexyl citrate is formed as an intermediate. Hydrolysis was concentration dependent, being faster at lower concentrations. Hydrolysis did not occur with 5 µmol/mL of serum. The half-life of hydrolysis for 50 nmol/mL trihexyl citrate in the rat liver cytosolic fraction was 1.2 minutes (The half-life was not given for the intestinal fraction.)

Aluminum citrate. The lipid bilayer permeation of neutral aluminum citrate was determined by measuring the flux across unilamellar phospholipid vesicles or liposomes, using 2 independent procedures.[23] The permeation of aluminum citrate was then compared to that of citric acid (as well as malic and lactic acids). Lipid bilayer permeation of 1.82 mmol/L aluminum citrate was slow; the permeability coefficient was, at most, 2×10^{-11} cm/s. Comparison of permeation of aluminum citrate to the acids indicated that the flux of aluminum citrate is limited by diffusion across the water–lipid interface. (The permeability coefficient for 6.0 mmol/L citric acid was 3.1×10^{-11} cm/s.)

Oral

Aluminum citrate. Eight male Sprague-Dawley rats were dosed by gavage with 100 mg aluminum/kg bw, as aluminum citrate, 6 days/wk for 4 weeks.[25] A control group was given tap water. Half of the animals were killed at the termination of dosing; the remaining animals were killed after a 5-week nontreatment period. The levels of aluminum in the cortex of the brain, the hippocampus, and the cerebellum were statistically significantly increased after 4 weeks of dosing with aluminum citrate, and there was no major difference between the animals killed at the termination of dosing and 5 weeks later. (In this study, groups of rats were also dosed with citric acid for 4 weeks or aluminum hydroxide for 9 weeks. The aluminum content in the cortex of the brain of rats dosed with citric acid was statistically significantly increased compared to controls. There were no statistically significant differences in aluminum content of the brain between control rats and those dosed with aluminum hydroxide.)

Ten female Sprague-Dawley rats were given drinking water with 80 mmol/L aluminum citrate for 8 months; a control group of 8 rats was given untreated water.[26] After 8 months of dosing, aluminum concentrations were statistically significantly increased in bone, spleen, liver, and kidneys, but not the brains, of treated animals.

Stearyl/distearyl citrate. Stearyl citrate is hydrolyzed readily to stearyl alcohol and citric acid in dogs and, to a lesser extent, in rats.[27] Stearyl citrate, predominantly as distearyl citrate, added to the feed of rats at a concentration of 2.5% to 10% was poorly absorbed (additional details were not provided).[18]

Isopropyl citrate. Isopropyl citrate, mostly as the monoisopropyl ester, was administered in the diet of 6 rats in a mono- and diglycerides vehicle at concentrations of $\leq$10%.[18] Isopropyl citrate was nearly completely absorbed (additional details were not provided).

Effect on Transdermal Absorption

Triethyl citrate. Triethyl citrate inhibited the transdermal absorption of viprostol, a synthetic prostaglandin E_2, through the skin of male hypertensive rats.[28] This effect was demonstrated by the statistically significant decrease in blood radioactivity levels following the topical application of [^{14}C]viprostol in triethyl citrate compared to those found with the use of petrolatum (pet) or

Table 9. Acute Toxicity Studies.

Ingredient	Animals[a]	No./group	Dose	LD_{50}	Reference
Dermal					
Citric acid					
Citric acid	Rabbits	10	5 g/kg tested	>5 g/kg	51
Triethyl citrate					
Triethyl citrate	Rabbits	4	Not stated	>5 g/kg	62
Triethyl citrate	Guinea pig	Not stated	Not stated	>10 mL/kg	71
Oral					
Tributyl citrate					
Tributyl citrate	Rats	5	10-30 mL/kg	No deaths reported	30
Tributyl citrate	Cats	4	30-50 mL/kg	No deaths reported	30
Trioctyldodecyl citrate					
Trioctyldodecyl citrate	Rats	10 (5/sex)	5 g/kg	No deaths reported	72
Inhalation					
Triethyl citrate	Rats	Not stated	6-h exposure to vapor	1300-3500 ppm	71
Intraperitoneal					
Monosodium citrate					
Monosodium citrate	White mice	Not stated	0.0477 mol/L solution	7.6 mmol/kg	73
Monosodium citrate	Albino rats	Not stated	0.381 mol/L solution	6.3 mmol/kg	73
Tributyl citrate					
Tributyl citrate	Swiss albino mice	Not stated	Chosen from a logarithmic scale	2900 mg/kg	31
Intravenous					
Monosodium citrate	White mice	Not stated	0.019 mol/L; rapid administration	0.23 mmol/kg	73
Monosodium citrate	White mice	20	0.25 mol/L administered at rate of 1.5 mmol/min (6 mL/min)	2.01 mmol/kg	73
Monosodium citrate	Rabbits	Not stated	0.477 mol/L; administered at a rate of 0.358 mmol/min (0.75 mL/min)	1.76 mm/kg	73

Abbreviation: LD_{50}, median lethal dose.
[a]Unless it is given, the sex of the animals was not stated.

silicone as the vehicle. A comparison of metabolic profiles also demonstrated slower hydrolysis of viprostol to free acid with the use of triethyl citrate as the vehicle.

Toxicological Studies

Single Dose (Acute) Toxicity

Acute toxicity studies are summarized in Table 9. Acute toxicity testing did not raise any toxicological concerns.

Repeated Dose Toxicity

Oral

Aluminum citrate. In a toxicokinetics study described previously, a group of 8 male Sprague-Dawley rats was dosed by gavage with 100 mg aluminum/kg bw, as aluminum citrate, 6 days/wk for 4 weeks.[25] A control group was given tap water. Half of the animals were killed at the termination of dosing; the remaining animals were killed after a 5-week nontreatment period. Body weights of test animals were similar to those of controls after 4 weeks of dosing. Body weights of treated animals decreased compared to controls during the recovery period, but the difference was not statistically significant.

In another toxicokinetics study described previously in this report, a group of 10 female Sprague-Dawley rats was given aluminum citrate in the drinking water at a concentration of 80 mmol/L for 8 months.[26] Final body weights of animals of the test group were statistically significantly decreased compared to the controls. Kidney function was not affected by dosing.

Isopropyl citrate ester mixture. A 6-week feeding study of an isopropyl citrate ester mixture consisting of 27% isopropyl citrate, 9% diisopropyl citrate, and 2% triisopropyl citrate, in a vehicle consisting of mono- and diglycerides (1:1) vegetable oil, was performed using rats.[29] Male rats had an average daily intake of 0.78 g and females 0.54 g of the citrate mixture, and no adverse effects were observed. (Additional details were not provided.)

Groups of 10 rats were fed diets containing 0%, 0.28%, 0.56%, or 2.8% of the above-mentioned isopropyl citrate ester mixture in the same vehicle (corresponding to 0%, 0.11%, 0.21%, and 1.06% isopropyl citrate ester content, respectively) for 2 years.[29] Again, no signs of toxicity were observed. Microscopic examination of select tissues did not reveal any test article-related changes.

Six-week dietary and 6-week gavage studies were performed in rabbits using the same isopropyl citrate ester mixture in the same vehicle.[29] Signs of toxicity were not observed in groups of 1 to 8 rabbits given feed containing 1.9% to 22.5% of the isopropyl citrate ester mixture or in groups of 1 to 3 rabbits dosed daily by gavage with 0%, 2.2%, 4.4%, or 9.2% of the

isopropyl citrate ester mixture. Selected tissues of the 8 high-dose males used in the feeding study were examined microscopically and no abnormalities were found.

Groups of 2 cocker puppies and 2 adult mongrel dogs were also fed a diet containing the isopropyl citrate ester mixture in vehicle.[29] Adverse effects were not observed when dogs were fed a diet containing 0.06% of the test article for 12 weeks.

Distearyl citrate ester mixture. A 6-week feeding study of a distearyl citrate ester mixture consisting of 12.5% stearyl citrate, 75% distearyl citrate, and 12.5% tristearyl citrate was performed using rats.[29] Male rats had an average daily intake of 1.32 g and females 1.06 g of the mixture and no adverse effects were observed. (Additional details were not provided.)

Groups of 10 rats were fed diets containing 0%, 0.5%, 2.0%, or 10.0% of the distearyl citrate ester mixture for 2 years.[29] No signs of toxicity were observed. Microscopic examination of select tissues did not reveal any test article-related changes.

In a 6-week dietary study in rabbits with the same distearyl citrate ester mixture, 2 groups of 8 rabbits were given feed containing 2% or 10% of the mixture.[29] No signs of toxicity were observed. Select tissues of the rabbits of the 10% group, including the liver, kidneys, heart, and brain, were examined microscopically. No abnormalities were found.

Groups of 2 cocker puppies and 2 adult mongrel dogs were also fed a diet containing the distearyl citrate ester mixture.[29] Adverse effects were not observed when dogs were fed a diet containing 3.0% of the test article for 12 weeks.

Tributyl citrate. Groups of 3 or 4 rats, number per sex not specified, were fed a diet containing 0%, 5%, or 10% tributyl citrate for 6 weeks.[30] No effect on body weight gain was observed in the 5% group. Body weight gains in the 10% group were decreased; the decrease may have been attributable to frequent diarrhea. No effects on blood counts were reported and no microscopic lesions were observed.

Two cats were dosed daily by gavage with 5 mL/kg tributyl citrate daily for 2 months, and 2 cats were used as negative controls.[30] No significant effects were observed.

Intraperitoneal

Tributyl citrate. A test group of 20 mice (sex not stated) was dosed by intraperitoneal injection with 580 mg/kg tributyl citrate in 3% acacia for 14 days while a group of 20 control mice was dosed with vehicle only.[31] Two animals per group were killed at the end of the study. Body weight gains were decreased in the test animals and the decrease was significant after 7 days. No significant changes in blood counts were observed and no microscopic lesions were observed.

Reproductive and Developmental Toxicity

Oral

Aluminum citrate. A group of 20 presumed pregnant rats were dosed daily by gavage with 1064 mg/kg bw aluminum citrate and 62 mg/kg bw citric acid, concurrently, on days 6 to 15 of gestation, and a negative control group of 20 gravid rats received distilled water only.[32] All animals were killed on day 20 of gestation. The actual numbers of gravid test and control rats were 15 and 17, respectively. Administration of aluminum citrate with citric acid was not maternally toxic, embryotoxic, or fetotoxic. A statistically significant increase in the absence of xiphoids was the only skeletal variation reported. The aluminum concentration in the maternal liver, kidney, brain, bone (femur), and placenta, as well as in the whole fetus, was determined. The aluminum concentration was statistically significantly increased in the liver, bone, and placenta of the test animals compared to controls; however, no aluminum was detected (detection limit 0.05 µg/g) in the whole fetuses of control or treated animals.

Distearyl citrate ester mixture. A multigeneration study was performed in which 4 generations of rats were fed a diet containing 0%, 1.9%, or 9.5% of the distearyl citrate ester mixture that was described earlier in this report.[29] Administration of the test article did not result in any reproductive or developmental effects or any general signs of toxicity.

In Vitro

Sodium citrate. The embryotoxic potential of sodium citrate was evaluated in a whole rodent embryo culture system using 9.5-day-old embryos from female Han Wistar rats without metabolic activation.[33] The no-effect concentration for all parameters evaluated, including crown-rump length and abnormalities, was >115 µmol/L sodium citrate.

Spermicidal Effects

Citric acid. The spermicidal effect of citric acid was determined by suspending human sperm in a solution of citric acid.[34] Addition of 0.1% citric acid to human sperm reduced pH and rendered sperm immotile within 30 minutes while 1% was almost instantly spermicidal. The effect on sperm penetration of cervical mucus was also evaluated by adding the acid to human cervical mucus in capillary tubes. Addition of 0.01% citric acid reduced, and addition of 0.1% completely abolished, sperm penetration.

Genotoxicity

Genotoxicity studies are summarized in Table 10. Citric acid and its salts and esters were mostly negative in in vitro and in vivo genotoxicity tests. Exceptions were weakly positive results in host-mediated assays with citric acid, equivocal results in an Ames test with aluminum citrate, and a weak dose-related response for sodium citrate in a suspension test in *Salmonella typhimurium* TA1537 that was not reproducible.

Antimutagenic Effects

Citric acid. The antimutagenic effect of citric acid was evaluated in an Ames test, with 4-nitro-1,2-phenylenediamine and sodium azide used as mutagens.[35] Using *S typhimurium* strain

Table 10. Genotoxicity Studies.

Concentration	Vehicle	Procedure	Test system	Results	Reference
In vitro					
Citric acid					
500-2000 μg/plate	Distilled water	Ames test, in triplicate; negative and positive controls	*S typhimurium* TA97, TA98, TA100, TA104, ±met act	Negative	74
≤5000 μg/plate	Phosphate buffer	Ames test	*S typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, ±met act	Negative	75
≤1000 μg/mL	Saline	Chromosome aberration assay	Chinese hamster fibroblast cells	Negative	75
6-600 μg/mL	Saline	Cytogenetic study	Human embryonic lung cultures, WI-38	Negative	76
1.0 mg/mL	Not stated	RK bacterial assay; was used as a nonmutagenic control	*E coli* CHY832	Negative	77
Aluminum citrate					
10-10 000 μg/plate	Water	Ames test	*S typhimurium* TA100, TA1535, TA97, TA98, TA102, TA104, ±met act; TA1537, without met act	Equivocal in TA97 w/met act	36
Ferric citrate					
≤25 000 μg/plate	Phosphate buffer	Ames test	*S typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, ±met act	Negative	75
≤500 μg/mL	Sodium CMC	Chromosome aberration assay	Chinese hamster fibroblast cells	Negative	75
≤2 mmol/L	Not stated	DNA strand break	Chinese hamster V79 cells	No reduction in double-stranded DNA	78
Monosodium citrate					
≤5000 μg/plate	Phosphate buffer	Ames test	*S typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, ±met act	Negative	75
≤3000 μg/mL	Saline	Chromosome aberration assay	Chinese hamster fibroblast cells	Negative	75
Potassium citrate					
0.001%-0.004%	DMSO	Ames test	*S typhimurium* TA1535, TA1537, TA1538, ±met act	Negative	79
0.001%-0.004% (*S typhimurium*)0.002%-0.004% (*S cerevisiae*)	DMSO	Suspension test	*S typhimurium* TA1535, TA1537, TA1538, *S cerevisiae* D4, ±met act	Negative	79
Sodium citrate (dihydrate)					
6.25×10^{-4}% to 25×10^{-4}%	DMSO	Ames test	*S typhimurium* TA1535, TA1537, TA1538, ±met act	Negative	80
6.25×10^{-4}% to 25×10^{-4}%	DMSO	Suspension test	*S typhimurium* TA1535, TA1537, TA1538, *S cerevisiae* D4	Weak dose-related response in *S typhimurium* TA1537 without activation, repeat trial neg; neg	80

(continued)

Table 10. (continued)

Concentration	Vehicle	Procedure	Test system	Results	Reference
				in *S cerevisiae*; negative w/ activation	
Triethyl citrate					
0.4%-1.6%	DMSO	Ames test	*S typhimurium* TA1535, TA1537, TA1538, ±met act	Negative	81
0.4%-1.6% (*S typhimurium*)0.425%-1.7% (*S cerevisiae*)	DMSO	Suspension test	*S typhimurium* TA1535, TA1537, TA1538, *S cerevisiae* D4; ±met act	Negative	81
Tributyl citrate					
Not given	Not given	Ames test	Not given	Negative	82
Not given	Not given	Chromosome aberration assay	Human peripheral blood lymphocytes	Negative	82
Triisostearyl citrate					
10-10 000 µg/plate	Ethanol	Ames test, in triplicate; negative and positive controls	*S typhimurium* TA1535, TA1537, TA98, TA100, ±met act	Negative	83
In vivo					
Citric acid					
1.2-120 mg/kg	Saline	Cytogenetic assay, oral	Rats	Negative	76
500, 3500 mg/kg (single dose); 300, 3000 mg/kg (1 dose/d; 5 days)	Saline	Cytogenetic assay, oral	Rats	Negative	76
1.2-120 mg/kg (single dose and 1 dose/d; 5 days)	Saline	Host-mediated assay, oral	Saccharomyces D3 mice	Weakly positive	76
3500 mg/kg (single dose and 1 dose/d; 5 days)	Saline	Host-mediated assay, oral	*S typhimurium* TA1530 and G46 mice	Neg (acute); weakly pos (subacute)	76
1.2, 12, 120 mg/kg (1 dose/d; 5 days)	Saline	Dominant lethal assay, oral, 1×/d for 5 days	Rats	Sig increase in preimplantation loss at week 4 in high-dose group	76
500, 3500 mg/kg (single dose); 300, 3000 mg/kg (1 dose/d; 5 days)	Saline	Dominant lethal assay, oral, 1 dose (acute) or 1×/d for 5 days (subacute)	Rats	Negative	76

Abbreviations: CMC, carboxymethyl cellulose; DMSO, dimethyl sulfoxide; met act, metabolic activation; neg, negative; pos, positive; *S cerevisiae*, *Saccharomyces cerevisiae*; w, with.

TA97, concentrations of 1 to 1000 μg/0.1 mL/plate citric acid inhibited the mutagenicity of 20 μg/0.1 mL/plate 4-nitro-1,2-phenylenediamine by 3.54% to 67.72% without metabolic activation and by 55.34% to 71.97% with metabolic activation. Using strain TA100, concentrations of 1 to 1000 μg/0.1 mL/plate citric acid inhibited the mutagenicity of 1.5 μg/0.1 mL/plate sodium azide by 15.47% to 50.65% without metabolic activation and 37.47% to 67.10% with metabolic activation.

Carcinogenicity

Aluminum Citrate

The National Toxicology Program has planned toxicity/carcinogenicity testing for aluminum citrate.[36] The rationale for testing is that aluminum is listed by the EPA as a drinking water contaminant with a high health research priority.

Irritation and Sensitization

Skin Irritation/Sensitization

Nonhuman and human skin irritation and sensitization studies are summarized in Table 11. In irritation studies in rabbits, 30% citric acid was not a primary irritant. 60% produced some erythema and edema that subsided with time, and undiluted citric acid produced mild to severe erythema and mild to moderate edema. Triethyl citrate, at concentrations up to 100%, was not an irritant in guinea pigs or rabbits, and trioctyldodecyl citrate applied neat was not a primary skin irritant in rabbits. In human studies, citric acid was not a dermal irritant at concentrations up to 5% aqueous (aq), and 20% triethyl citrate was not irritating in humans. Sodium citrate did not produce any immediate (nonimmunologic contact urticaria) reactions. In sensitization testing, a cuticle cream containing 4% citric acid was not an irritant or a sensitizer in humans; 2.5% aq citric acid produced positive results in skin prick test in 3 of the 91 patients with urticaria or angioedema. Triethyl citrate, applied undiluted during epidermal induction, was a strong sensitizer in a guinea pig maximization test but 20% in pet was not a primary irritant or sensitizer in human studies. Trioctyldodecyl citrate was a mild sensitizer in a local lymph node assay when applied neat but the same concentration was not an irritant or sensitizer in human studies. Tributyl citrate (concentration not stated) was not a sensitizer in animal studies. In human studies, 25% tristearyl citrate and 100% triisostearyl citrate were not irritants or sensitizers in repeated insult patch tests.

Ocular Irritation

Ocular irritation studies are summarized in Table 12. Citric acid was predicted to be a moderate/severe to severe/extreme ocular irritant in in vitro studies, and it was minimally irritating to rabbit eyes at a concentration of 10% and mildly irritating at a concentrations 30%. In in vitro studies, tri-isostearyl citrate was predicted to be nonirritating to eyes. Triethyl citrate, 33.3%, did produce irritation in rabbit eyes and undiluted trioctyldodecyl citrate was nonirritating.

Miscellaneous Studies

Effects in Skin

Citric acid. The effect of 1 mol/L (16%, w/w) citric acid on skin cell renewal and irritation (as stinging) was determined at a pH of 3, 5, and 7. The dansyl chloride method was used to determine skin cell renewal and irritation was evaluated subjectively as stinging in the nasal fold area; stinging was scored on a scale of 0 to 4 every minute for 15 minutes.[37] (It is not stated, but the assumed maximum score is 60.) Citric acid test product of 2 mg/cm^2 was applied to the test area on the volar forearm of humans 2×/daily. The vehicle consisted of 15% ethanol (specially denatured 40), 5% ethoxydiglycol, 5% butylene glycol, and water. Cell renewal was measured in at least 8 patients; citric acid increased cell renewal by 16.1%, 12.8%, and 3% at pH 3, 5, and 7, respectively. Using a minimum of 10 patients, the irritation scores for 1 mol/L citric acid at pH 3, 5, and 7 were 38, 35.4, and 23.6, respectively.

The effect of 5% citric acid on skin cell renewal and irritation was also evaluated at the same pHs.[38] Cell renewal was greater at this concentration; 18%, 14%, and 8% increases were seen with 5% citric acid at pH 3, 5, and 7, respectively. Irritation scores (as stinging) were 2.3, 2.1, and 1.1 (on a scale of 1-5) at pH 3, 5, and 7, respectively. (Details of application were not provided.)

Five male patients participated in a 30-day study to evaluate the effects of citric acid on skin morphology.[39] Cream formulations containing 10%, 20%, or 25% citric acid were evaluated, and 0.2 mL of each cream were applied to a 2 × 2 cm^2 area of the ventral forearm of each patient. A fourth site on the forearm was used as an untreated control. Occlusive patches, 3×/wk, were applied during week 1 and nonocclusive patches, 3×/wk, were applied during weeks 2 to 3. Open applications were made daily during week 4. At the end of dosing, a 3-mm punch biopsy was taken from each site. Irritation was observed with the 20% and 25% formulations. (Details as to the extent of irritation was not provided, other than it was "visible.") Microscopically, an increase in viable epidermal thickness that increased with dose was observed at all dose levels. a "substantial" increase in Langerhans cells was observed with the 20% and 25% citric acid creams, and glycosaminoglycan (GAG) content was "markedly" increased at the sites dosed with 20% and 25% citric acid compared to that seen at the untreated and 10% citric acid sites.

A 20% citric acid lotion, pH 3.5, was applied twice daily for 3 months to photodamaged skin of the forearm of 6 female patients.[40] The lotion vehicle without citric acid was applied to the contralateral arms as a control. A 4-mm punch biopsy specimen was taken from each site after 3 months of application. Application of the lotion containing citric acid produced a statistically significant increase in skinfold thickness, with a 16.3% increase from baseline recorded. The skinfold thickness

Table 11. Dermal Irritation and Sensitization.

Test article	Concentration	Test pop	Procedure	Results	Reference
Nonhuman					
Irritation					
Citric acid					
Citric acid	30% aq	3 NZW rabbits	Draize test, 0.5 mL applied for 4 h to intact and abraded; occlusive patch	Not a primary irritant; PII = 84	84
Citric acid	Not stated	Rabbits	Acute dermal irritation/corrosion study	Slightly irritating; avg erythema score = 0.33	85
Citric acid	60% pure	NZW rabbits, 5M/3F	0.5 mL; applications to 1 animal for 3 min, to 1 for 60 min, to the remainder for 4 h	3 min: very slight erythema 60 min: very slight erythema 4 h: very slight moderate to severe erythema, very slight moderate edema, subsided to well-defined erythema and no edema after 48 h	86
Citric acid	100%	10 rabbits	5 g/kg were applied in an acute study (details not provided)	Mild (n = 3), moderate (n = 4), and severe (n = 2) erythema; mild (n = 8) and moderate (n = 2) edema	51
Citric acid	15%	32 male Wistar rats	Evan blue test: 2% Evan blue was injected iv into the tail of rats; 0.1 mL was then injected intradermally to a site on the back; animals were killed after 0.5, 1, 3, and 6 h	Statistically significantly more dye was extracted with citric acid compared to saline	87
Triethyl citrate					
Triethyl citrate	40%, 70%, 100% in ethanol	4F guinea pigs/gp	24 h, 8 mm occlusive patch; test sites scored 24 and 48 h after patch removal	Barely perceptible erythema at 24 h in 1 animal of the 100% group; no irritation with 40% or 70%	88
Triethyl citrate	0.05%-1.0% in 0.01% DBS/saline	Guinea pigs, 4 mol/L/gp	Intradermal injection, 0.1 mL; test sites scored after 24 h	Faint pink reaction at all test sites with all concentrations	88
Triethyl citrate	100%	4 rabbits	5 g/kg were applied in an acute study (details not provided)	No irritation	62
Triethyl citrate	15% and 33.3% in alcohol SDA 39C	3 albino rabbits	0.5 mL applied to a 2 × 2 (unites not given area of intact and abraded skin for 24 h with an occlusive covering)	Not a primary irritant; PII = 0	62
Triethyl citrate	33.3% in pet	3 albino rabbits	As above	Not a primary irritant; PII = 0	62
Trioctyldodecyl citrate					
Trioctyldodecyl citrate	neat	6 rabbits (sex not specified)	0.5 mL applied to intact and abraded skin for 24 h under an occlusive patch	Not a primary skin irritant; PII = 0.00	72
Sensitization					
Triethyl citrate					
Triethyl citrate	induction: intradermal%, 2.5% in 0.01% DBS/saline; epidermal, 100%challenge: 50% in absolute eth	9 guinea pigs	Magnusson-Kligman GPMT; FCA was used at intradermal induction; occlusive patches were used during intradermal induction and at challenge	Strong sensitizer; 9/9 animals sensitized after 2 challenges; primarily intense erythema, with some moderate and diffuse erythema, was observed	88
Tributyl citrate					
Tributyl citrate	Not provided	Not provided	GPMT or LLNA (addl details not provided)	Negative	82
Trioctyldodecyl citrate					
Trioctyldodecyl citrate	0, 10, 50, 100% (w/v) in acetone/olive oil (4:1, v/v)	5 mice	LLNA; 25 µL/ear were applied daily for 3 days; untreated and positive (α-hexylcinnamic aldehyde) control were used	Neat material was considered a mild sensitizer; the SI for the concentrations tested ranged from 1.1 to 3.1	72
Human					
Irritation					
Citric acid					
Citric acid	0.3 N solution (vehicle not specified)	Not specified	Stinging potential was evaluated by applying 0.1-0.2 mL to an abraded site on the forearm for ≤5 min; sig. change measured as difference from first to last day of dosing	Citric acid produced the most painful stinging response: citric, acetic >> aconitic > tartaric > ascorbic; citric acid has scored quite low when intercompared to other acids for primary irritancy	89

(continued)

Table 11. (continued)

Test article	Concentration	Test pop	Procedure	Results	Reference
Citric acid	5% aq, pH 2	20 patients, 14F/6M	50 μL applied to the back using 12 mm occlusive patch each AM; each PM, either the same patch or 0.5% aq SLS was applied; procedure repeated for 4 days; irritation was measured by visual scoring, TEWL, and skin color reflectance	No irritation with citric acid alone; exposure with SLS caused a clear irritant reaction; however, this reaction was less than that seen 1× daily exposure to SLS	90
Citric acid	5% aq, pH 4	As above	As above	No irritation with citric acid alone; exposure with SLS caused a clear irritant reaction; however, this reaction was less than that seen 1× daily exposure to SLS	90
Citric acid, in hand cleansers (A and B; % citric acid not given)	Neat	12 patients/group	Use test; product was applied ≥20/d for 2 wk; sc hydration was measured with a corneometer; TEWL measured with an evaporation meter; sig determined as above	Δ erythema: A, ~0.3; B, ~0.7 TEWL: A, ~4 g/m^2/h ($P \leq 0.5$); B, ~1.25 g/m^2/h Δ sc hydration: A, ~−1; B, ~−1.9	91
Hand cleansers as above (A and B), plus a third cleanser (not def)	Neat	8 patients/group	Forearm wash test; each group received 2 products to apply simultaneously; forearms were washed for 1 min 2×, then rinsed for 30 sec; sig changes measured as above	Δ erythema: A, ~0.7 ($P \leq 0.5$); B, ~0.45 TEWL: A, ~11 g/m^2/h ($P \leq 0.5$); B, 8 g/m^2/h ($P \leq 0.5$) Δ sc hydration: A, ~−9.5($P \leq 0.5$); B, ~−8	91
Hand cleansers as above (A and B), 2 addl cleanser (not def)	10%	40 patients	Patch test; 50 μL of each cleanser applied using 12 mm Finn chambers; 48 h	Δ erythema: A, ~2.7 ($P \leq 0.5$); B, ~2.25 ($P \leq 0.5$) TEWL: A, 14 g/m^2/h; B, ~7.9 g/m^2/h, diff btwn A and B ($P \leq 0.5$) Δ sc hydration: A, ~−7.9 ($P \leq 0.5$); B, ~−7.7 ($P \leq 0.5$)	91
Citric acid	1% aq	133 oral disease patients	48 h patch test, occlusive	No positive reactions	92
Citric acid	2.5% aq	49 atopic; 56 nonatopic patients	20 min occlusive application	No immediate (nonimmunologic contact urticaria) reactions	93
Citric acid	Not stated (most likely 100%)	702 contact dermatitis patients	Finn chambers were applied the back using Scanpor tape; 48 h	No reactions	94
Sodium citrate					
Sodium citrate	10% aq	49 atopic; 56 nonatopic patients	20 min occlusive application	No immediate (nonimmunologic contact urticaria) reactions	93
Triethyl citrate					
Triethyl citrate	20% in pet	22 patients	48 closed patch test	Not irritating	62
Sensitization					
Citric acid					
Citric acid	4% in a cuticle cream	56 patients	HRIPT; semiocclusive patches applied 3×/wk for 3 wk; a challenge patch was applied after 2 wk	Not an irritant or a sensitizer	95
Citric acid	2.5% aq	91 patients w/chronic urticaria or angioedema	Skin prick test	Positive results in 3 patients; 1 of the positive reactors also reacted to benzoic and propionic acids	96
Triethyl citrate					
Triethyl citrate	4.8% in a blush	106 patients	HRIPT; 0.2 g applied to a ¾ × ¾-sq in occlusive patch and then moistened; applied 3×/wk for 3 wk; a challenge patch was applied after 2 wk	Not a dermal irritant or a sensitizer	97
Triethyl citrate	Concentration range tested not specified (vehicle—alcohol 39°C)	41 patients 5 males and 36 females	HRIPT; 0.5 mL applied to a Webril patch affixed to an elastic bandage; nine 24-h patches were applied during induction; challenge patches were applied to the test site and an untested site	Not a primary irritant or sensitizer; no effects observed with 15%	62
Triethyl citrate	Concentration ranged tested not specified (vehicle —alcohol 39°C)	41 patients 10 males and 31 females	HRIPT; as above	Not a primary irritant or sensitizer; no effects observed with 33.33%	62
Triethyl citrate	Concentration range tested not specified (vehicle—pet)	45 patients 10 males and 35 females	HRIPT; as above, except that 0.4 mL was applied	Not a primary irritant or sensitizer; no effects observed with 33.33%	62

(continued)

Table 11. (continued)

Test article	Concentration	Test pop	Procedure	Results	Reference
Triethyl citrate	Concentration range tested not specified (vehicle—alcohol, SDA 39 C)	26 patients	Modified maximization study: *induction*: 5 alternate 48-h occlusive patches applied to the back or forearm, with 2.5% SLS pretreatment; *challenge*: 48-h semiocclusive patch, with 2.5% SLS pretreatment	Not a sensitizer according to the Kligman scale; irritant effects with 15% at induction ranged from mild erythema to erythema and edema with vesiculation and/or ulceration; rxns at challenge included minimal to well-defined erythema; no sensitization at 15%	62
Triethyl citrate	Concentration range tested not specified (vehicle—pet)	25 patients	As above	1 patient was not patched during challenge due to rxns to substances during induction; rxns at induction included minimal erythema to erythema and edema; rxns at challenge included minimal to well-defined erythema; not a sensitizer according to the Kligman scale; no effects at 33.33%	62
Triethyl citrate	Concentration range tested not specified (vehicle—pet)	22 patients	Maximization test: *induction*: 5 alternate 48-h occlusive patches applied to the forearm, with 5% aq pretreatment with the first patch only; *challenge*: 48-h semiocclusive patch, with 5% SLS pretreatment (occlusive)	No effects observed with 20%	62
Triethyl citrate	Neat	59 patients	HRIPT; 0.4 mL, 20× 20 mm Webril pad applied with a 40 × 40 mm adhesive square; 9 induction patches	Not an irritant or a sensitizer	98
Tristearyl citrate					
Tristearyl citrate	25% in olive oil; heated until soluble	110 patients	HRIPT; 0.2 mL applied to a 1-sq in pad of a semiocclusive patch; induction patches applied 3×/wk for 3 wk; a challenge patch was applied after 2 wk	Not a primary irritant or sensitizer	99
Triisostearyl citrate					
Triisostearyl citrate	15.5% in a lip gloss	110 patients	HRIPT; 0.2 g applied to a 1-sq in pad of a semi-occlusive patch; induction patches applied 3×/wk for 3 wk; a challenge patch was applied after 2 wk	Not an irritant or a sensitizer	100
Triisostearyl citrate	Neat	114 patients	HRIPT; 150 µL applied to a 2-cm^2 absorbent pad of an occlusive patch; induction patches applied 4×/wk for 3 wk; 4 challenge applications were made on a previously untreated site	Not an irritant or a sensitizer	67
Trioctyldodecyl citrate					
Trioctyldodecyl citrate	Neat	105 patients	HRIPT; 150 µL applied to a 2-cm^2 absorbent pad under a 4-cm^2 occlusive covering; induction patches applied 4×/wk for 3 wk; 4 challenge applications were made on a previously untreated site	Not an irritant or a sensitizer	72

Abbreviations: addl, additional; DBS, dodecylbenzenesulfonate; F, female; FCA, Freund complete adjuvant; GPMT, guinea pig maximization test; HRIPT, human repeated insult patch test; iv, intravenously; LLNA, local lymph node assay; pet, petrolatum; PII, primary irritation index; rxn, reaction; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; Δ, change.

Table 12. Ocular Irritation Studies.

Test article	Concentration/dose	Animals/gp	Method	Results	Reference
Alternative studies					
Citric acid					
Citric acid	2% in NaCl	–	Luminescent bacteria toxicity test (Microtox test)	Moderate/severe ocular irritant; EC_{50} = 14 mg/L	101
Citric acid	Undiluted	–	EYTEX assay	Severe/extreme irritant; EDE > 51	102
Triisostearyl citrate					
Triisostearyl citrate	10% in corn oil	–	MatTek EpiOcular in vitro toxicity assay; 100 µL	Nonirritating; ET_{50} > 256 min	103
Nonhuman studies					
Citric acid					
Citric acid (hydrate)	5.0% (0.26 mol/L); pH 2.1	6 NZW rabbits	Modified Draize study; test material was placed directly on central portion of cornea; eyes rinsed in 1 gp	No corneal opacity in rinsed or unrinsed eyes; conjunctivitis in all animals through day 7 (details not given)	104
Citric acid	10% and 30% aq	3 NZW rabbits	0.1 mL; Draize eye irritation study	10%: PII = 9.3; minimally irritating 30%: PII = 16.0; mildly to moderately irritating	105
Citric acid	Not given	Rabbits	Acute eye irritation/corrosion study	Avg scores (24-72 h): cornea = 2.8; iris = 0.0; conjunctiva = 1.7	85
Triethyl citrate					
Triethyl citrate	15% and 33.3% in alcohol SDA 39C	3 NZW rabbits	0.1 mL; Draize eye irritation study	Both concentrations: conjunctival irritation and corneal involvement, which did not clear by day 7	62
Triethyl citrate	33.3% in pet	3 NZW rabbits	0.1 mL; Draize eye irritation study	Conjunctival irritation and corneal involvement cleared on day 7	62
Trioctyldodecyl citrate					
Trioctyldodecyl citrate	Neat	6 rabbits	0.1 mL; Draize eye irritation study	Nonirritating; MMTS = 0.00	72

Abbreviations: aq, aqueous; avg, average; EC_{50}, concentration causing a 50% reduction in light; EDE, EYTEX/Draize equivalent; ET_{50}, percentage of viability 50%; gp, group; MMTS, maximum mean total score; NZW, New Zealand white.

of the vehicle-treated skin decreased slightly. Viable epidermis thickness also increased in a statistically significant manner, increasing 40% when compared to untreated skin. A statistically significant increase in GAG content was evidenced by a 2.5-fold increase in epidermal hyaluronic acid staining, a 57% increase in dermal hyaluronic acid staining, and a 66% increase in dermal chondroitin sulfate staining, as compared to skin treated with vehicle only. (Although the percentage of increase in staining was greater for chondroitin sulfate, staining for hyaluronic acid was approximately double that of chondroitin sulfate in both vehicle and citric acid-treated sites.)

Seven patients with moderate to severe photoaged skin applied a lotion containing 25% citric acid, pH 3.5, to 1 forearm and a placebo lotion to the other forearm twice daily for 6 months.[41] (Similar lotions containing glycolic or lactic acid were also evaluated.) Skin thickness measurements were performed in triplicate throughout the study. The 2-skin layer thickness of the forearm treated with citric acid (and the other AHAs) increased 25% while the thickness of the control forearm decreased 2%; the difference between the citric acid and the control sites was statistically significant. (There was no statistically significant difference in skin thickness among the 3 AHAs tested.) Microscopically, the mean epidermal thickness of skin and the mean thickness of papillary dermis in samples of skin treated with the citric acid lotion were statistically significantly greater than controls. (Total number of samples examined microscopically was not given.) There was no indication of inflammation. The amount of ground substance was variably increased in the citric acid-treated samples. Collagen fibers appeared to be increased in treated skin samples, but there was not a statistically significant difference in collagen fiber density in the papillary dermis between AHA-treated and -untreated sites.

It has been hypothesized that AHAs have the following mechanism of action.[24] In the stratum corneum, a low concentration of AHAs diminish corneocyte cohesion. In keratinocytes, AHAs stimulate epidermal proliferation, possibly by improving energy and redox status of the keratinocytes. In fibroblasts, high concentrations of AHA in an appropriate vehicle are thought to induce epidermolysis and epidermal separation and impact the papillary dermis and reticular dermis, leading to dermal changes that include the synthesis of new collagen.

Case Report

Citric acid. A woman reported difficulty in breathing and severe facial pain 4 hours after a professionally administered cosmetic peel procedure with a product containing 10% citric acid (and other compounds that were not identified).[41] The facial peel was applied for 4 hours. The patient also had first- and second-degree burns to the face and anterior neck. Permanent facial and neck scars, but no airway pathology, resulted.

Cough Reflex

Citric acid. Citric acid was used as a tussive agent in cough challenge testing.[43] Ten humanswere exposed to incremental doses of citric acid (10-1000 mmol/L) using an air-driven nebulizer. Using the mean cough frequency, a statistically significant dose–response relationship was observed. Individuals had different threshold and maximum tolerable concentrations; using interpolated values, the concentration that caused 5 coughs was 141.3 mmol/L citric acid. Using 10 Dunkin Hartley guinea pigs exposed to 0.9% saline and then, 10 minutes later, a single challenge of 30 to 300 mmol/L citric acid for 2 minutes, the calculated concentration producing 5 coughs (in 10 minutes) was 74.1 mmol/L citric acid.

The cough reflex to citric acid is produced by irritation of the larynx and the trachea and thought to be mediated by receptors that are distributed mainly in the larynx and upper airways.[44] In humans, the cough reflex was decreased with higher inspiratory flow rates as opposed to lower rates. The researchers were not able to definitively state a reason the decrease was seen but did state an important factor may be laryngeal deposition of the aerosol.

The mechanism of irritant properties was examined by comparing the cough response of isotonic citric acid in saline, isotonic sodium citrate, sodium citrate in saline, isotonic D-glucose, and distilled water.[45] All solutions were nebulized and inhaled by 7 patients for 1-minute periods. Cough occurred in response to inhalation of every test article except sodium citrate in saline (616 mOsm/L). The mean cough frequency (coughs/min) was 11.4 for 0.69% citric acid in 0.79% saline (308 mOsm/L), 12.5 for sodium citrate (308 mOsm/L), 18.1 for D-glucose (308 mOsm/L), and 15.7 for water (0 mOsm/L).

Citric acid induced airway constriction in anesthetized Hartley guinea pigs.[46] A citric acid aerosol was generated from a 0.6-mol/L citric acid solution and each animal received 50 breaths of 4 mL of the solution using a nebulizer. At 2 to 3 minutes following exposure to citric acid, the aerosol induced significant airway constriction that persisted to the end of the study (20 minutes following administration).

In another study, anesthetized guinea pigs were administered 10% weight/volume (w/v) aq aerosol citric acid for 1 minute using a nebulizer; airway resistance increased 79% and lung compliance decreased 68%.[47] In anesthetized guinea pigs in which the vagal nerve had been cut, a 5% increase in resistance and compliance was seen following exposure to citric acid. In conscious guinea pigs exposed to a 10% w/v aq aerosol of citric acid for 2 minutes using a glass nebulizer (particle size, 0.5-4 μm), the animals coughed 1 to 2 times in the first 30 seconds, and then a short period of hyperventilation was observed. The researchers theorized that the bronchoconstriction was due to an increase in airway resistance and involved parasympathetic innervation.

Anesthetic Effects

Triethyl and tributyl citrate. The corneal reflex in rabbit eyes was temporarily eliminated upon instillation of 3 drops of a 5%

suspension of triethyl or tributyl citrate in 3% acacia; the number of animals used was not stated.[30] The anesthetic effect was confirmed by the intradermal administration of 0.1 mL of a 2% solution of triethyl or tributyl citrate into an area of the shaved back of guinea pigs; again, the number of animals used was not stated. Triethyl citrate resulted in insensitivity to pricking of the area lasting 12 to 20 minutes while tributyl citrate produced a "deadened area" for a period greater than 2 hours.

Summary

Citric acid is an α-hydroxytricarboxylic acid that is reported to function in cosmetics as a chelating agent, pH adjuster, or fragrance ingredient. Citric acid can also be classified as a β-hydroxy acid. The 12 inorganic salts are reported to have many diverse functions while the 20 alkyl esters are reported to function mostly as skin-conditioning agents although they can have other functions. Citric acid is used in almost every category of cosmetic product and has 6795 reported uses. Citric acid is reported to be used at concentrations up to 39% in products that are diluted for (bath) use and up to 4% in leave-on products. With the exception of sodium, tributyl, and triethyl citrate, all other in-use ingredients have less than 50 uses. Triisostearyl citrate is used at up to 80% in lipstick formulations. Trioctyldodecyl and tricaprylyl citrate are used at concentrations of 30% and 27%, respectively, in leave-on formulations; all other in-use ingredients are used at ≤12%.

Citric acid, calcium citrate, ferric citrate, manganese citrate, potassium citrate, sodium citrate, diammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate are GRAS direct food additives. These ingredients, plus magnesium citrate, distearyl citrate, tristearyl citrate, and tributyl citrate are FDA-approved indirect food additives.

Citric acid is ubiquitously found in nature in virtually all organisms as an intermediate of the Krebs cycle. Orally administered citric acid is well absorbed and largely metabolized. Oral administration of aluminum citrate to male Sprague-Dawley rats, 6 days/wk for 4 weeks, resulted in a statistically significant increase in levels of aluminum in the brain in 1 study. In another study in which Sprague-Dawley rats were given aluminum citrate in the drinking water for 8 months, aluminum levels were increased in other parts of the body but not in the brain. Distearyl citrate, when added to the diet of rats, was poorly absorbed while nearly complete absorption was observed when isopropyl citrate was administered in the diet of rats.

The dermal median lethal dose values for citric acid and triethyl citrate were >5 g/kg in rabbits. Results of oral, inhalation, and other parenteral single-dose studies with various citrates did not indicate any notable toxic effects in mice, rats, rabbits, or dogs. Administration of 80 mmol/L aluminum citrate in water for 8 months did not affect the body weights of rats. Repeated oral dosing with an isostearyl citrate ester mixture or a distearyl citrate ester mixture did not have adverse effects on rats, rabbits, or dogs. Repeated oral dosing with tributyl citrate did not have an adverse effect on rats (10% in the diet for 6 weeks) or cats (5 mL/kg for 2 months).

Oral administration of 1064 mg/kg bw aluminum citrate concurrent with 62 mg/kg bw citric acid to rats was not maternally-, embryo-, or fetotoxic; the aluminum concentration was statistically significantly increased in the liver, bone, and placenta of the test animals but no aluminum was detected in the control or treated-group fetuses. Dietary administration of up to 9.5% of a distearyl citrate ester mixture did not produce any reproductive or developmental effects in a multigenerational study.

Citric acid and its salts and esters gave mostly negative reports in in vitro and in vivo genotoxicity tests. Exceptions were weakly positive results in in vitro and in vivo host-mediated assays with citric acid, equivocal results in an Ames test with aluminum citrate, and a weak dose-related response in a suspension test with sodium citrate in *S typhimurium* TA1537 that was not reproducible. Citric acid had antimutagenic effects, inhibiting the mutagenicity of 4-nitro-1,2-phenylenediamine and sodium azide.

In irritation studies in rabbits, 30% citric acid was not a primary irritant, 60% produced some erythema and edema that subsided with time, and undiluted citric acid produced mild to severe erythema and mild to moderate edema. Triethyl citrate, at concentrations up to 100%, was not an irritant in guinea pigs or rabbits, and trioctyldodecyl citrate applied neat was not a primary skin irritant in rabbits. In human studies, citric acid was not a dermal irritant at concentrations up to 5% aq and 20% triethyl citrate was not irritating in humans. Sodium citrate did not produce any immediate (nonimmunologic contact urticaria) reactions.

In sensitization testing, a cuticle cream containing 4% citric acid was not an irritant or a sensitizer in humans; 2.5% aq. citric acid produced positive results in skin prick test in 3 of the 91 patients with urticaria or anigoedema. Triethyl citrate, applied undiluted during epidermal induction, was a strong sensitizer in a guinea pig maximization test but 20% in pet was not a primary irritant or sensitizer in human studies. Trioctyldodecyl citrate was a mild sensitizer in a local lymph node assay when applied neat but the same concentration was not an irritant or sensitizer in human studies. Tributyl citrate (concentration not stated) was not a sensitizer in animal studies. In human studies, 25% tristearyl citrate and 100% triisostearyl citrate were not irritants or sensitizers in repeated insult patch tests.

Citric acid was predicted to be a moderate/severe to severe/extreme ocular irritant in in vitro studies, and it was minimally irritating to rabbit eyes at a concentration of 10% and mildly irritating at a concentration of 30%. In in vitro studies, triisostearyl citrate was predicted to be nonirritating to eyes. Triethyl citrate, 33.3%, did produce irritation in rabbit eyes, and undiluted trioctyldodecyl citrate was nonirritating.

Citric acid, ≥5%, increased cell renewal and epidermal thickness in human skin, and there appeared to be a greater increase at higher concentrations and/or lower pH of citric acid. Citric acid was a tussive agent in human inhalation challenge tests and induced airway restriction in animals. The cough reflex to citric acid is produced by irritation of the larynx and the trachea and is thought to be mediated by receptors that are

distributed mainly in the larynx and upper airways. Triethyl and tributyl citrate had an anesthetic effect in rabbit eyes.

Discussion

The Panel considered that the oral safety of citric acid, calcium citrate, ferric citrate, manganese citrate, potassium citrate, sodium citrate, diammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate has been well substantiated in that these ingredients are GRAS direct food additives. Therefore, the focus of this safety assessment was on the dermal toxicity of these ingredients. Although there are data gaps, the chemical structures, physicochemical properties, and functions and concentrations in cosmetics allow grouping these ingredients together and extending the available toxicological data to support the safety of the entire group.

Because citric acid and some of its salts and esters can be used in products that may be aerosolized, the Panel discussed the issue of incidental inhalation exposure. The limited inhalation data address the cough reflex induced by inhalation exposure to citric acid using a nebulizer so the induction of the cough reflex was not relevant to cosmetic exposure. Since inhalation data were limited, the Panel considered other available data to characterize the potential for citric acid and some of its salts and esters to cause systemic toxicity, irritation, or sensitization. They noted that as discussed earlier, many of these ingredients are GRAS ingredients and therefore oral toxicity was not a concern with these GRAS ingredients, that these ingredients gave mostly negative reports in in vitro and in vivo genotoxicity tests, and that they were not irritants or sensitizers in clinical testing.

The maximum reported concentrations of citric acid used in a spray product is 0.7%, of a salt is 0.2% sodium citrate, of an ester is 4% trioctyldodecyl citrate, and in deodorants is 2% triethyl citrate. The Panel noted that 95% to 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. However, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. Inhaled droplets/particles deposited in the nasopharyngeal and bronchial regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. Nevertheless, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects.

The Panel discussed whether citric acid or any of its salts or alkyl esters would be irritants. Available repeated insult patch testing at the highest leave-on concentration of 4% citric acid demonstrated an absence of both dermal irritation and sensitization, suggesting that these ingredients would not be irritants in formulation.

Although citric acid can be considered an AHA, it is also a β-hydroxy acid. Structurally, citric acid is a tricarboxylic acid, and as such, has a unique functionality and is chemically and biologically distinct from the AHAs considered in the CIR safety assessment of AHAs (ie, glycolic and lactic acid). Therefore, the concerns that stem from the mode of action of AHAs was not considered relevant to citric acid and its inorganic salts and alkyl esters.

Conclusion

The Panel concluded that citric acid and the inorganic citrate salts and alkyl citrate esters, listed subsequently, are safe in the present practices of use and concentration.

Citric acid
Inorganic salts:
- aluminum citrate;
- calcium citrate*;
- copper citrate*;
- diammonium citrate;
- disodium cupric citrate*;
- ferric citrate;
- magnesium citrate;
- manganese citrate*;
- monosodium citrate;
- potassium citrate;
- sodium citrate;
- zinc citrate.

Alkyl esters:
- isodecyl citrate;
- isopropyl citrate*;
- stearyl citrate;
- dilauryl citrate;
- distearyl citrate*;
- tributyl citrate;
- tri-C12-13 alkyl citrate;
- tri-C14-15 alkyl citrate;
- tricaprylyl citrate;
- triethyl citrate;
- triethylhexyl citrate;
- trihexyldecyl citrate*;
- triisocetyl citrate;
- triisopropyl citrate*;
- trilauryl citrate*;
- trioctyldodecyl citrate;
- trioleyl citrate*;
- triisostearyl citrate;
- tristearyl citrate*;
- ethyl citrates.

Were ingredients in this group not in current use (as indicated by *) to be used in the future, the expectation is that they would be used at concentrations comparable to others in this group.

Auhtors' Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. Fiume MM, Bergfeld WF, Belsito DV, et al. Final report on the safety assessment of glycolic acid, ammonium, calcium, potassium, and sodium glycolates, methyl, ethyl, propyl, and butyl glycolates, and lactic acid, ammonium, calcium, potassium, sodium and TEA-lactates, methyl, ethyl, isopropyl, myristyl, and cetyl lactates. *Int J Toxicol*. 1998;17(1):1-241.
2. Food and Drug Administration (FDA). Guidance: labeling for cosmetics containing alpha hydroxy acids. Guidance for industry, labeling for topically applied cosmetic products containing alpha hydroxy acids as an ingredient; 2005. http://www.fda.gov/Cosmetics/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ucm090816.htm. Accessed June 7, 2011.
3. Anastassiadis S, Morgunov IG, Kamzolova SV, Finogenova TV. Citric acid production patent review. *Recent Pat Biotechnol*. 2008;2(2):107-123.
4. Merck & Co., Inc. The Merck Index; 2010. http://themerckindex.cambridgesoft.com/themerckindex/Forms/Home/ContentArea/Home.aspx. *Accessed April* 8, 2011.
5. Berovic M, Legisa M. Citric acid production. *Biotechnol Annu Rev*. 2007;13:303-343.
6. de Guertechin, Luis Oldenhove. Surfactants: classification. In: Barel AO, Paye M, Maibach HI, eds. *Handbook of Cosmetic Science and Technology*. 3rd ed. New York: Informa Healthcare; 2009:769-770.
7. Gottschalck TE, Breslawec HP. *International Cosmetic Ingredient Dictionary and Handbook*. 14th ed. Washington, DC: Personal Care Products Council; 2012.
8. Food and Drug Administration (FDA). *Frequency of use of cosmetic ingredients. FDA Database*. Washington, DC: FDA; 2011. Updated February.
9. Personal Care Products Council. Concentration of use by FDA product category. Unpublished data submitted by the Council on November 1, 2011; 2011:9.
10. Personal Care Products Council. Updated concentration of use by FDA product category: Citric acid and its salts and esters. Unpublished data submitted by the Council of January 28, 2011; 2011:9.
11. Bremmer HJ, Prud'homme de Lodder LCH, Engelen JGM. Cosmetics Fact Sheet: to assess the risks for the consumer; Updated version for ConsExpo 4. Report No. RIVM 320104001/2006; 2006:1-77.
12. Johnsen MA. The influence of particle size. *Spray Technol Mark*. 2004;14:(11):24-27.
13. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
14. Rothe H. Special Aspects of Cosmetic Spray Evalulation. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, DC; 2011.
15. European Commission. European Commission Health and Consumers Cosmetic Cosing database; 2011. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple. Accessed February 25, 2011.
16. European Commission. European Commission Health and Consumers Cosmetic Cosing [Cosmetics Directive (v.1)]—Annex III/24; 2011. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=28271&back=1. Accessed February 25, 2011.
17. Food and Drug Administration (FDA). Code of Federal Regulations, Title 21; 2011. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm. Accessed March 14, 2011.
18. Life Sciences Research Office. Evaluation of the health aspects of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. Prepared for the Bureau of Foods of the Food and Drug Administration, Contract No. FDA 223-75-2004; 1977.
19. Organisation for Economic Co-operation and Development (OECD). SIDS Initial Assessment Report—Citric Acid (CAS No. 77-92-9). United Nations Environment Programme (UNEP) Chemicals; 2001. http://www.chem.unep.ch/irptc/sids/OECDSIDS/77929.pdf. Accessed March 13, 2011.
20. Hamm LL. Renal handling of citrate. *Kidney Int*. 1990;38(4):728-735.
21. Tracor-Jitco, Inc. *Scientific Literature Reviews on Generally Recognized as Safe (GRAS) Food Ingredients: Citric Acid*. Alexandria, VA: National Technical Information Service; 1974.
22. Fouda HG. Safety assessment of citroflex plasticizers in vitro by serum, liver and intestinal enzymes. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association on December 4, 1998; 1982:31.
23. Akeson MA, Munns DN. Lipid bilayer permeation by neutral aluminum citrate and by three alpha-hydroxy carboxylic acids. *Biochim Biophys Acta*. 1989;984(2):200-206.
24. Berardesca E. Alpha hydroxy acids. *Handb Cosmet Sci Technol*; 2001. CAPLUS AN 2001:697206 (Conference; General Review).
25. Slanina P, Falkeborn Y, Frech W, Cedergren A. Aluminum concentrations int he brain and bone of rats fed citric acid, aluminum citrate or aluminum hydroxide. *Food Chem Toxic*. 1984;22(5):391-397.
26. Vittori D, Nesse A, Pérez G, Garbossa G. Morphologic and functional alterations of erythroid cells induced by long-term ingestion of aluminum. *J Inorg Biochem*. 1999;76(2):113-120.
27. Madhavi DL, Salunkhe DK. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, eds. *Food Science and Technology (New York), 71*. New York, NY: Basel, Switzerland: Marcel Dekker, Inc.; 1996:267-359.

28. Nicolau G, Dahlin DC, Kohlbrenner M, et al. Skin metabolism and transdermal absorption of viprostol, a synthetic PGE_2 analog, in the rat: effect of vehicle. *Skin Pharmacol.* 1989;2(1): 22-29.
29. Deuel HJ Jr, Greenberg SM, Calbert CE, Baker R, Fisher HR. Toxicological studies on isopropyl and stearyl citrates. *Food Res.* 1951;16(3):258-280.
30. Finkelstein M, Gold H. Toxicology of the citric acid esters: tributyl citrate, acetyl tributyl citrate, triethyl citrate, and acetyl triethyl citrate. *Toxicol Appl Pharmacol.* 1959;1(3):283-298.
31. Meyers DB, Autian J, Guess WL. Toxicity of plastics used in medical practice. II. Toxicity of citric acid esters used as plasticizers. *J Pharm Sci.* 1964;53(7):774-777.
32. Gomez M, Domingo JL, LLobet JM. Developmental toxicity evaluation of oral aluminum in rats: influence of citrate. *Neurotoxicol Teratol.* 1991;13(3):323-328.
33. Bechter R, Brouillard JF. The effects of different chemical forms of a test compound on embryotoxicity, distribution and metabolism in vitro. *Toxicol In Vitro.* 1988;2(3):181-188.
34. Brown-Woodman PDC, Post EJ, Chow PYW, White IG. Effects of malonic, maleic, citric, and caffeic acids on the motility of human sperm and penetration of cervical mucus. *Int J Fertil.* 1985;30(3):38-44.
35. Bala S, Grover IS. Antimutagenicity of some citrus fruits in *Salmonella typhimurium*. *Mutat Res.* 1989;222(141):148.
36. National Toxicology Program. Salmonella study overview with aluminum citrate. Study A14572; 2003. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=A14572&cas%5Fno=31142%2D56%2D0&activetab=detail. Accessed April 19, 2011.
37. Smith WP. Comparative effectiveness of a-hydroxy acids on skin properties. *Int J Cosmet Sci.* 1996;18(2):75-83.
38. Smith WP. Hydroxy acids and skin aging. *Cosmet Toilet.* 1994; 109(9):41-48.
39. Green BA, Wildnauer RH. Effect of 10%, 20%, and 25% alpha-hydroxyacid (citric acid) formulations on skin morphology. In: *58th Annual Meeting of the American Academy of Dermatology, San Francisco, CA, March 10-15, 2000.* Cited in RIFM (2011) RIFM data synopsis on citric acid.
40. Bernstein EF, Underhill CB, Lakkakorpi J, et al. Citric acid increases viable epidermal thickness and glycosaminoglycan content of sun-damaged skin. *Dermatol Surg.* 1997;23(8): 689-694.
41. Ditre CM, Griffin TD, Murphy GF, et al. Effects of α-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. *J Am Acad Dermatol.* 1996;34(2 pt 1):187-195.
42. Ghadishah D, Gorchynski J. Airway compromise after routine alpha-hydroxy facial peel administration. *J Emerg Med.* 2002; 22(4):353-355.
43. Laude EA, Higgins KS, Morice AH. A comparative study of the effects of citric acid, capasicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulm Pharmacol.* 1993;6(3): 171-175.
44. Barros MJ, Zammattio SJ, Rees PJ. Importance of inspiratoyr flow rate in the cough response to citric acid inhalation in normal subjects. *Clin Sci.* 1990;78(5):521-525.
45. Lowry RH, Wood AM, Higenbottam TW. Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. *Clin Sci.* 1988;74(4):373-376.
46. Lai YL, Huang PC. Reactive oxygen species in sustained airway constriction induced by citric acid aerosol inhalation. *Eur J Pharmacol.* 2002;452(2):229-233.
47. Allott C, Evan DP, Marshall PW. A model ofr irritant-induced bronchoconstriction in the spontaneously breathing guinea-pig. *Br J Pharmacol.* 1980;71(1):165-168.
48. Archer Daniels Midland Co. Citric acid anhydrous, USP/FCC. Unpublished data submitted by the Council on November 19, 2010; 2010:1.
49. ACD/Labs. Advanced Chemistry Development (ACD/Labs) Software. 2011. (11.02): As cited in Chemical Abstracts Services Registry. Date Accessed 2011.
50. American Chemistry Council. U.S. High Production Volume (HPV) Chemical Challenge Program. Robust summaries for acetic acid and salts category; 2001. http://www.epa.gov/HPV/pubs/summaries/acetisalt/c13102rt.pdf. Accessed April 8, 2011.
51. Research Institute for Fragrance Materials (RIFM). RIFM data synopsis on citric acid. Unpublished document submitted by RIFM on March 21, 2011; 2011:39.
52. Archer Daniels Midland Co. Solubility fo citric acid in water. Unpublished data submitted by the Council on November 19, 2010; 2010:1.
53. Archer Daniels Midland Co. pH of citric acid-sodium citrate solutions. Unpublished data submitted byt the Council on November 19, 2010. 1.
54. Food and Drug Administration (FDA). Code of Federal Regulations, 21CFR184.1195 Calcium citrate; 2010. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?FR=184.1195&CFRPart=&FRSearch=. Accessed April 17, 2011.
55. European Commission—European Chemicals Bureau. IUCLID dataset—sodium dihydrogen citrate; 2000. http://ecb.jrc.ec.europa.eu/iuclid-datasheet/18996355.pdf. Accessed March 13, 2011.
56. Archer Daniels Midland Co. Potassium citrate, USP/FCC. Unpublished data submitted by the Council on November 19, 2010; 2010:1.
57. National Institute for Occupational Safety and Health. International Chemical Safety Cards: trisodium citrate anhydrous; 1994. http://www.cdc.gov/niosh/ipcsneng/neng1218.html. Accessed March 14, 2011.
58. European Commission—European Chemicals Bureau. IUCLID dataset—trisodium citrate; 2010. http://ecb.jrc.ec.europa.eu/iuclid-datasheet/68042.pdf. Accessed March 13, 2011.
59. Research Institute for Fragrance Materials (RIFM). RIFM data synopsis on sodium citrate. Unpublished document submitted by RIFM on March 21, 2011; 2011:11.
60. Clayton GD, Clayton FE, eds. *Patty's Industrial Hygiene and Toxicology.* New York: John Wiley & Sons, Inc.; 1994.
61. Gooding CM, Vahlteich HW, Neal RH. Citric acid esters. US 2518678. August 15, 1950.
62. Research Institute for Fragrance Materials (RIFM). RIFM data synopsis on triethyl citrate. Unpublished document submitted by RIFM on March 21, 2011; 2011:33.

63. National Institute for Occupational Safety and Health. International Chemical Safety Cards: Triethyl citrate; 1994. http://www.cdc.gov/niosh/ipcsneng/neng1350.html. Accessed March 14, 2011.
64. Morflex, Inc. Citroflex™ citric acid esters. Technical Bulletin 101. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association on December 4, 1998; 1998:15.
65. Salit NI, Sadykov AS. Esters of citric and malic acid. *Zhurnal Obshchei Khimii*. 1963;33(8):2746-2748.
66. Personal Care Products Council. Information on trisostearyl citrate and triictyldodecyl citrate. Unpublished data submitted by the Council on January 4, 2011; 2011:1.
67. Product Investigations, Inc. Determination of the irritating and sensitizing propensities of EX-1028 (triisostearyl citrate) on human skin. Report: PII No. 22097. Unpublished data submitted by the Council on January 4, 2011; 2007:12.
68. Archer Daniels Midland Co. Process stages: potassium citrate, USP/FCC. Unpublished data submitted by the Council on November 19, 2010; 2010:1.
69. Environmental Protection Agency. EPA R.E.D. Facts: Citric acid. NTIS No. PB92-221837; 1992.
70. Amidon GE, Peck GE, Block LH, et al. Proposed new USP general information chapter, excipient performance. *Pharmacopeial Forum*. 2007;33(6):1311-1323.
71. Opdyke DLJ. Monographs on fragrance raw materials. Triethyl citrate. *Food Cosmet Toxicol*. 1979;17(4):389-390.
70. Lubrizol. G-66 Guerbet Ester (trioctyldodecyl citrate) toxicology studies. TOX-017. Tox summary data submitted by the Council on January 4, 2011; 2005:2.
71. Gruber CM Jr, Halbeisen WA. A study on the comparative toxic effects of citric acid and its sodium salts. *J Pharmacol Exp Ther*. 1948;94(1):65-67.
74. Al-Ani FY, Al-Lami SK. Absence of mutagenic activity of acidity regulators in the Ames Salmonella/microsome test. *Mutat Res*. 1988;206(4):467-470.
75. Ishidate M Jr, Sofuni T, Yoshikawa K, et al. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol*. 1984;22(8):623-636.
76. Litton Bionetics, Inc. Mutagenic evaluation of compound FDA 71-54, citric acid. NTIS No. PB-245 463; 1975.
77. Hayes S, Gordon A, Sadowski I, Hayes C. RK bacterial test for independently measuring chemical toxicity and mutagenicity: short-term forward selection assay. *Mutat Res*. 1984;130(2):97-106.
78. Hartwig A, Schlepegrell R. Induction of oxidative DNA damage by ferric iron in mammalian cells. *Carcinogenesis*. 1995;162(12):3009-3013.
79. Litton Bionetics, Inc. Mutagenic evaluation of compound. FDA 75-4. 006100-05-6, Potassium Citrate, NF. FCC Granular. NTIS #PB254518; 1975.
80. Litton Bionetics, Inc. Mutagenic evaluation of compound FDA 75-12. 006132-04-3, Sodium Citrate, USP, FCC hydrous, granular. NTIS No. PB254510; 1975.
81. Litton Bionetics, Inc. Mutagenic evaluation of compound FDA 75-10. 000077-93-0, Triethyl Citrate, FCC. NTIS No. PB257866; 1976.
82. Wolfreys AM, Basketter DA. Mutagens and sensitizers—an unequal relationship? *J Toxicol Cutaneous Ocul Toxicol*. 2004;23(3):197-205.
83. NOTOX B.V. Evaluation of the mutagenic activity of EX-1028 (triisostearyl citrate) in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat). NOTOX Project No. 484965. Unpublished data submitted by the Council on January 4, 2011; 2007:26.
84. F. Hoffmann-LaRoche Ltd. Unpublished report, dermal irritation test, occlusive patch. Unpublished data cited in OECD 2001; 1984.
85. Kowalski RL, Hartnagel RE. Unpublished report; acute dermal and ocular irritation/corrosion study in rabbits. Unpublished data cited in OECD 2001; 1991.
86. Hill Top Biolabs, Inc. D.O.T. corrosivity potential study in rabbits of: citric acid solution, 60%. Unpublished data cited in American Chemistry Council (2001); 1992.
87. Sousa SM, Bramante CM, Taga EM. Biocompatibility of EDTA, EGTA and citric acid. *Braz Dent J*. 2005;16(1):3-8.
88. Unilever Limited. Sensitization potential of Citroflex A2, Citroflex A4, a nd Citroflex 2 (triethyl citrate) tested in guinea pigs. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association on May 12, 1999; 1976:31.
89. Laden K. Studies on irritancy and stinging potential. *J Soc Cosmet Chem*. 1973;24(6):385-393.
90. Schliemann-Willers S, Fuchs S, Kleesz P, Grieshaber R, Elsner P. Fruit acids do not enhance sodium lauryl sulphate-induced cumulative irritant contact dermatitis in vivo. *Acta Derm Venereol*. 2005;85(3):206-210.
91. Spoo J, Wigger-Alberti W, Berndt U, Fischer T, Elsner P. Skin cleansers: Three test protocols for the assessment of irritancy ranking. *Acta Dermato Venereol*. 2002;82(1):13-17.
92. Torgerson RR, Davis MDP, Bruce AJ, Farmer SA, Rogers RS. Contact allergy in oral disease. *J Am Acad Dermatol*. 2007;57(2):315-321.
93. Lahti A. Nonimmunologic contact urticaria. *Acta Dermatol Venereol Suppl*. 1980;60(91):1-49.
94. Reider N, Issa A, Hawranek T, et al. Absence of contact sensitization to *Aloe veraa* (L.) Burm. f. *Contact Dermatitis*. 2005;53(6):332-334.
95. Clinical Research Laboratories Inc. Repeated insult patch test of a cuticle cream containing 4% citric acid. CRL Study Number: CRL134907-2. Unpublished data submitted by Personal Care Products Council; 2007.
96. Malanin G, KAlimo K. The results of skin testing with food additives and the effect of an elimination diet in chronic ad recurrent urticaria and recurrent angioedema. *Clin Exp Allergy*. 1989;19(5):539-543.
97. Consumer Product Testing Co. Repeated insult patch test of a powder blush containing 4.8% triethyl citrate. Experiment Reference Number: C02-0969.02. Unpublished data submitted by Personal Care Products Council; 2002.
98. Hill Top Research. Repeated insult patch test on Citroflex 2 liquid (triethyl citrate), Citroflex A-2 liquid, and Citroflex A-4 liquid. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association on December 4, 1998; 1978:3.
99. Consumer Product Testing Co. Repeated insult patch test on test material C-SAT 020029 (tristearyl citrate). Experiment Ref. No. C02-0279.01. Unpublished data submitted by the Council on December 10, 2010; 2002:14.

100. Consumer Product Testing Co. Repeated insult patch test of a lip gloss containing 15.5% Triisostearyl Citrate. Experiment Reference Number: C09-099 1.01. Unpublished data submitted by Personal Care Products Council; 2009.
101. Bulich AA, Tung KK, Scheibner G. The luminescent bacteria toxicity test: its potential as an in vitro alternative. *J Biolumin Chemilumin*. 1990;5(2):71-77.
102. Gordon VC. Utilization of biomacromolecular *in vitro* assay systems in the prediction of *in vivo* toxic responses. *Lens Eye Toxic Res*. 1992;9(3-4):211-227.
103. Consumer Product Testing Co. The MatTek corporation EpiOcular™ tissue model *in vitro* toxicity testing system. Experiment Ref. No. V04-0142-3. Unpublished data submitted to the Council on January 4, 2011; 2004:5.
104. Murphy JC, Ostenberg RE, Seabaugh VM, Bierbower GW. Ocular irritancy responses to various pHs of acids and bases with and without irrigation. *Toxicology*. 1982;23(4):281-291.
105. F. Hoffmann-LaRoche Ltd. Unpublished report; Draize ocular irritation study in rabbits. Unpublished data cited in OECD 2001.

SAFETY DATA SHEET

Revision Date 19-Jan-2018 **Revision Number** 3

1. Identification

Product Name **Triethyl citrate**

Cat No. : **AC375060000; AC375060010; AC375060025; AC375060050; AC375062500**

CAS-No 77-93-0
Synonyms No information available

Recommended Use Laboratory chemicals.
Uses advised against Not for food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Acros Organics
One Reagent Lane
Fair Lawn, NJ 07410

Emergency Telephone Number
For information **US** call: 001-800-ACROS-01 / **Europe** call: +32 14 57 52 11
Emergency Number **US:**001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No.**US:**001-800-424-9300 / **Europe:**001-703-527-3887

2. Hazard(s) identification

Classification
Classification under 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Based on available data, the classification criteria are not met

Label Elements
None required

Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Triethyl citrate	77-93-0	>95

4. First-aid measures

Eye Contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.
Skin Contact	Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.
Inhalation	Move to fresh air. Get medical attention immediately if symptoms occur.
Ingestion	Clean mouth with water and drink afterwards plenty of water. Get medical attention if symptoms occur.
Most important symptoms and effects	None reasonably foreseeable.
Notes to Physician	Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media	Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Unsuitable Extinguishing Media	No information available
Flash Point	151 °C / 303.8 °F
Method -	No information available
Autoignition Temperature	No information available
Explosion Limits	
Upper	No data available
Lower	No data available
Sensitivity to Mechanical Impact	No information available
Sensitivity to Static Discharge	No information available

Specific Hazards Arising from the Chemical
Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products
Carbon monoxide (CO) Carbon dioxide (CO_2)
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
0	1	0	N/A

6. Accidental release measures

Personal Precautions	Ensure adequate ventilation. Use personal protective equipment.
Environmental Precautions	Should not be released into the environment. See Section 12 for additional ecological information.
Methods for Containment and Clean Up	Sweep up or vacuum up spillage and collect in suitable container for disposal.

7. Handling and storage

Handling	Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation.
Storage	Keep containers tightly closed in a dry, cool and well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limitsestablished by the region specific regulatory bodies.

Engineering Measures None under normal use conditions.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection No protective equipment is needed under normal use conditions.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Liquid
Appearance	No information available
Odor	Odorless
Odor Threshold	No information available
pH	No information available
Melting Point/Range	-46 °C / -50.8 °F
Boiling Point/Range	294 °C / 561.2 °F @ 760 mmHg
Flash Point	151 °C / 303.8 °F
Evaporation Rate	No information available
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	0.7 mmHg @ 122 °C
Vapor Density	No information available
Specific Gravity	1.136
Solubility	Soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	35.2 mPa.s (25°C)
Molecular Formula	C12 H20 O7
Molecular Weight	276.29

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Stable under recommended storage conditions.

Conditions to Avoid Incompatible products. Excess heat.

Incompatible Materials Strong oxidizing agents

Hazardous Decomposition Products Carbon monoxide (CO), Carbon dioxide (CO_2)

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Triethyl citrate	5900 mg/kg (Rat)	>5000 mg/kg (Rabbit)	1300 ppm/6h (Rat)

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation No information available

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Triethyl citrate	77-93-0	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects Not mutagenic in AMES Test

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure None known

STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

.

Persistence and Degradability Soluble in water Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its water solubility.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT	Not regulated
TDG	Not regulated
IATA	Not regulated
IMDG/IMO	Not regulated

15. Regulatory information

All of the components in the product are on the following Inventory lists: X = listed

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Triethyl citrate	X	X	-	201-070-7	-		X	X	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA Not applicable

California Proposition 65 This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Slight risk, Grade 1

16. Other information

Prepared By	Regulatory Affairs Thermo Fisher Scientific Email: EMSDS.RA@thermofisher.com
Revision Date	19-Jan-2018
Print Date	19-Jan-2018
Revision Summary	This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS