Toxicological profile for

Phenylacetic acid

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

2-Phenylacetic acid (PubChem)

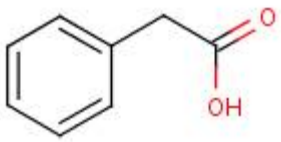
1.2. Synonyms

2-Phenylacetic acid; 4-09-00-01614 (Beilstein Handbook Reference); AI3-08920; Acetic acid, phenyl-; BRN 1099647; Benzenacetic acid; Benzeneacetic acid; Benzylcarboxylic acid; EINECS 203-148-6; FEMA No. 2878; HSDB 5010; Kyselina fenyloctova [Czech]; NSC 125718; Phenylacetic acid; Phenylacetic acid (natural); Phenylethanoic acid; Phenyllacetic acid; UNII-ER5I1W795A; alpha-Toluic acid (ChemIDplus)

1.3. Molecular formula

C8-H8-O2 (ChemIDplus)

1.4. Structural Formula



(ChemIDplus)

1.5. Molecular weight (g/mol)

136.149 (ChemIDplus)

1.6. CAS registration number

103-82-2

1.7. Properties

1.7.1. Melting point

(°C): 76.5 (EPISuite, 2017); 76-78 (EFSA, 2009); 76.7 (ChemIDplus); 74-79 or 150 (ChemSpider)

1.7.2. Boiling point

(°C): 265.5 (EPISuite, 2017); 265 (ChemSpider)

1.7.3. Solubility

1.66E+004 mg/L at 20°C (EPISuite, 2017); 1.73E+004 mg/L at 25°C (HSDB, 2018; PubChem); 0.16 g/100 mL at 20°C [1600 mg/L] (PubChem)

1.7.4. pKa

4.31 (at pH 5 10% free acid; at pH 7 0.1% free acid) (HSDB, 2018)

1.7.5. Flashpoint

(°C): 132 (closed cup) (ChemSpider; ICSC, 2006; PubChem); 190 (closed cup) (GSBL, 2021)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): 543 (ICSC, 2006)

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

3.8x10-3 mm Hg at 25°C (EPISuite, 2017); 0 mm Hg (PubChem)

1.7.11. log Kow

1.41 (ChemSpider; EPISuite)

2. General information

2.1. Exposure

“Probable Routes of Human Exposure:

Occupational exposure to phenylacetic acid may occur through inhalation and dermal contact with this compound at workplaces where phenylacetic acid is produced or used. Monitoring data indicate that the general population may be exposed to phenylacetic acid via inhalation of tobacco and wood smoke, ingestion of food and drinking water, and dermal contact with consumer products containing phenylacetic acid (SRC).”

As taken from HSDB, 2018.

Cosmetics	Yes (Merck, 1996)	Food	Yes 1.3 - 20 ppm (max) (Burdock, 2010)
Environment	Yes (HSDB, 2018)	Pharmaceuticals	Yes (Martindale, 1993)
In tobacco naturally	Yes	In the burnt part	Yes (Lloyd, 1976)

EXPOSURE			
Inhalation	Cough. Sore throat.	Use local exhaust or breathing protection.	Fresh air, rest.
Skin	Redness.	Protective gloves.	Rinse skin with plenty of water or shower.
Eyes	Redness. Pain.	Wear safety goggles	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then refer for medical attention.
Ingestion		Do not eat, drink, or smoke during work.	Rinse mouth. Give one or two glasses of water to drink.

As taken from Phenylacetic acid (ICSC, 2006) available at http://www.inchem.org/documents/icsc/icsc/eics1260.htm

“Body Burden:

Phenylacetic acid was present in 47.3% of the 54 samples of normal human expired air from an average concentration of 0.161 ng/L(1). [(1) Krotoszynski BK et al; J Anal Toxicol 3: 225-34 (1979)] **PEER REVIEWED**

Other Environmental Concentrations:

Phenylacetic acid has been identified as a chemical component of tobacco smoke, tobacco and substitute tobacco smoke(1). Phenylacetic acid was qualitatively found in cigarette smoke condensate(2). Burn emission rates of phenylacetic acid in mg/g of organic carbon were found to be 0.055 for red oak(3), 0.286 for paper birch(3), 0.130 for white pine(3), 0.047 for balsam(1), 0.044 for yellow poplar(2), 0.055 for mockernut hickory(4), 0.171 for loblolly pine(4), and 0.228 for slash pine(4). Phenylacetic acid was also detected in emissions from red maple(1), hemlock(3), balsam fir(3), white ash(4), and sweet gum(4).[(1) Rodgman A, Perfetti TA; The Chemical Components of Tobacco and Tobacco Smoke 2nd, ed., Baco Raton, FL: CRC Press p. 1236, 2078 (2013) (2) Arnarp J et al; Acta Chemica Scandinavica 43: 381-5 (1989). Available from, as of Jun 14, 2017: https://www.ncbi.nlm.nih.gov/pubmed/?term=2485642 (3) Fine PM et al; Environ Sci Technol 35: 2665-75 (2001) (4) Fine PM et al; Environ Sci Technol 36: 1442-1451 (2002)] **PEER REVIEWED**. .”

As taken from HSDB, 2018.

Based on the Maximised Survey-derived Daily Intake (MSDI) approach, EFSA estimated daily intakes per person to be 240 µg in Europe and 60 µg in the USA (EFSA, 2009).

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

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	2.00	4.76	Meat products	0.80	1.30
Baked goods	6.76	12.32	Nonalcoholic beverages	1.09	2.12
Frozen dairy	4.09	8.61	Soft candy	7.20	13.88
Gelatins, puddings	2.83	5.10	Sweet sauce	1.00	20.00
Hard candy	3.17	3.17			

Estimated intake from flavouring use: 0.001002 mg/kg bw/day.

As taken from Burdock, 2010.

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

Phenylacetic acid is listed as a fragrance ingredient by the US EPA InertFinder Database (2022) and IFRA.

“It is used in perfume, as a precursor in manufacture of penicillin G, fungicide, flavoring, and laboratory reagent. It is also used in production of drugs of abuse.

According to the 2012 TSCA Inventory Update Reporting data, 1 reporting facility estimates the number of persons reasonably likely to be exposed during the manufacturing, processing, or use of phenylacetic acid (benzeneacetic acid) in the United States may be as low as 100-499 workers and as high as 100-499 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 and 2016 Chemical Data Reporting information on chemical production and use in the United States. Available from, as of June 12, 2017: http://www.epa.gov/chemical-data-reporting] **PEER REVIEWED**."

As taken from HSDB, 2018.

“Industrial Processes with risk of exposure: Farming (Pesticides)”.

As taken from Haz-Map, 2020.

Phenylacetic acid (CAS RN 103-82-2) is used as a flavour enhancer in oral non-medicinal natural health products (Health Canada, 2022).

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%
benzaldehyde	168250767	5.34	261419252	7.48
phenylacetic acid	2957776492	93.90	3172589297	90.82
Total ion chromatogram	3150022356	100	3493387028	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	Chemical class	Mol. Wt. (M) Bp or mp (°C)	Max. Cig. Appln. Level (ppm)	Purity of Sample Pyrolysed	Composite of Pyrolysate (Compound, %)	Max. Level in Smoke (microg)
Phenylacetic acid CAS 103-82-2	Acid	M=136 bp 265	65	98%	Phenylacetic acid 51.6 Benzaldehyde 38.5 Ethylphenyl acetate	11 13 2 0.4

					6.0 Diphenylethanone 1.1 Benzoic acid 1.0 Dibenzyl 0.3 Phenylstyrene or stilbene 0.2 Benzyl phenylacetate 0.2 Benzohenone 0.2 Toulene 0.2 Acetophenone 0.1 4 Minor products, 1 unidentified 0.8	0.3 0.1 0.07 0.07 0.07 0.07 0.03 0.3

In a pyrolysis study, 30.6% of phenylacetic acid added to cigarettes was transferred intact to the smoke. (Purkis et al. 2011).

Phenylacetic acid has been identified as a chemical component of tobacco smoke, tobacco and substitute tobacco smoke(1). Phenylacetic acid was qualitatively found in cigarette smoke condensate(2). Burn emission rates of phenylacetic acid in mg/g of organic carbon were found to be 0.055 for red oak(3), 0.286 for paper birch(3), 0.130 for white pine(3), 0.047 for balsam(1), 0.044 for yellow poplar(2), 0.055 for mockernut hickory(4), 0.171 for loblolly pine(4), and 0.228 for slash pine(4). Phenylacetic acid was also detected in emissions from red maple(1), hemlock(3), balsam fir(3), white ash(4), and sweet gum(4).[(1) Rodgman A, Perfetti TA; The Chemical Components of Tobacco and Tobacco Smoke 2nd, ed., Baco Raton, FL: CRC Press p. 1236, 2078 (2013) (2) Arnarp J et al; Acta Chemica Scandinavica 43: 381-5 (1989). Available from, as of Jun 14, 2017: https://www.ncbi.nlm.nih.gov/pubmed/?term=2485642 (3) Fine PM et al; Environ Sci Technol 35: 2665-75 (2001) (4) Fine PM et al; Environ Sci Technol 36: 1442-1451 (2002)] **PEER REVIEWED**. ."

As taken from HSDB, 2018.

2.3. Ingredient(s) from which it originates

Reported found among the constituents of a few essential oils: tobacco, Rosa centifolia, Bulgarian rose, orange floweres absolute, neroli and Mentha arvensis of Japanese origin; also reported present among the volatile consitituents of cocoa. Also reported found in guava, papaya, raspberry, strawberry, cooked potato, tomato, peppermint oil, pepper, rye bread, cheddar cheese, Swiss cheese, gruyere cheese, boiled mutton, beer, cognac, cider, sherry, grape wines, white wine, sake, cocoa, tea, honey soy protein, passion fruit, starfruit, mango, mushroom, malt, wort, roasted chicory root, naranjilla ftuit, choke berry, sea buckthorn and Chinese quince.

As taken from Burdock, 2010.

"Naturally occurs in some essential oils, honeys and teas" (Haz-Map, 2020).

Natural Pollution Sources:

Phenylacetic acid occurs in Japanese peppermint oil, neroli oil, and in trace amounts in rose oils(1). Phenylacetic acid was found as a volatile component in yellow box honey at 0.2 mg/kg and blue gum honey at 0.1 mg/kg(2). Phenylacetic acid was identified as a flavor compound in pine sprout tea and pine needle tea(3). [(1) Bauer K, Garbe D; Ullmann's Encyclopedia of Industrial Chemistry.

Gerhartz W (ed) 5th ed Deerfield Beach, FL: VCH Publishers Vol A11: 141-250 (1985) (2) Darcy BR et al; J Agric Food Chem 45: 1834-43 (1997) (3) Kim KY, Chung HJ; J Agric Food Chem 48: 1269-72 (1997)] **PEER REVIEWED**

Food Survey Values:

Phenylacetic acid was found as a volatile component in yellow box honey at 0.2 mg/kg and blue gum honey at 0.1 mg/kg(1). Phenylacetic acid was identified as a flavor compound in pine sprout tea and pine needle tea(2). Phenylacetic acid was detected in the volatiles of Rambutan fruit and in the juice at a concentration of 131.67 ug/L(3). [(1) Darcy BR et al; J Agric Food Chem 45: 1834-43 (1997) (2) Kim KY, Chung HJ; J Agric Food Chem 48: 1269-72 (1997) (3) Ong PKC et al; J Agric Food Chem 46: 611-615 (1998)] **PEER REVIEWED**

Phenylacetic acid was identified as a volatile component of rotten mussels (left out at room temperature for 10 days) at 2.35 ug/g wet weight, but not in fresh mussels(1).[(1) Yashuhara A; Journal of Chromatography 409: 251-58 (1987)] **PEER REVIEWED**

As taken from HSDB, 2018. Present in roasted chicory root and jasmine essence.

As taken from Khan IA and Abourashed EA, 2010.

“Phenylacetic acid occurrence in plants(1).Genus species Family Common name(s) Part Concn (ppm) Camellia sinensis Theaceae Tea Leaf 1850-3130 Mentha x piperita Lamiaceae Peppermint Leaf 0.001-0.01 Zea mays Poaceae Corn Pollen or Spore not reported Polianthes tuberosa Agavaceae Tuberose Flower not reported Juglans nigra Juglandaceae Black Walnut Seed not reported Citrus aurantium Rutaceae Bitter Orange Flower; Plant not reported Ligustrum japonicum Oleaceae Ligustri Fructus Flower not reported Piper methysticum Piperaceae Kava-Kava Rhizome not reported Prunus dulcis Rosaceae Almond Flower not reported [(1) US Dept Agric; US Dept Agric, Agric Res Service. 1992-2017. Dr. Duke's Phytochemical and Ethnobotanical Databases. Phenylacetic-Acid. Available from, as of June 14, 2017: https://phytochem.nal.usda.gov/phytochem/search] **PEER REVIEWED**.”

As taken from HSDB, 2018.

“The aim of this work was to undertake a detailed analysis on chemical constituents of brown propolis, originating from four different states (Bahia, Minas Gerais, Paraná and Sergipe) of Brazil. The volatile profile was determined by using HS-SPME-GC–MS along with the determination of total phenolic compounds content, flavonoids and antioxidant activity. A total of 315 volatile compounds were identified, however, several of them have not been reported so far in the Brazilian brown propolis. The terpenes represented the major class with 40.92–84.66% of the total area in the chromatograms. PCA analysis of the majority of compounds successfully indicated the volatile profile of each propolis sample according to their geographical origin. The analysis of volatile compounds and its characterization also varied significantly and confirmed that these depended on the geographical area of collection of propolis. The data generated in this work may help in establishing criteria for quality control and tracking the specific region of propolis production in different states of Brazil.” As taken from Olegário LS et al. 2019. Food Res. Int. 123, 481-502. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31284998/

“Gardenia jasminoides Ellis, which belongs to the Rubiaceae family, is a widely used traditional Chinese medicine. Although effect of Gardenia jasminoides Ellis has been widely reported, its anti-inflammatory role in intestinal mucosal injury induced by LPS remains unclear. In the present study, we investigated the effects of decoction extracted from Gardenia jasminoides on the morphology and intestinal antioxidant capacity of duodenum induced by LPS in mice. Further analysis was carried out in the expression of inflammatory and anti-inflammatory cytokines. Nuclear factor-kappa B (NF-κB) was determined by Western blot. Gardenia jasminoides water extract was qualitative analyzed by high-performance liquid chromatography coupled with electro spray ionization quadrupole time-of-flight mass spectrometry. The results showed that Gardenia decoction markedly

inhibited the LPS-induced Tumor necrosis factor (TNF)-α, Interleukin (IL)-6, IL-8, and IL-1 production. It was also observed that Gardenia decoction attenuated duodenum histopathology changes in the mouse models. Furthermore, Gardenia decoction inhibited the expression of NF-κB in LPS stimulated mouse duodenum. These results suggest that Gardenia decoction exerts an anti-inflammatory and antioxidant property by up-regulating the activities of the total antioxidant capacity (T-AOC), the total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px). Gardenia decoction is highly effective in inhibiting intestinal mucosal damage and may be a promising potential therapeutic reagent for intestinal mucosal damage treatment." As taken from Cui Y et al. 2019. Front. Pharmacol. 10, 180. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30983991/

Phenylacetic acid is a human metabolite (PubChem).

3. Status in legislation and other official guidance

FDA Requirements:

Phenylacetic acid is a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: a) they are used in the minimum quantity required to produce their intended effect, and otherwise in accordance with all the principles of good manufacturing practice, and 2) they consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, prior-sanctioned for such use, or regulated by an appropriate section in this part. [21 CFR 172.515 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2017: http://www.ecfr.gov] **PEER REVIEWED**

The following chemical has been specifically designated by the Administrator of the Drug Enforcement Administration as subject to the provisions of this part and parts 1309 and 1313 of this chapter. Phenylacetic acid, its esters and its salts are List 1 chemicals; DEA Code: 8791. [21 CFR 1310.02(a)(8); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 5, 2017: http://www.ecfr.gov] **PEER REVIEWED**

Odor Threshold:

Malodorous substance. Off site odor is the primary environmental concern. The odor threshold is undoubtedly very low but is not quantified. [European Chemicals Bureau; IUCLID Dataset, Phenylacetic acid (103-82-2) (2000 CD-ROM edition).] **PEER REVIEWED**

NFPA Hazard Classification:

Health: 2. 2= Materials that, on intense or continued (but not chronic) exposure, could cause temporary incapacitation or possible residual injury, including those requiring the use of respiratory protective equipment that has an independent air supply. These materials are hazardous to health, but areas may be entered freely if personnel are provided with full-face mask self-contained breathing apparatus that provides complete eye protection. [National Fire Protection Association; Fire Protection Guide to Hazardous Materials. 14TH Edition. Quincy, MA 2010, p. 325-97] **PEER REVIEWED**

Flammability: 1. 1= This degree includes materials that must be preheated before ignition will occur, such as Class IIIB combustible liquids and solids and semi-solids whose flash point exceeds 200 deg F (93.4 deg C), as well as most ordinary combustible materials. Water may cause frothing if it sinks below the surface of the burning liquid and turns to steam. However, a water fog that is gently applied to the surface of the liquid will cause frothing that will extinguish the fire. [National Fire Protection Association; Fire Protection Guide to Hazardous Materials. 14TH Edition. Quincy, MA 2010, p. 325-97] **PEER REVIEWED**

Instability: 0. 0= This degree includes materials that are normally stable, even under fire exposure conditions, and that do not react with water. Normal fire fighting procedures may be used. [National Fire Protection Association; Fire Protection Guide to Hazardous Materials. 14TH Edition. Quincy, MA 2010, p. 325-97] **PEER REVIEWED**

As taken from HSDB, 2018.

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives PHENYLACETIC ACID	
COE No.:	672
FEMA No.:	2878
JECFA No.:	1007
Chemical names:	PHENYLACETIC ACID
Synonyms:	BENZYLCARBOXYLIC ACID; alpha-TOLUIC ACID
Functional class:	FLAVOURING AGENT
Latest evaluation:	2002
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 913-JECFA 59/53
Specifications:	COMPENDIUM ADDENDUM 10/FNP 52 Add.10/56
Tox monograph:	FAS 50-JECFA 59/215

As taken from JECFA, 2003. Evaluation of phenylacetic acid available at http://www.inchem.org/documents/jecfa/jeceval/jec_1886.htm

“The group of phenethylalcohol, aldehyde, acid, and related acetals and esters was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food, their rapid absorption, metabolic detoxication, and excretion in humans and other animals, their low level of flavor use, the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of phenethyl alcohol, aldehyde, acid, and related acetals and esters as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.”

As taken from Adams TB et al. (2005).

There are REACH dossiers on phenylacetic acid (ECHA, 2021).

Phenylacetic acid (CAS RN 103-82-2) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Phenylacetic acid (CAS RN 103-82-2) is included on the US FDA’s inventory of “Substances Added to Food (formerly EAFUS)” as a flavoring agent or adjuvant and is included under 21 CFR section 172.515 (Synthetic flavoring substances and adjuvants) (FDA, 2022a,b).

Listed in the US EPA InertFinder Database (2022) as approved for fragrance use pesticide products.

Phenylacetic acid is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). The 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 2020 CDR list are available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do

Phenylacetic acid is listed as authorised for use as a flavouring substance in all categories of flavoured food in the EU under regulation (EU) no. 872/2012.

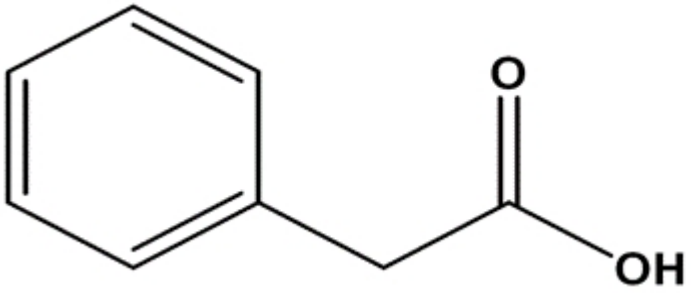
As taken from European Commission, 2012

Phenylacetic acid has been given GRAS (generally recognized as safe) status by FEMA (FEMA no. 2878) (Hall and Oser, 1965).

Phenylacetic acid (CAS RN 103-82-2) is included on the New Zealand Inventory of Chemicals ,does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006) and is classified according to the New Zealand Authorities (NZ EPA CCID).

Benzeneacetic acid (CAS 103-82-2) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” (“the exposure band for this chemical was based in part on volume data arising from a NICNAS call for information”) (AICIS, 2013).

FL No	**08.038**
In register	Yes
Flavis name	Phenylacetic acid
Chemical group number	15
Chemical group name	Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters.
CAS No	103-82-2
CE No	672
JECFA N0	1007
FEMA No	2878
Systematic name	Acetic acid-, phenyl-
Synonyms	alpha-Toluic acid; Benzylcarboxylic acid
Evaluation	FGE.53

Needed Tox	
Remarks	
Ref short	
Reference	
Structure	

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

Phenylacetic acid (CAS RN 103-82-2) is classified as an NHP [Natural Health Product] for medicinal use under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations. It should be noted that this ingredient is subject to additional regulatory requirements in accordance with the Precursor Control Regulations since it is listed on Schedule VI Part 1 of the Controlled Drugs and Substances Act (Health Canada, 2022).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Phenylacetic acid was administered to man, 14 species of sub-human primates and 11 non-primates. Four amino-acid conjugates...phenylacetylglutamine, phenylacetylglycine, phenylacetyltaurine and diphenylacetylornithine were identified in urine... [The Chemical Society. Foreign Compound Metabolism in Mammals Volume 3. London: The Chemical Society, 1975., p. 569] **PEER REVIEWED**

Yields benzoic acid in rats. /from table/ [Goodwin, B.L. Handbook of Intermediary Metabolism of Aromatic Compounds. New York: Wiley, 1976., p. 12] **PEER REVIEWED**

Esterases in human liver microsomes hydrolyzed ... phenylacetate (Vmax 57 +/ - 8 umol/min/g tissue), /and/ ... esterases found in the human liver cytosol /also/ hydrolyzed ... phenylacetate (Vmax 37 +/- 2.9 umol/min/g tissue) ... .. Human plasma esterase hydrolyzed ... phenylacetate (Vmax 250 +/ - 17 umol/min/ml). ... Phenylacetate hydrolysis involved arylesterase in plasma, both arylesterase and carboxylesterase in liver microsomes and carboxylesterase in liver cytosol. Plasma hydrolysis is less important and overall esterase activity is lower in humans than in the rat which is therefore a poor model. /Phenylacetate/ [McCracken NW et al; Biochem Pharmacol 46 (7): 1125-9 (1993)] **PEER REVIEWED** PubMed Abstract

... In freshly prepared and cryopreserved /guinea pig/ liver slices, phenylacetic acid was the main metabolite of 2-phenylethalamine. In freshly prepared liver slices, phenylacetic acid was completely inhibited by disulfiram (inhibitor of aldehyde dehydrogenase), whereas isovanillin (inhibitor of aldehyde oxidase) inhibited acid formation to a lesser extent and allopurinol (inhibitor of xanthine oxidase) had no effect. In cryopreserved liver slices, isovanillin inhibited phenylacetic acid by 85%,

whereas disulfiram inhibited acid formation to a lesser extent and allopurinol had no effect. In liver slices, 2-phenylethylamine is rapidly oxidized to phenylacetic acid, via phenylacetaldehyde, by aldehyde dehydrogenase and aldehyde oxidase with no contribution from xanthine oxidase. [Panoutsopoulos GI; In Vivo 18 (6): 779-86 (2004)] **PEER REVIEWED** PubMed Abstract

The dose limiting toxicity and pharmacokinetics of phenylacetic acid (phenylacetate) were studied in 17 patients with advanced solid tumors who received single iv bolus doses followed by a 14 day continuous iv infusion of the drug in a phase I trial. Phenylacetic acid displayed nonlinear pharmacokinetics with evidence for induction of drug clearance. Ninety-nine percent of phenylacetic acid elimination was accounted for by conversion to phenylacetylglutamine which was excreted in the urine... [Thibault A et al; Cancer Res 54: 1690-94 (1994)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018.

Phenyl acetate (or phenylacetate) is a carboxylic acid ester that has been found in the biofluids of patients with nephritis and/or hepatitis as well as patients with phenylketonuria (PKU), an inborn error of metabolism. Phenyl acetate has been identified as a uremic toxin according to the European Uremic Toxin Working Group (PMID: 22626821). Excess phenylalanine in the body can be disposed of through a transamination process leading to the production of phenylpyruvate. The phenylpyruvate can be further metabolized into a number of products. Decarboxylation of phenylpyruvate gives phenylacetate, while a reduction reaction gives phenyllactate. The phenylacetate can be further conjugated with glutamine to give phenylacetyl glutamine. All of these metabolites can be detected in serum and urine of PKU patients. Phenyl acetate is also produced endogenously as the metabolite of 2-Phenylethylamine, which is mainly metabolized by monoamine oxidase to form phenyl acetate. 2-phenylethylamine is an "endogenous amphetamine" which may modulate central adrenergic functions, and the urinary phenyl acetate levels have been postulated as a marker for depression. (PMID: 17978765 , 476920 , 6857245). Phenylacetate is also found in essential oils, e.g. neroli, rose oil, free and as esters' and in many fruits. As a result it is used as a perfumery and flavoring ingredient. Phenyl acetate is a microbial metabolite.

As taken from HMDB.

“Phenylacetic acid (PAA) represents a substructure of a class of nonsteroidal anti-inflammatory carboxylic acid-containing drugs capable of undergoing metabolic activation in the liver to acylcoenzyme A (CoA)- and/or acyl glucuronide-linked metabolites that are proposed to be associated with the formation of immunogenic, and hence potentially hepatotoxic, drug-protein adducts. Herein, we investigated the ability of PAA to undergo phenylacetyl-S-acyl-CoA thioester (PA-CoA)-mediated covalent binding to protein in incubations with freshly isolated rat hepatocytes in suspension. Thus, when hepatocytes were incubated with phenylacetic acid carboxy-(14)C (100 microM) and analyzed for PA-CoA formation and covalent binding of PAA to protein and over a 3-h time period, both PA-CoA formation and covalent binding to protein increased rapidly, reaching 1.3 microM and 291 pmol equivalents/mg protein after 4 and 6 min of incubation, respectively. However, the covalent binding of PAA to protein was reversible and decreased by 72% at the 3-h time point. After 3 h of incubation, PAA was shown to be metabolized primarily to phenylacetyl-glycine amide (84%). No PAA-acyl glucuronide was detected in the incubation extracts. PA-CoA reacted readily with glutathione in buffer, forming PA-S-acyl-glutathione; however, this glutathione conjugate was not detected in hepatocyte incubation extracts. Coincubation of hepatocytes with lauric acid led to a marked inhibition of PA-CoA formation and a corresponding inhibition of covalent binding to protein. SDS-polyacrylamide gel electrophoresis analysis showed the formation of two protein adducts having molecular masses of approximately 29 and approximately 33 kDa. In summary, PA-CoA formation in rat hepatocytes leads to the highly selective, but reversible, covalent binding to hepatocyte proteins, but not to the transacylation of glutathione” (Grillo and Lohr, 2009).

4.2. Absorption, distribution and excretion

“Phenylacetic acid... /is/ rapidly absorbed from human buccal tissues or membranes. [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754] **PEER REVIEWED**

Man excreted 93%...as glutamine conjugate... . New world monkeys excreted conjugates of glutamine, glycine and taurine, while old world species excreted large proportion of free acid and only glutamine and taurine conjugates. Non-primate species excreted only glycine connjugate. [The Chemical Society. Foreign Compound Metabolism in Mammals Volume 3. London: The Chemical Society, 1975., p. 569] **PEER REVIEWED**

Distribution of conjugates in 24 hr urine samples showed marked species variation. [The Chemical Society. Foreign Compound Metabolism in Mammals Volume 3. London: The Chemical Society, 1975., p. 569] **PEER REVIEWED**”.

As taken from HSDB, 2018.

Phenylacetic acid is rapidly absorbed from the gastro-intestinal tract.

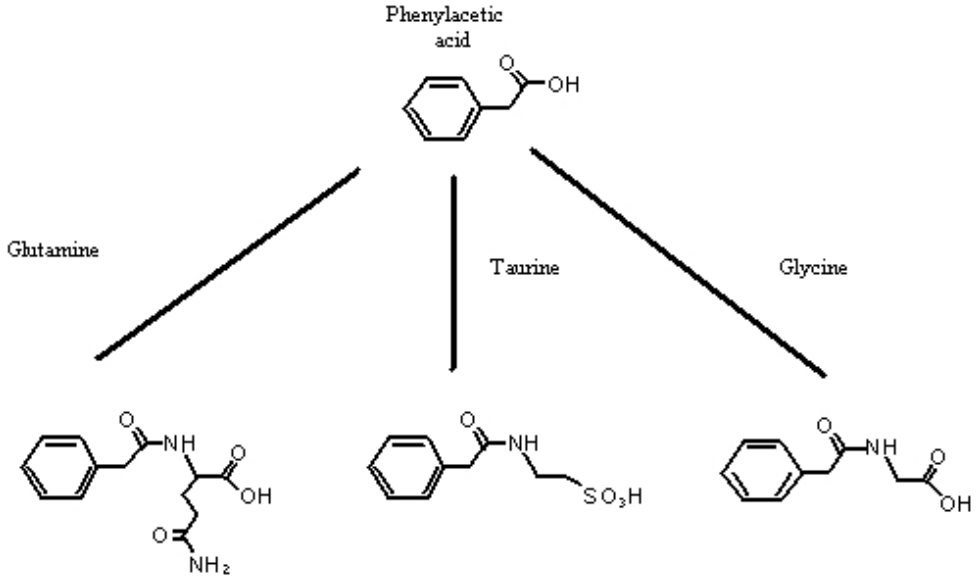
“Significant concentrations of free phenylacetic acid have been found after high doses (Teuchy et al., 1971; James et al., 1972).”

As taken from WHO Food Additives Series 50, available at http://www.inchem.org/documents/jecfa/jecmono/v50je11.htm

“Phenylacetic acid was administered to man, 14 species of sub-human primates and 11 non-primates. Four amino-acid conjugates...phenylacetylglutamine, phenylacetylglycine, phenylacetyltaurine and diphenylacetylornithine were identified in urine.”

As taken from HSDB, 2018.

“Although phenylacetic acid has been studied extensively, investigations conducted before 1950 on human metabolism (Shiple & Sherwin, 1922; Power & Sherwin, 1927; Ambrose et al., 1933; Wagreich et al., 1940) failed to account for the endogenous level of 250–500 mg/kg per day of phenylacetic acid conjugated with glutamine (Stein et al., 1954) present in human urine and did not adequately characterize the array of urinary conjugates that were formed from phenylacetic acid. More recent work demonstrates that conjugation is both dose-dependent and species-specific. The major metabolic options available to phenyl acetic acid are conjugation with glucuronic acid, glycine, taurine or glutamine and elimination as the free acid.”



“In carnivores (e.g., dogs, cats and ferrets), glycine conjugation predominates, with no detectable glutamine conjugation. Likewise, in rodents and lagomorphs (rabbits), phenylacetic acid is excreted primarily as the glycine conjugate. Unconjugated phenylacetic acid and minute amounts of taurine conjugates are also excreted. In rats, > 94% of a dose of 80 mg/kg bw of phenylacetic acid given by intraperitoneal injection was excreted as the glycine conjugate (James et al., 1972).”

“Clearly, the nature of the amino acid used for conjugation is a function of species. The sources and amounts of available amino acids alter the conjugating ability of different species. In humans, endogenous sources of glutamine include those from waste urea nitrogen.”

“The capacity for glutamine conjugation was studied in three control subjects and three patients with phenylketouria, each of whom was given a single 80-mg dose of [carboxy-^{14}C]phenylacetic acid. The average excretion of phenylacetylglutamine (measured as mmol/g creatinine) by the patients with phenylketouria was approximately five times that of two of the control subjects, indicating that the glutamine conjugation mechanism can cope with large amounts of phenylacetic acid (James & Smith, 1973). The mechanism for conjugation of glutamine with phenylacetic acid probably involves formation of a phenylacetic acid–coenzyme A intermediate. Perfusion of human kidney for 1 h or incubation of human liver homogenate with [^{14}C]glutamine and phenylacetyl–coenzyme A resulted in yields of the 5 and 13% of the respective radioactive conjugates (Moldave & Meister, 1957).”

“The capacity for glutamine conjugation has been studied in three volunteers and three patients exhibiting phenylketonuria (James et al., 1973). Each subject was given a single 80 mg dose of [carboxy-^{14}C]-phenylacetic acid. The average excretion of phenylactylglutamine (measured as mmoles/g creatinine) by phenylketonurics was approximately five times that of the control subjects indicating that the glutamine conjugation mechanism is able to cope with large amounts of phenylacetic acid produced by phenylketonurics. The mechanism for conjugation of glutamine with phenylacetic acid probably involves formation of a phenylacetic acid coenzyme-A (CoA) intermediate (Moldave and Meister, 1957). On hour perfusion of human kidney or incubation of human liver homogenate with [^{14}C]-glutamine and phenylacetyl-CoA results in the formation of the radioactive conjugate in the respective yields of 5% and 13%.” As taken from Food and Chemical Toxicology, 43, 2005, pp. 1179-1206

“Phenylacetic acid was present in 47.3% of the 54 samples of normal human expired air from an average concentration of 0.161 ng/L”

“Man excreted 93%...as glutamine conjugate... . New world monkeys excreted conjugates of glutamine, glycine and taurine, while old world species excreted large proportion of free acid and only glutamine and taurine conjugates. Non-primate species excreted only glycine conjugate.”

“Distribution of conjugates in 24 hr urine samples showed marked species variation.[”As taken from HSDB, 2018.

“The distribution and type of conjugation are virtually unaffected by continued ingestion of phenylacetic acid. One person fed 34 doses of 1000–10 000 mg of the acid over 97 days excreted > 90% of the administered dose as the phenylacetylglutamine conjugate (Ambrose et al., 1933). Like humans, Old and New World monkeys conjugate phenylacetic acid with glutamine and, to a lesser extent, taurine; however, significant quantities of acid (1–44%) are excreted free.”

“Phenyl acetic is conjuguated and primarily excreted in the urine.”

“Phenylacetic acid is a normal component of human urine (250–500 mg/24 h), forming mainly from the breakdown of phenylalanine by intestinal bacteria (Seakins, 1971) or by oxidative deamination of endogenous phenethylamine (Richter, 1938; Seakins, 1971).”

“Two volunteers, each fed a 300-mg dose of S-phenethyl-amine, excreted 60–62% of the administered dose as conjugated phenylacetic acid in the urine within 2–4.5 h (Richter, 1938;

Seakins, 1971). Furthermore, > 80% of [^{14}C]-S-phenethylamine fed to mice was rapidly excreted from urine as conjugated [^{14}C]phenylacetic acid (Block, 1953)."

"In an earlier study, 53% of a dose of 1300 mg bw of phenylacetic acid administered to rabbits by gavage was isolated from the 24-h urine as an ether-soluble acid. No appreciable quantity (< 0.5%) of free phenylacetic acid was recovered (Bray et al., 1946). In another study, only 0.4–3.1% of an oral dose of 740 mg/kg bw phenylacetic acid was excreted unconjugated in the urine of rabbits within 6 h (Tulane & Lewis, 1933)."

"More than 98% of a single oral dose of 80 mg of [carboxy-^{14}C]phenylacetic acid administered to each of three healthy volunteers and two patients with phenylketouria was excreted in the urine within 24 h as the glutamine conjugate (In a third patient with phenylketouria in this study, 12% of the dose was excreted as the glutamine conjugate, but this was attributed to incomplete collection of urine.) (James et al., 1973). Two male volunteers excreted 98% of an oral dose of 1 mg/kg bw of [carboxy-^{14}C]phenylacetic acid in their urine within 24 h (James et al., 1972), with > 90% of the dose recovered as the glutamine conjugate. The results of studies with radiolabelled phenylacetic acid indicate that it is rapidly absorbed and quantitatively excreted within 24 h."

"Two men excreted an average of 91% and 7% of an oral dose of 1 mg/kg bw of [carboxy-^{14}C]phenylacetic acid within 24 h as glutamine and taurine conjugates, respectively. In contrast to most animals, humans have only traces of the glycine conjugate (James et al., 1972)."

"In rodents, endogenous unconjugated phenylacetic acid can be found at concentrations at which glycine conjugation is capacity-limited, presumably by the supply of endogenous glycine (Gregus et al., 1993). Only small amounts of the glycine conjugate enter the bile: < 10% of a dose of phenylacetic acid of 100 mg/kg bw delivered to the small bowel via an indwelling catheter was collected from the bile of rats over 4 h (Koss & Lamprecht, 1968)."

"Uremic toxins are compounds normally excreted in urine that accumulate in patients with chronic kidney disease as a result of decreased renal clearance. Phenylacetic acid (PAA) has been identified as a new protein bound uremic toxin. The purpose of this study was to investigate in vitro the interaction between PAA and human serum albumin (HSA) at physiological and pathological concentrations. We used ultrafiltration to show that there is a single high-affinity binding site for PAA on HSA, with a binding constant on the order of 3.4 × 10(4) M(-1) and a maximal stoichiometry of 1.61 mol per mole. The PAA, at the concentration reported in end-stage renal patients, was 26% bound to albumin. Fluorescent probe competition experiments demonstrated that PAA did not bind to Sudlow's site I (in subdomain IIA) and only weakly bind to Sudlow's site II (in subdomain IIIA). The PAA showed no competition with other protein-bound uremic toxins such as p-cresyl-sulfate or indoxyl sulfate for binding to serum albumin. Our results provide evidence that human serum albumin can act as carrier protein for phenylacetic acid." As taken from Saldanha JF et al. 2016. Biochimie 125, 53-8. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26945842

"Phenylacetic acid is detected in human faecal samples. Phenylacetic acid occurs as a protein-bound uremic compound in humans.

Phenylacetic acid, the major metabolite of phenylethylamine, has been identified and quantitated in rat brain regions by capillary column high-resolution gas chromatography mass spectrometry. Its distribution is heterogeneous and correlates with that of phenylethylamine. The values obtained were (ng/g +/- SEM): whole brain, 31.2 +/- 2.7; caudate nucleus, 64.6 +/- 6.5; hypothalamus, 60.1 +/- 7.4; cerebellum, 31.3 +/- 2.9; brainstem, 33.1 +/- 3.3, and the "rest," 27.6 +/- 3.0.[Durden DA, Boulton AA; J Neurochem 38 (6): 1532-6 (1982)] **PEER REVIEWED**.

Conjugated and unconjugated phenylacetic acid and m- and p-hydroxyphenylacetic acid have been determined in the plasma of normal healthy subjects after fasting, consumption of a meal and ingestion of deuterium labelled amine precursors, by high-resolution gas chromatography--high resolution mass spectrometry with selected ion monitoring of their trifluoroethyl-pentafluoropropionyl derivatives. We observed that all three conjugated acids are higher in fasting

than in non-fasting subjects, and unconjugated phenylacetic acid was lower. Ingestion of deuterium-labelled amine precursors resulted in the appearance in the blood of the correspondingly labelled acids, a peak in the concentrations being reached about 1 hr after consumption. Conjugated and unconjugated acids as expected increased following the consumption of a meal. Unconjugated phenylacetic acid was significantly higher in females than in males. Most values tended to increase with age, with male unconjugated and conjugated m-hydroxyphenylacetic acid and female conjugated phenylacetic and m-hydroxyphenylacetic acids increasing significantly.[Davis BA et al; J Chromatogr 230 (2): 219-30 (1982)] **PEER REVIEWED**."

"**Background:** Elevated plasma ammonia is central to the pathogenesis of hepatic encephalopathy. Sodium phenylacetate or glycerol phenylbutyrate is approved for urea cycle disorders, but limited clinical data are available for hepatic encephalopathy. Phenylacetic acid (PAA) plasma exposure has been reported to correlate with neurologic adverse events in patients with cancer but not in patients with urea cycle disorders or hepatic encephalopathy. Ornithine phenylacetate, an intravenous dosage form of the L-ornithine salt of phenylacetate, is under development for hepatic encephalopathy.

Objective: This analysis summarized the pharmacokinetics and safety of ornithine phenylacetate to support the dosing strategy and to assist with the monitoring and management of neurologic adverse events in a global clinical development program.

Methods: Phenylacetic acid and phenylacetylglutamine (PAGN) pharmacokinetic data and adverse events from five clinical studies were included in the analysis. Hepatic and renal dysfunction were assessed by baseline Child-Pugh score and creatinine clearance, respectively. Predicted plasma exposures of PAA at the occurrence of neurologic adverse events were used for exposures and neurologic adverse event analysis.

Results: Phenylacetic acid exhibited nonlinear pharmacokinetics. Phenylacetic acid exposure was 35% higher in Child-Pugh C than in Child-Pugh B. No significant pharmacokinetic difference was identified between Caucasian and Asian subjects after body weight adjustment. Phenylacetylglutamine renal clearance decreased by five-fold in severe renal impairment compared with subjects with normal renal function. Renal dysfunction significantly elevated PAGN plasma concentrations; however, elevated PAGN due to reduced excretion of PAGN did not change PAA exposure and plasma ammonia levels. No correlation was observed between PAA plasma exposure and neurologic adverse events in patients with stable cirrhosis or acute hepatic encephalopathy.

Conclusions: Dose adjustment should be considered for patients with low body weight and severely impaired hepatic function. Phenylacetic acid plasma exposure was not correlated with neurologic adverse events in the ornithine phenylacetate target patient population."

As taken from Wang K et al. 2021.

4.3. Interactions

Type: Biochemical or cellular interactions

Remark: The in vivo formation of (1–14C) acetyl–coenzyme A from D–(3–14C)3–hydroxybutyrate in the brain of the suckling rat was not affected by postnatal exposure to phenyl acetate. However, utilization of the generated acetyl–coenzyme A was significantly inhibited in certain metabolic reactions, namely synthesis of fatty acids and of sterols, but not in others as the Krebs cycle reactions that lead to the production of dicarboxylic amino acids. The incorporation of D–(U–14C) glucosamine into N–acetylneuraminic acid bound to glycoproteins was appreciably diminished in the rat pup previously exposed to maternal phenylketonuria induced by phenyl acetate. During the period of very rapid development of the brain, interference by phenyl acetate and/or its metabolites with certain critical biosynthetic pathways that require acetyl–coenzyme A would significantly contribute to retarded maturation of the brain that occurs in phenylketonuria.

Source: ELF ATOCHEM UK Ltd WIDNESS (22)

As taken from IUCLUD Dataset (2000), Phenylacetic acid (103-82-2).

“This work was initiated to determine whether toxicity generated through inhibition of mitochondrial fuel metabolism is similar to high glucose/palmitate (HG/PA)-induced glucolipotoxicity. Influx of glucose and free fatty acids into the tricarboxylic acid (TCA) cycle was inhibited by treatment with the pyruvate carboxylase (PC) inhibitor phenylacetic acid (PAA) and carnitine palmitoyl transferase-1 (CPT-1) inhibitor etomoxir (Eto), or knockdown of PC and CPT-1. Treatment of PAA/Eto or knockdown of PC/CPT-1 induced apoptotic death in INS-1 beta cells. Similar to HG/PA treatment, PAA/Eto increased endoplasmic reticulum stress responses but decreased the Akt signal. JNK inhibitor or chemical chaperone was protective against both PAA/Eto- and HG/PA-induced cell death. All attempts to reduce [Ca2+](i), stimulate lipid metabolism, and increase the TCA cycle intermediate pool protected PAA/Eto-induced death as well as HG/PA-induced death. These data suggest that signals induced from impaired mitochondrial fuel metabolism play a critical role in HG/PA-induced glucolipotoxicity.” As taken from Lee JH et al. 2014. Mol. Cell. Endocrinol. 383, 48-59. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24333689?dopt=AbstractPlus

“We show that testicular orphan nuclear receptor 4 (TR4) increases the expression of pyruvate carboxylase (PC) gene in 3T3-L1 adipocytes by direct binding to a TR4 responsive element in the murine PC promoter. While TR4 overexpression increased PC activity, oxaloacetate (OAA) and glycerol levels with enhanced incorporation of (14)C from (14)C-pyruvate into fatty acids in 3T3-L1 adipocytes, PC knockdown by short interfering RNA (siRNA) or inhibition of PC activity by phenylacetic acid (PAA) abolished TR4-enhanced fatty acid synthesis. Moreover, TR4 microRNA reduced PC expression with decreased fatty acid synthesis in 3T3-L1 adipocytes, suggesting that TR4-mediated enhancement of fatty acid synthesis in adipocytes requires increased expression of PC gene.” As taken from Park SS et al. 2014. FEBS Lett. 588, 3947-3953. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25240193

“Uremic toxins are compounds normally excreted in urine that accumulate in patients with chronic kidney disease as a result of decreased renal clearance. Phenylacetic acid (PAA) has been identified as a new protein bound uremic toxin. The purpose of this study was to investigate in vitro the interaction between PAA and human serum albumin (HSA) at physiological and pathological concentrations. We used ultrafiltration to show that there is a single high-affinity binding site for PAA on HSA, with a binding constant on the order of 3.4 × 10(4) M(-1) and a maximal stoichiometry of 1.61 mol per mole. The PAA, at the concentration reported in end-stage renal patients, was 26% bound to albumin. Fluorescent probe competition experiments demonstrated that PAA did not bind to Sudlow's site I (in subdomain IIA) and only weakly bind to Sudlow's site II (in subdomain IIIA). The PAA showed no competition with other protein-bound uremic toxins such as p-cresyl-sulfate or indoxyl sulfate for binding to serum albumin. Our results provide evidence that human serum albumin can act as carrier protein for phenylacetic acid.” As taken from Saldanha JF et al. 2016. Biochimie 125, 53-8. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26945842

“Tendinopathy is a rare but serious complication of quinolone therapy. Risk factors associated with quinolone-induced tendon disorders include chronic kidney disease accompanied by the accumulation of uremic toxins. Hence, the present study explored the effects of the representative uremic toxins phenylacetic acid (PAA) and quinolinic acid (QA), both alone and in combination with ciprofloxacin (CPX), on human tenocytes in vitro. Tenocytes incubated with uremic toxins +/- CPX were investigated for metabolic activity, vitality, expression of the dominant extracellular tendon matrix (ECM) protein type I collagen, cell-matrix receptor β1-integrin, proinflammatory interleukin (IL)-1β, and the ECM-degrading enzyme matrix metalloproteinase (MMP)-1. CPX, when administered at high concentrations (100 mM), suppressed tenocyte metabolism after 8 h exposure and at therapeutic concentrations after 72 h exposure. PAA reduced tenocyte metabolism only after 72 h exposure to very high doses and when combined with CPX. QA, when administered alone, led to scarcely any cytotoxic effect. Combinations of CPX with PAA or QA did not cause greater cytotoxicity than incubation with CPX alone. Gene expression of the pro-inflammatory cytokine IL-

1β was reduced by CPX but up-regulated by PAA and QA. Protein levels of type I collagen decreased in response to high CPX doses, whereas PAA and QA did not affect its synthesis significantly. MMP-1 mRNA levels were increased by CPX. This effect became more pronounced in the form of a synergism following exposure to a combination of CPX and PAA. CPX was more tenotoxic than the uremic toxins PAA and QA, which showed only distinct suppressive effects." A taken from Popowski E et al. 2020. Int. J. Mol. Sci. 21(12), 4241. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32545914/

"Fermentation of dietary fiber and metabolism of polyphenols by the gut microbiota produce short chain fatty acids (SCFAs) and some simple phenolic acids, both of which have been independently shown to have anti-inflammatory effects and to improve gut health. While synergistic interactions between the two colonic metabolite groups have been speculated, direct evidence on whether and how these compounds work together towards collective anti-inflammatory effect and gut health remain unclear. In this study, the most important SCFA butyric acid (BtA) and three phenolic metabolites benzoic acid (BzA), phenylacetic acid (PAA) and phenylpropionic acid (PPA) were selected and assessed for the anti-inflammatory effects and the underlying mechanisms in a TNF-α-induced Caco-2 cell model. Significantly stronger inhibition of TNF-α-induced IL-8 production was found in cells pre-treated with different concentrations of mixtures of BtA and the phenolic metabolites than individual compounds. Further study showed that the synergistic effect was via significant down-regulation of the gene expressions of IL-8, TNF-α and VCAM-1, and of phosphorylation of JNK, p38 and IκBα, cellular signaling mediators of the MAPK and NF-κB pathways. Although these are results of a cell model, they do provide significant insights into the synergistic anti-inflammatory effects between SCFAs and phenolic metabolites. As both metabolite groups are simultaneously present in the gut, our findings therefore also suggest a potential synergistic effect between the two colonic metabolite groups in maintaining gut health." As taken from Zheng S et al. 2021. Food Res. Int. 139, 109833. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33509458/

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
guinea pig	LD50	oral	2250mg/kg (2250mg/kg)		Voprosy Pitaniya. Problems of Nutrition. Vol. 33(5), Pg. 48, 1974.
mouse	LD50	intraperitoneal	2270mg/kg (2270mg/kg)		Farmaco, Edizione Scientifica. Vol. 13, Pg. 286, 1958.
mouse	LD50	oral	2250mg/kg (2250mg/kg)		Voprosy Pitaniya. Problems of Nutrition. Vol. 33(5), Pg. 48, 1974.
mouse	LD50	subcutaneous	1500mg/kg (1500mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 116, Pg. 154, 1958.
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 13, Pg. 901, 1975.
rat	LD50	intraperitoneal	1600mg/kg (1600mg/kg)		Bollettino Chimico Farmaceutico. Vol. 112, Pg. 53, 1973.

rat	LD50	oral	2250mg/kg (2250mg/kg)		Voprosy Pitaniya. Problems of Nutrition. Vol. 33(5), Pg. 48, 1974.

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

Acute Oral Toxicity

Type:	LD50
Species:	rat
Value:	> 5000 mg/kg bw
Method:	other
Remark:	A value 2250 mg/kg is given by a Russian author. However he gave exactly the same values for acute orale LD50 in mice and guinea pig (cited by RTECS). Consequently these values are questionable, taking into account the other figures found in scientific papers (5141, 5142, 5143).
Source:	ELF ATOCHEM UK Ltd WIDNESS
	(13)

Acute Dermal Toxicity

Type:	LD50
Species:	rabbit
Value:	> 5000 mg/kg bw
Method:	other
Year:	1975 GLP: no data
Source:	ELF ATOCHEM UK Ltd WIDNESS

	(13)

Acute Toxicity, other Routes

Type:	LD50
Species:	rat
Route of admin.:	i.p.
Value:	= 1600 mg/kg bw
Year:	1973 GLP: no data
Remark:	Calculation by Litchfield and Wilcoxon's method; 6–8 animals per dose; 8 days observations.
Source:	ELF ATOCHEM UK Ltd WIDNESS
	(14)

Type:	LD50
Species:	mouse
Route of admin.:	i.p.
Value:	= 2270 mg/kg bw
Year:	1958 GLP: no data
Test substance:	no data
Source:	ELF ATOCHEM UK Ltd WIDNESS
	(15)

Type:	LD50
Species:	mouse
Route of admin.:	s.c.
Value:	= 1500 mg/kg bw
Year:	1958 GLP: no
Source:	ELF ATOCHEM UK Ltd WIDNESS
	(16)

As taken from IUCLID Dataset (2000), Phenylacetic acid (103-82-2).

Human case:

“Phenylacetic acid given orally at a single dose of 20 mg/kg bw to each of five male volunteers resulted in a two- to fourfold increase in urinary indole-3-acetic acid concentration (Tashian, 1960). Presumably, the phenylacetic acid deactivated tryptophan decarboxylase, resulting in increased conversion of tryptophan to indole-3-acetic acid.”

As taken from WHO Food Additives Series 50, available at http://www.inchem.org/documents/jecfa/jecmono/v50je11.htm

“In study of acute effects in mice, ip injection of 300 mg/kg proved toxic; 11 of 15 experimental animals died. Time to death varied from 10 min to 10 days.” As taken from HSDB, 2018.

“An 18–23% reduction in the plasma tripeptide concentration was observed within a few hours of ingestion of a 4000-mg dose of phenylacetic acid in a volunteer (Shiple & Sherwin, 1922).”

“Other studies have been conducted in rodents and humans on the effects of phenylacetic acid on various biochemical parameters, mostly associated with phenylketouria. Phenylacetic acid administered orally to fasted guinea-pigs at a dose of 380, 500 or 750 mg/kg bw resulted in a reduction in blood sugar to 13%, 44% and 60% of the initial value, respectively, within 2–4 h. Rabbits were as sensitive as guinea-pigs to the hypoglycaemic action of phenylacetic acid. Phenylacetic acid administered intravenously at a dose of 62 mg/kg bw also produced a marked reduction in blood sugar in a cat, which was sustained for at least 5 h (Stewart, 1962).”

Species	**Route**	**Dose data**
Rat	Oral	LD_{50}: > 5000mg/kg bw
Rat	Gavage	LD_{50}: 2200 mg/kg bw
Mouse	Gavage	LD_{50}: 2200 mg/kg bw
Guinea pig	Gavage	LD_{50}: 2250 mg/kg bw

5.2. Repeated dose toxicity

Repeated Dose Toxicity

Species:	rat
Sex:	no data
Strain:	Fischer 344
Route of admin.:	other: S.C and I.P
Exposure period:	28 days
Frequency oftreatment:	continuous s/c release by osmotic minipump and i.p injection once day
Post. obs. period:	0 day

Doses:	550 mg/kg/d (s/c); 300 mg/kg/d (i.p)
Control Group:	yes, concurrent vehicle
Method:	other
Year:	1994 GLP: no data
Test substance:	no data
Remark:	– 50 rats were used. – treatment with phenylacetate extented survival when started on the day of tumor inoculation (P = 0.01) or 7 days after (P =0.03), without adverse effects. Phenylacetate levels in serum after 7 days was in the therapeutic range as determined in vitro: 2.45 mM (ca 333.2 mg/l) and 3.1 mM in cerebrospinal fluid. Electron microscopy demonstrated marked hypertrophy and organization of the rough endoplasmic reticulum, indicating cell differenciation. In addition in vitro studies demonstrated dose–dependent inhibition of the rate of tumor proliferation and restoration of anchorage dependency, a marker of phenotypic reversion. Route of ministration: i.p + sc.
Source:	ELF ATOCHEM UK Ltd WIDNESS(17)

As taken from IUCLID Dataset (2000), Phenylacetic acid (103-82-2).

5.3. Reproduction toxicity

“Day 10 rat embryos (4 or 8 somites) were cultured in rat serum to which tested compounds were added. After 26 hr of culture, embryos were scored morphologically…. and /phenylacetic acid/ induced dose-related embryotoxicity above 0.3 mg/ml. These results suggest a possible role for PKU-related phe-metabolites in the induction of congenital malformations.”

“In teratogenic study with rats, administration of phenylacetic acid on 12th day of embryogenesis affected body weight, retarded skeletal ossification, and caused embryos to be resorbed at twice rate of controls. Dosage was 0.2% of LD50, or 3.2 mg/kg.”

“The effects of phenylacetate (PA) on fetal brain growth were examined in pregnant rats receiving a 20% case in diet with 1.0, 1.5, 2.0 or 2.5% PA. Control rats were fed the 20% casein diet ad libitum or restricted to daily consumption of 9 and 6 g. In experimental groups of rats total food intake during pregnancy decreased however the decrease was not so large as we had expected. By plotting the fetal brain weight (Y, mg) against maternal food intake (X, g/21 days) in control groups the following hyperbolic regression equation was obtained: $Y = -4243/X + 124.6$ (n: 18, $r = 0.80$, $p < 0.001$). Similar plots for excess PA rats fell below this line, indicating that prenatal fetal brain growth was impaired by an excess in PA per se specifically as well as by decreased food intake nonspecifically. Administering the excess PA diets resulted in decreases in total RNA total protein and RNA/DNA ratio in the fetal brain whereas total DNA was unchanged showing the impairment of protein synthesis not proliferation. No remarkable changes in concentration and pattern of free amino acids in maternal plasma in excess PA groups were observed. Using a relation between total food intake during pregnancy and fetal brain weight the extent of specific and nonspecific effects of excess PA and phenylalanine on brain growth was compared.”

As taken from HSDB, 2018.

“In screening studies, low doses of phenethyl alcohol (No. 987) and phenylacetic acid (No. 1007) were reported to have teratogenic effects resembling fetal alcohol syndrome (Mankes et al., 1983, 1984, 1985). These results were contradicted by those of a study in which phenethyl alcohol given to pregnant rats at high doses at critical periods of embryogenesis did not cause any visible anomalies in embryonal development (Burdock et al., 1987). More recent, comprehensive studies with high doses of phenethyl alcohol given orally (Bottomley et al., 1987) or dermally (Palmer et al., 1986) indicated that this group of substances has little reproductive or developmental toxicity.”

As taken from WHO Food Additives Series 50, available at http://www.inchem.org/documents/jecfa/jecmono/v50je11.htm

Developmental Toxicity/Teratogenicity:

Species:	rat
Sex:	female
Route of admin.:	oral feed
Exposure period:	21 days
Frequency of treatment:	ad libitum or restricted daily consumption of 9 and 6 g
Duration of test:	21 days
Doses:	1 – 1.5 – 2 or 2.5% PA in 20% casein diet
Control Group:	other: 20% casein diet
Method:	other
Remark:	Rats receiving 20% casein diet, were submitted approximately to 0.5% PA, corresponding to 5000 ppm, 500 mg/kg body weight/day.
Result:	In experimental groups of rats total food intake during pregnancy decreased, however the decrease was not so large as we had expected. By plotting the fetal brain weight (Y, mg) against maternal food intake (X, g/21 days) in control groups the following hyperbolic regression equation was obtained: $Y = -4243/X + 124.6$ (n: 18; $r = 0.08$; $p < 0.001$). Similar plots for excess PA rats fell below this line, indicating that prenatal fetal brain growth was impaired by an excess in PA per se specifically, as well as by decreased food intake nonspecifically. Administering the excess PA diets resulted in decreases in total RNA, total protein and RNA/DNA ratio in the fetal brain, whereas total DNA was unchanged, showing the impairment of protein synthesis, not proliferation. No remarkable changes in concentration and pattern of free amino acids in maternal plasma in excess PA groups were observed.
Source:	ELF ATOCHEM UK Ltd WIDNESS(18)

Species:	other: mouse embryo
Route of	other: whole embryo culture

admin.:	
Exposure period:	24 hours
Duration of test:	24 hours
Doses:	0.01 mM to 10 mM
Control Group:	no
Year:	1990 GLP:
Remark:	The embryotoxic concentration 10 mM (1.5 mg/ml), toxic concentration for man following IV perfusion. In rat, after 800 mg/kg/day, the serie concentration was only 0.33 mg/ml.
Result:	The aim of this study was to det. the teratogenicity of phenylalanine (Phe) and Phe metabolites in neurulating mouse embryos. Therefore, the system of whole embryo culture was employed and D9 (neurulating) mouse embryos were exposed to Phe, phenylethylamine (PEA), phenylpyruvic acid (PPA), phenylacetic acid (PAA), 2–OH phenylacetic acid (2–OHPAA), and phenyllactic acid (PLA) at concns. ranging from 0.01 mM to 10 mM for 24 h. After 24 h, embryos were examined for morphology abnormalities and protein content. Phe at 1 and 6 mM concns. was not teratogenic; however, 10 mM inhibited cranial neural tube closure in 82% of the embryos. PEA was the most toxic factor and concns. of 1 and 10 mM were embryo–lethal, whereas neural tube closure defects (NTDs) were obsd. in 67% of the embryos at 0.1 mM. 2–OH PAA was the second most toxic metabolite with concns of 1 and 10 mM producing NTDs in 10 and 100% of the embryos, resp. PLA and PAA produced no NTDs at concns of 1 mM 60% at 5 mM, and 100% at 10 mM. Finally, PPA produced approx. 50% TDs at both 1 mM and 10mM concns. PLA, PAA, 2–OH PAA and PPA produced a significant redn. in embryonic protein, and PEA and 2–OH PAA reduced yolk sac protein values. PEA, 2–OH PAA, PAA, and PLA also produced craniofacial abnormalities, i.e., incomplete expansion of the forebrain, collapse of the optic vesicle, and hypoplasia of the mandible and/or the maxilla. The results showed that the relative teratogenicity of Phe and its metabolites differs and suggested that a metabolite of Phe and/or a combinaison of Phe and one or more of its metabolites play a role in the phenylketonuric embryopathy.
Source:	ELF ATOCHEM UK Ltd WIDNESS (19)[Denno KM & Sadler DW, 1990]
Species:	other: rat embryo
Route of admin.:	other: whole embryo culture
Exposure period:	26 hours
Duration of test:	26 hours
Year:	1991
Remark:	The toxic effects at the considerable concentration 1 mg/ml are confirmed.

Result:	Mothers with untreated PKU can have offspring whose prenatal development is affected by the mothers' disease. These congenital malformations are associated with accumulation of^PHE and its metabolites in maternal blood. We have studied the possible embryotoxicity of PHE and four PKU– related metabolites, phenylpyruvic acid (phepyr.), phenyllactic acid (phelac), o–hydroxyphenylacetic acid (hypheac) and phenylacetic acid (pheac) in the postimplantation whole embryo culture systeme. Day 10 rat embryos (4 or 8 somites) were cultured in rat serum to which tested compounds were added. After 26 h of culture, embryos were scored morphologically. PHE was not embryotoxic in the system, even in the presence of maternal hepatocytes to facilitate metabolism of PHE. HPLC – analysis of medium after coculture revealed the formation of tyrosine, but no PKU – related metabolites were detected. Phepyr induced some growth retardation at 1 mg/ml, whereas phelac had no effect. Hypheac and pheac induced dose–related embryotoxicity above 0.3 mg/ml. These results suggest a possible role for PKU–related phe metabolites in the induction of congenital malformations.
Source:	ELF ATOCHEM UK Ltd WIDNESS(20)
Species:	rat
Sex:	female
Route of admin.:	unspecified
Exposure period:	during pregnancy
Doses:	0,2 the LD50
Remark:	Russian publication.
Result:	Tests were set up in 75 albino rats from which 737 embryos had been obtained. Subject to determination were: the numbers of live and dead embryos, their weight, lenght, anatomic changes in the internal organs, skeletal structure and also the RNA and DNA content in their liver. Investigations showed the phenylacetic acid, phenylethyl and cinnamic alcohols administered to pregnant rats in a dose of 0.2 LD50 during critical periods of embryogenesis to produce an embryotoxic effect of differing intensity. Most pronounced proved the action of phenylacetic acid, which manifested itself in that the test embryos, as contrasted to the control ones, demonstrated on the 20th day of embryogenesis a lagging body weight gain, retarded skeletal ossification, a reduced RNA content in the liver, the number of resorbed embryos being twice as great.
Source:	ELF ATOCHEM UK Ltd WIDNESS (21)

As taken from IUCLID Dataset (2000), Phenylacetic acid (103-82-2).

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Sex/Duration	Toxic Effects	Reference
TDLo - Lowest	Oral	Rodent - rat	450 mg/k	female 4 day(s) after	Reproductive - Effects on	VPITAR Voprosy Pitaniya. Problems of Nutrition. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-10, 1932-41; V.11- 1952- Volume(issue)/page/year:

published toxic dose			g	conception	Embryo or Fetus - fetotoxicity (except death, e.g., stunted fetus) Reproductive - Specific Developmental Abnormalities - musculoskeletal system	32,50,1973
TDLo - Lowest published toxic dose	Oral	Rodent - rat	19500 mg/kg	female 14 day(s) pre-mating - 4 day(s) post-birth	Reproductive - Maternal Effects - other effects	HPV133 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzeneethanol.pdf http://www.epa.gov/HPV/pubs/summaries/phnethal/c13895tc.htm Volume(issue)/page/year: -,-,2002
TDLo - Lowest published toxic dose	Oral	Rodent - rat	39000 mg/kg	female 14 day(s) pre-mating - 4 day(s) post-birth	Reproductive - Maternal Effects - other effects Reproductive - Effects on Newborn - viability index (e.g., # alive at day 4 per # born alive)	HPV133 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzeneethanol.pdf http://www.epa.gov/HPV/pubs/summaries/phnethal/c13895tc.htm Volume(issue)/page/year: -,-,2002

As taken from RTECS, 2017.

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	5.0	COSMOS TTC (NON-CANCER)

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

NOAEL/LOAEL Owner	Original NOAEL	Original LOAEL	Critical Sites	Critical Effects
PAFA	5.0 mg/kg bw/day	45.0 mg/kg bw/day		OFFSPRING DEVELOPMENTAL

Safety Evaluation Comments: no comments available.

Source Document: no source document available

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

“Increased abortions, delayed bone growth, decreased fetal weight, and altered development of the nervous system were observed in laboratory animals exposed to high doses during pregnancy.”

As taken from HSDB, 2018.

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which phenylacetic acid was part of the test mixture include EMT000312 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

No.	Agent	End-point	Test object	Maximum concentration	Result	Reference
In vitro						
1007	Phenylacetic acid	Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	1000 mg	Negative[a]	Heck et al. (1989)
1007	Phenylacetic acid synthesis	Unscheduled DNA	Rat hepatocytes	500 mg	Negative	Heck et al. (1989)
1007	Phenylacetic acid	Mutation	Mouse lymphoma L5178Y TK+/- cells	1500 mg	Negative[a]	Heck et al. (1989)

“Phenylacetic acid has been tested for his ability to induce reverse mutation in various strains of Salmonella typhimurium (e.g., TA98, TA100, TA1535, TA1537 and TA1538) in the presence or absence of an exogenous metabolic activation system. It was not mutagenic when tested at concentrations up to 5000 µg/ml or 50 mg/plate (Oda et al., 1979; Florin et al., 1980; Rapson et al., 1980; Ishidate et al., 1984; Heck et al., 1989; Kato et al., 1989; Fujita et al., 1994).”

“No increase in unscheduled DNA synthesis was noted when rat hepatocytes were incubated with phenylacetic acid (Heck et al., 1989).”

Tests of the ability of phenylacetic acid to induce mutation in Mouse lymphoma L5178Y $Tk^{+/-}$ cells were inconclusive.

As taken from WHO Food Additives Series 50, available at http://www.inchem.org/documents/jecfa/jecmono/v50je11.htm

Other In vitro Test:

Of phenylacetic acid (PEAA), phenylpyruvic acid and homogentisic acid, PEAA was found to be the most effective in inhibiting carnitine biosynthesis in rats. Following 60 min, a single intraperitoneal dose of PEAA the relative conversion rate, i. e. the hydroxylation, of tracer [Me-(3)H]butyrobetaine to [Me-(3)H]carnitine decreased from 62.2+/-6.00% to 39.4+/-5.11% (means+/-S.E.M., P<0.01) in the liver, in the only organ doing this conversion in rats. The conversion of loading amount of unlabeled butyrobetaine to carnitine was also markedly reduced. The impaired hydroxylation of butyrobetaine was reflected by a reduced free and total carnitine levels in the liver and a reduced total carnitine concentration in the plasma. PEAA decreased the hepatic level of glutamic acid and alpha-ketoglutaric acid (alpha-KG), suggesting a mechanism for the reduced flux through the butyrobetaine hydroxylase enzyme, because alpha-KG is an obligatory co-enzyme. [Fischer GM et al; Biochim Biophys Acta 1501 (2-3): 200-10 (2000)] **PEER REVIEWED** PubMed Abstract

In cultured /embryonic rat/ cortical neurons, cytoplasmic free calcium concentration ([Ca(2+)](i)) decreased dramatically when treatment with phenylalanine (Phe) and phenyllactic acid, while phenylacetic acid treatment immediately increased [Ca(2+)](i), which began to decrease after 3 min. [Yu YG et al; Neurochem Res 32 (8): 1292-301 (2007)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018.

“Background: 4-hydroxyphenylacetic acid (HO-PAA) is produced by intestinal microbiota from L-tyrosine. High concentrations in human fecal water have been associated with cytotoxicity, urging us to test HO-PAA's effects on human colonocytes. We compared these effects with those of phenylacetic acid (PAA), phenol and acetaldehyde, also issued from amino acids fermentation. Methods: HT-29 Glc-/+ human colonocytes were exposed for 24 h to metabolites at concentrations between 350 and 1000 μM for HO-PAA and PAA, 250-1500 μM for phenol and 25-500 μM for acetaldehyde. We evaluated metabolites'cytotoxicity with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide and DNA quantification assays, reactive oxygen species (ROS) production with H2DCF-DA, and DNA damage with the comet assay. We measured cell oxygen consumption and mitochondrial complexes activity by polarography. Results: Although HO-PAA displayed no cytotoxic effect on colonocytes, it decreased mitochondrial complex I activity and oxygen consumption. This was paralleled by an increase in ROS production and DNA alteration. Cells pretreatment with N-acetylcysteine, a ROS scavenger, decreased genotoxic effects of HO-PAA, indicating implication of oxidative stress in HO-PAA's genotoxicity. PAA and phenol did not reproduce these effects, but were cytotoxic towards colonocytes. Last, acetaldehyde displayed no effect in terms of cytotoxicity and mitochondrial metabolic activity, but increased DNA damage. Conclusions: Several bacterial metabolites produced from amino acids displayed deleterious effects on human colonocytes, in terms of genotoxicity (HO-PAA and acetaldehyde) or cytotoxicity (PAA and phenol). General significance: This study helps understanding the consequences of intestinal microbiota's metabolic activity on the host since amino acids fermentation can lead to the formation of compounds toxic towards colonic epithelial cells.” As taken from Armand L et al. 2019. Biochim. Biophys. Acta Gen. Subj. 1863(8), 1292-1301. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31034912/

5.5. Cytotoxicity

Phenylacetate (PA) and phenylbutyrate (PB) are aromatic fatty acids that are presently undergoing evaluation as potential antineoplastic agents. In vitro, PA and PB cause differentiation or growth inhibition of malignant cells. Clinical trials of these drugs as single agents indicate that they are not myelosuppressive; therefore, combinations with other chemotherapy agents may be possible. The goals of this study were to determine whether PA and PB (a) are cytotoxic to malignant B cells from patients with non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia and (b) exhibit additive or synergistic induction of apoptosis when administered to myeloma cell lines in

combination with conventional drugs. In the clinical specimens, cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and percent apoptosis was measured using 7-aminoactinomycin D and flow cytometry. Viability was decreased by > 50% in 7% (1/15) of non-Hodgkin's lymphoma samples treated with 5 mM PA, 27% treated with 1 mM PB, and 60% treated with 2 mM PB. Likewise, viability was decreased by > 50% in 44% (4/9) of chronic lymphocytic leukemia samples treated with 5 mM PA, 67% treated with 1 mM PB, and 100% treated with 2 mM PB. Studies in the myeloma cell lines demonstrated that PB treatment induced activation of caspases 3, 7, and 9 accompanied by cleavage of their substrates and internucleosomal DNA degradation. Combinations of PA or PB with conventional drugs (cytarabine, topotecan, doxorubicin, etoposide, chlorambucil, melphalan, fludarabine, carboplatin, and cisplatin) were examined for synergism (combination index < 1 in median effect analysis) in inducing apoptosis of both the MY5 and 8226 human myeloma cell lines. At concentrations that killed > 50% of cells, most combinations were additive; however, PB was synergistic with cytarabine, etoposide, and topotecan, with the combination index < 1 at each of the 50, 75, and 95% apoptosis levels. These observations indicate that PA and PB can induce apoptosis in malignant B cells and enhance the cytotoxicity of agents used in the treatment of these malignancies (Witzig TE et al., 2000).

Phenylketonuria is a genetic defect that, without strict dietary control, results in the accumulation of phenylalanine (Phe) in body fluids. If a low-Phe diet is not maintained during pregnancy, the offspring of phenylketonuric women are born with mental retardation and microcephaly. Primary cultures of rat cerebellar granule cells, rat cortical astrocytes, human fetal astrocytes, and human neuroblastoma (SY5Y) cells and human astrocytoma (1321N1) cells were used to test the hypothesis that the microencephaly may be a result of neuronal cell death and reduced astrocyte proliferation. Exposure to Phe or to six Phe metabolites [phenylacetic acid (PAA), phenyllactic acid, hydroxyphenylacetic acid, phenylpyruvic acid, phenylethylamine (PEA), and mandelic acid] did not result in astroglial or neuronal cell cytotoxicity. Treatment of 1321N1 cells, human fetal astrocytes, or rat astrocytes with 5 mM Phe for 24 h decreased DNA synthesis 19 +/- 4, 30 +/- 4, and 60 +/- 6%, respectively. This effect was concentration dependent, and flow cytometry revealed that Phe treatment resulted in the accumulation of cells in the G(0)/G(1) phase of the cell cycle. In addition, in 1321N1 cells, exposure to 5 mM PAA, and in rat astrocytes, exposure to 0.5 mM PEA inhibited cell proliferation 42 +/- 4 and 55 +/- 4%, respectively. These metabolites also resulted in the accumulation of cells in the G(0)/G(1) phase of the cell cycle. In human fetal astrocytes, 0.5 mM PEA and 0.5 mM PAA resulted in a 41 +/- 12 and 52 +/- 11% reduction proliferation, respectively (Oberdoerster J et al., 2000).

“Background: 4-hydroxyphenylacetic acid (HO-PAA) is produced by intestinal microbiota from L-tyrosine. High concentrations in human fecal water have been associated with cytotoxicity, urging us to test HO-PAA's effects on human colonocytes. We compared these effects with those of phenylacetic acid (PAA), phenol and acetaldehyde, also issued from amino acids fermentation. Methods: HT-29 Glc-/+ human colonocytes were exposed for 24 h to metabolites at concentrations between 350 and 1000 µM for HO-PAA and PAA, 250-1500 µM for phenol and 25-500 µM for acetaldehyde. We evaluated metabolites'cytotoxicity with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide and DNA quantification assays, reactive oxygen species (ROS) production with H2DCF-DA, and DNA damage with the comet assay. We measured cell oxygen consumption and mitochondrial complexes activity by polarography. Results: Although HO-PAA displayed no cytotoxic effect on colonocytes, it decreased mitochondrial complex I activity and oxygen consumption. This was paralleled by an increase in ROS production and DNA alteration. Cells pretreatment with N-acetylcysteine, a ROS scavenger, decreased genotoxic effects of HO-PAA, indicating implication of oxidative stress in HO-PAA's genotoxicity. PAA and phenol did not reproduce these effects, but were cytotoxic towards colonocytes. Last, acetaldehyde displayed no effect in terms of cytotoxicity and mitochondrial metabolic activity, but increased DNA damage. Conclusions: Several bacterial metabolites produced from amino acids displayed deleterious effects on human colonocytes, in terms of genotoxicity (HO-PAA and acetaldehyde) or cytotoxicity

(PAA and phenol). General significance: This study helps understanding the consequences of intestinal microbiota's metabolic activity on the host since amino acids fermentation can lead to the formation of compounds toxic towards colonic epithelial cells." As taken from Armand L et al. 2019. Biochim. Biophys. Acta Gen. Subj. 1863(8), 1292-1301. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31034912/

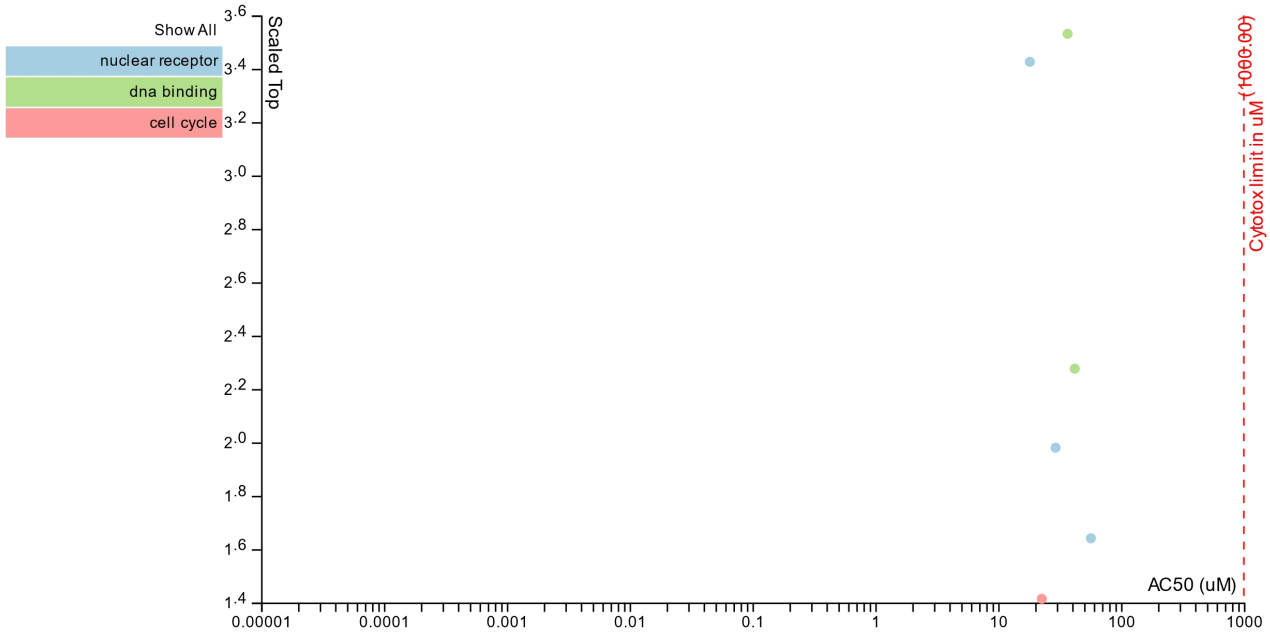
High-throughput Assay Data

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

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

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

Figure 1



5.6. Carcinogenicity

"Phenylacetic acid did not promote tumor formation when given to rabbits iv and sc for 40 days."

As taken from HSDB, 2018.

The increasing incidence of melanoma and the poor responsiveness of disseminated disease to conventional treatments call for the development of new therapeutic approaches. Phenylacetate, a nontoxic differentiation inducer, can suppress the growth of other neuroectodermal tumors, i.e.,

gliomas, in laboratory models and in humans. This finding led us to explore the efficacy of phenylacetate and related aromatic fatty acids in melanoma. Phenylacetate and phenylbutyrate were found to a) induce selective cytostasis and maturation of cultured human melanoma cells, b) modulate the expression of genes implicated in tumor metastasis (type IV collagenase and tissue inhibitor of metalloproteinases-2) and immunogenicity (HLA class I); and c) enhance the efficacy of other agents of clinical interest, including retinoids, interferon-alpha, suramin, and 5-aza-2'-deoxycytidine. Reflecting on the phenotypic heterogeneity of melanoma, the degree of biologic alterations induced by phenylacetate/phenylbutyrate varied significantly among the tumor cell lines tested. Although losing invasive capacity and tumorigenicity in athymic mice, poorly differentiated cells exhibited only a marginal change in morphology, remained amelanotic, and resumed growth after treatment was discontinued. By contrast, treatment of melanoma cells that were in a more advanced stage of maturation resulted in profound alterations in cell growth, morphology, and pigmentation consistent with terminal differentiation. The in vitro antitumor activity was observed with nontoxic, pharmacologic concentrations of phenylacetate and phenylbutyrate, suggesting potential clinical use of these drugs in the treatment of melanomas. (Liu L et al., 1994).

To assess the possible carcinogenicity of phenoxy herbicides and related chlorophenols and dioxins, the International Agency for Research on Cancer is coordinating an international collaborative study of workers exposed to these compounds in their production or use. Four British cohorts of chemical manufacturers which have been recruited to the survey are described. They comprise a total of 2239 men employed during 1963-85. These subjects were traced to 31 December 1987 through the National Health Service Central Register and the National Insurance Index, and their mortality compared with that in the national population. Two deaths were from non-Hodgkin's lymphoma with 0.87 expected. Both deaths occurred more than 10 years after first exposure to phenoxy compounds. One further non-Hodgkin's lymphoma was registered in a living subject with probable exposure to phenoxy compounds. No cases of soft tissue sarcoma or Hodgkin's disease were recorded (Coggon D et al., 1991).

Anti-Tumoral Effect:

Phenylbutyrate (PB) and its metabolite phenylacetate (PA) demonstrate anticancer activity in vitro through promotion of cell differentiation, induction of apoptosis through the p21 pathway, inhibition of histone deacetylase, and in the case of PB, direct cytotoxicity. We studied the pharmacokinetics, metabolism, and cerebrospinal fluid (CSF) penetration of PA and PB after intravenous (i.v.) administration in the nonhuman primate. METHODS: Three animals received 85 mg/kg PA and 130 mg/kg PB as a 30-min infusion. Blood and CSF samples were obtained at 15, 30, 35, 45, 60 or 75 min, and at 1.5, 2.5, 3.5, 5.5, 6.5, 8.5, 10.5 and 24.5 h after the start of the infusion. Plasma was separated immediately, and plasma and CSF were frozen until HPLC analysis was performed. RESULTS: After i.v. PA administration, the plasma area under the concentration-time curve (AUC) of PA (median +/- SD) was 82 +/- 16 mg/ml.min, the CSF AUC was 24 +/- 7 mg/ml.min, clearance (Cl) was 1 +/- 0.3 ml/min per kg, and the AUCCSF:AUCplasma ratio was 28 +/- 19%. After i.v. PB administration, the plasma PB AUC was 19 +/- 3 mg/ml.min, the CSF PB AUC was 8 +/- 11 mg/ml.min, the PB Cl was 7 +/- 1 ml/min per kg, and the AUCCSF:AUCplasma ratio was 41 +/- 47%. The PA plasma AUC after i.v. PB administration was 50 +/- 9 mg/ml.min, the CSF AUC was 31 +/- 24 mg/ml.min, and the AUCCSF:AUCplasma ratio was 53 +/- 46%. CONCLUSIONS: These data indicate that PA and PB penetrate well into the CSF after i.v. administration. There may be an advantage to administration of PB over PA, since the administration of PB results in significant exposure to both active compounds. Clinical trials to evaluate the activity of PA and PB in pediatric central nervous system tumors are in progress (Berg S et al. 2001).

“Phenylacetate (PA) inhibits the growth of tumor cells in vitro and in vivo and shows promise as a relatively nontoxic agent for cancer treatment. A recent report shows that prolonged exposure of cells to low concentrations of PA can enhance the radiation response of brain tumor cells in vitro, opening up the possibility of using this drug to improve the radiation therapy of brain tumor patients. We investigated the cytotoxicity produced by sodium phenylacetate (NaPA) alone and in

combination with X-rays in SF-767 human glioblastoma cells and in two medulloblastoma cell lines, Masden and Daoy. Exposure of all three cell lines to relatively low concentrations of NaPA for up to 5 days did not enhance the subsequent cell killing produced by X-irradiation. However, enhanced cell killing was achieved by exposing either oxic or hypoxic cells to relatively high drug concentrations (> 50-70 mM) for 1 h immediately before X-irradiation. Because central nervous system toxicity can occur in humans at serum concentrations of approximately 6 mM PA, translation of these results into clinical trials will likely require local drug-delivery strategies to achieve drug concentrations that can enhance the radiation response. The safety of such an approach with this drug has not been demonstrated (Ozawa T et al., 1999).

“Phenylacetic acid exposure did not increase the number of tumors caused by known cancer agents in laboratory animals. Data on the potential of phenylacetic acid exposure alone to cause cancer in laboratory animals were not available. The potential for phenylacetic acid to cause cancer in humans has not been assessed by the U.S. EPA IRIS program, the International Agency for Research on Cancer, or the U.S. National Toxicology Program 14th Report on Carcinogens.”

5.7. Irritation/immunotoxicity

Skin, Eye and Respiratory Irritations:

Inhalation: cough, sore throat. Skin: redness. Eyes: redness. Pain

[International Program on Chemical Safety/Commission of the European Communities; International Chemical Safety Card on Phenylacetic acid (June 2006). Available from, as of June 27, 2017: http://www.inchem.org/pages/icsc.html] **PEER REVIEWED**

As taken from HSDB, 2018.

“A rapid screening protocol was used to evaluate the potential immunotoxicity of flavouring ingredients, including phenylacetic acid. The protocol incorporated key elements of the National Toxicology Program’s tier testing strategy, including measurement of body weight, lymphoid organ weight and cellularity, as well as functional tests of the humoral (antibody plaque-forming cells) response to sheep erythrocytes and cell-mediated immunity to Listeria monocytogenes bacterial challenge. Decreases in body weight, spleen and/or thymus weight or a decrease in spleen cellularity may be indicative of depressed immune competence. The number of antibody-producing plasma cells, the result of antigen-driven B-cell differentiation, after immunization with a T-cell-dependent antigen such as sheep red blood cells, provides information about the functional integrity of, and communication among, several cell populations important in antibody-mediated immunity, including T cells, B cells and macrophages. The Listeria model system was selected because the pathogenesis of this infection and the host’s immune response are similar in mice and humans. The model system is useful for assessing immunosuppression since both immunocompetent T cells and macrophages are required to control infection and supply protective immunity. Phenylacetic acid administered orally to groups of 10–20 female CD-1 or $B6C3F_1$ mice, aged 6–8 weeks, at doses as high as 100 mg/kg per day had no effect on spleen weight, thymus weight, spleen cellularity, anti-sheep red blood cell response or Listeria mortality (Vollmuth et al., 1989).”

“Phenylacetic acid was shown to be devoid of immunomodulatory effects in a testing strategy to evaluate the effects of 35 commonly used flavouring ingredients on humoral and cell-mediated immune responses. Female CD-1 or B6C3F1 mice were given phenylacetic acid at a dose of 250, 500 or 1000 mg/kg bw per day intragastrically for 5 days. L. monocytogenes bacterial challenge was conducted to assess cell-mediated immunity and the antibody response to sheep erythrocytes was determined as a measure of humoral immunity. Body weights, lymphoid organ weights and spleen cellularity were also measured. Cyclophosphamide served as a positive control.

Phenylacetic acid did not modulate the cell-mediated or humoral response at any dose tested (Gaworski et al., 1994)."

As taken from WHO Food Additives Series 50, available at http://www.inchem.org/documents/jecfa/jecmono/v50je11.htm

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration into the eye	Rodent - rabbit	100 mg/24H	Moderate	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: OTS0559218

As taken from RTECS, 2017.

"Its acute effect on skin is slight irritation. /from table/[Patty, F. (ed.). Industrial Hygiene and Toxicology: Volume II: Toxicology. 2nd ed. New York: Interscience Publishers, 1963., p. 1839] **PEER REVIEWED**"

As taken from HSDB, 2018.

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Phenyl Acetic 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 Phenyl Acetic 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	19.5	JTI KB Study Report (s)
In vitro cytotoxicity	19.5	JTI KB Study Report (s)

Phenylacetic acid inhibits activity of coenzyme A. [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754] **PEER REVIEWED**

... In this study, inhibitors of Ca2+ ATPase were isolated from ultrafiltrate of patients with end-stage renal failure. They were identified as dimethylguanosine, phenylethylamine, and phenylacetic acid by chromatography and mass spectrometry. Ca2+ ATPase activity was measured spectrophotometrically as the difference in hydrolysis of ATP in the presence and absence of Ca2+ with different concentrations of ATP and the isolated substances. All of the identified compounds are sufficiently lipophilic to penetrate the blood-brain barrier and to accumulate in cerebral tissue. The inhibitory effects of these agents were additive. The apparent K(m) values for ATP and Ca2+ were not altered by these substances, suggesting a noncompetitive mechanism of inhibition. In plasma of healthy subjects, the substances were not detectable. [Jankowski J et al; J Am Soc Nephrol 9 (7): 1249-57 (1998)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018.

“BACKGROUND: The activation of polymorphonuclear leucocytes (PMNLs) causes inflammation and as a result cardiovascular disease, which is a main risk factor for increased morbidity and mortality in patients with chronic kidney disease. Toxins accumulating in uraemic patients play a major role in modulating essential PMNL functions and apoptosis, the latter being crucial for a coordinated resolution of inflammation. One uraemic toxin is phenylacetic acid (PAA). We therefore investigated whether PAA contributes to the deranged immune response in uraemia by modulating PMNL activities.....PAA significantly enhanced the stimulation of PMNL oxidative burst by Escherichia coli, phagocytosis of E. coli by PMNLs and the expression of CD11b and CD18 at the PMNL surface. PAA significantly decreased PMNL apoptosis resulting in an increased percentage of viable cells. PAA affected neither the oxidative burst stimulated by phorbol-12-myristate-13-acetate nor PMNL chemotaxis. CONCLUSIONS: PAA increases the activation of various PMNL functions and the expression of surface activation markers, while it attenuates PMNL apoptotic cell death. Therefore, PAA may contribute to the inflammatory state and consequently to increased cardiovascular risk in uraemic patients.”As taken from Cohen G et al. 2013. Nephrol. Dial. Transplant. 28(2), 421-9. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23229930

“Cranberry consumption has shown prophylactic effects against urinary tract infections (UTI), although the mechanisms involved are not completely understood. In this paper, cranberry phenolic compounds and their potential microbial-derived metabolites (such as simple phenols and benzoic, phenylacetic and phenylpropionic acids) were tested for their capacity to inhibit the adherence of uropathogenic Escherichia coli (UPEC) ATCC53503 to T24 epithelial bladder cells. Catechol, benzoic acid, vanillic acid, phenylacetic acid and 3,4-dihydroxyphenylacetic acid showed anti-adhesive activity against UPEC in a concentration-dependent manner from 100-500 uM, whereas procyanidin A2, widely reported as an inhibitor of UPEC adherence on uroepithelium, was only statistically significant ($p < 0.05$) at 500 uM (51.3% inhibition). The results /show/ the anti-adhesive activity of some cranberry-derived phenolic metabolites against UPEC in vitro, suggesting that their presence in the urine could reduce bacterial colonization and progression of UTI.[de Llano DG et al; Int J Mol Sci 16 (6): 12119-30 (2015)] **PEER REVIEWED*.

NO prevents atherogenesis and inflammation in vessel walls by inhibition of cell proliferation and cytokine-induced endothelial expression of adhesion molecules and proinflammatory cytokines. Reduced NO production due to inhibition of either eNOS or iNOS may therefore reinforce atherosclerosis. Patients with end-stage renal failure show markedly increased mortality due to atherosclerosis. In the present study we tested the hypothesis that uremic toxins are responsible for reduced iNOS expression. LPS-induced iNOS expression in mononuclear leukocytes was studied using real-time PCR. The iNOS expression was blocked by addition of plasma from patients with end-stage renal failure, whereas plasma from healthy controls had no effect. Hemofiltrate obtained

from patients with end-stage renal failure was fractionated by chromatographic methods. The chromatographic procedures revealed a homogenous fraction that inhibits iNOS expression. Using gas chromatography/mass spectrometry, this inhibitor was identified as phenylacetic acid. Authentic phenylacetic acid inhibited iNOS expression in a dose-dependent manner. In healthy control subjects, plasma concentrations were below the detection level, whereas patients with end-stage renal failure had a phenylacetic acid concentration of 3.49 +/- 0.33 mmol/L (n = 41). It is concluded that accumulation of phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. That mechanism may contribute to increased atherosclerosis and cardiovascular morbidity in patients with end-stage renal failure.[Jankowski J et al; J Clin Invest 112 (2): 256-64 (2003)] **PEER REVIEWED**."

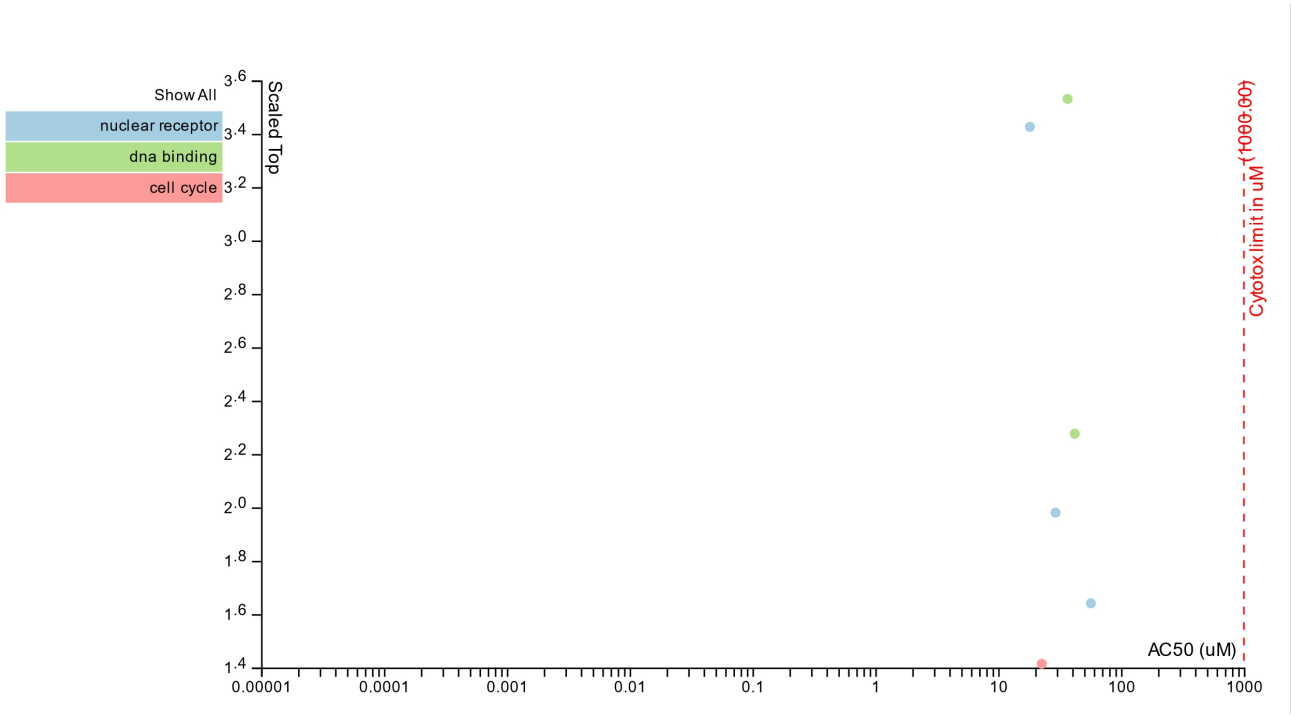
High-throughput Assay Data

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

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

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

Figure 1



"Associated with the inhibition of p21(ras) isoprenylation."

As taken from ChemIDplus.

6. Functional effects on

6.1. Broncho/pulmonary system

Skin, Eye and Respiratory Irritations:

Inhalation: cough, sore throat. Skin: redness. Eyes: redness. Pain

[International Program on Chemical Safety/Commission of the European Communities; International Chemical Safety Card on Phenylacetic acid (June 2006). Available from, as of June 27, 2017: http://www.inchem.org/pages/icsc.html] **PEER REVIEWED**

As taken from HSDB, 2018.

6.2. Cardiovascular system

“Other studies in rodents and humans have investigated the effects of phenylacetic acid on various biochemical parameters, mostly associated with phenylketonuria. Although blood sugar levels were reduced in guinea pigs (12-60%) fed 380-750 mg/kg bw of phenylacetic acid, no effect on glucose leveles was observed over a 2-6h period when a volunteer was fed a 3000 mg dose (ca. 50 mg/kg bw (FDA, 1993))(Stewart, 1962). Phenylacetic acid administered orally to fasted guinea pigs in dose of 375, 500 or 750 mg/kg bw resulted in a reduction in blood sugar of 12%, 44% and 60% of the initial values, respectively, within 2-4h (Stewart, 1962). Rabbits were as sensitive as guinea pigs to the hypoglycemic action of phenylacetic acid (Stewart, 1962). Orally administered phenylacetic acid (62.5 mg/kg bw) also produced a drastic reduction in blood sugar in cats, which was sustained for at least 5h (Stewart, 1962).” As taken from Food and Chemical Toxicology, 43, 2005, pp. 1179-1206

“Tumor necrosis factor (TNF)-α and oxidative stress are considered to play crucial roles in atherosclerosis and vascular calcification. "Uremic toxins" detected in patients with chronic kidney disease (CKD) could cause impaired signal transduction and dysfunction in many organs. Since phenylacetic acid (PAA), identified as one of the uremic toxins, has an inhibiting property of monocytes as well as osteoblastic cells, we examined the effects of PAA on TNF-α secretion and oxidative stress in vascular endothelial cells. In human aortic endothelial cells, TNF-α secretion was assessed after treatment with PAA using an ELISA kit and following the manufacturer's instructions. For determination of reactive oxygen species (ROS), 8-hydroxydeoxyguanosine (8-OHdG) in the culture medium was measured in the presence or absence of PAA. Treatment with PAA in aortic endothelial cells for 24 h significantly stimulated TNF-α secretion in a dose-dependent manner ranging between 0.5 and 5 mM. On the other hand, the 8-OHdG level in the culture medium was significantly increased in the cells incubated with 1 mM PAA for 12 h. To determine if PAA-induced TNF-α secretion is mediated by ROS production, the effect of free radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) was examined. It was found that PAA-induced TNF-α secretion was significantly inhibited by TEMPOL. Our findings indicate that PAA stimulates TNF-α secretion at least in part through ROS production in aortic endothelial cells. The plasma PAA level was reported to be approximately 3.5 mM in end-stage CKD patients, whereas it was <5 μM in healthy subjects; thus, PAA could be involved in the pathological changes of the vasculature in CKD” (Morita et al, 2011).

6.3. Nervous system

“In a study of the effect of various phenyl-substituted acids on glycosyl transferase activity in cerebral spinal fluid (CSF), 1h incubation of human CSF with phenylacetic acid (2-10mM) produced up to 91% inhibition of galactosyl transferase activity utilized in cerebral glycoprotein synthesis. The authors hypothesized taht inhibition of these enzymes may play a key role in pathgenesis of

phenylketonuria (Ko et al., 1973).” As taken from Food anc Chemical Toxicology, 43, 2005, pp. 1179-1206

“BACKGROUND: Resistance to antiepileptic drugs and the intolerability in 20-30% of the patients raises demand for developing new drugs with improved efficacy and safety. Acceptable anticonvulsant activity, good tolerability, and inexpensiveness of docosahexaenoic acid (DHA) make it as a good candidate for designing and development of the new anticonvulsant medications. METHODS: Ten DHA-based molecules were screened based on in silico screening of DHA-like molecules by root-mean-square deviation of atomic positions, the biological activity score of Professional Association for SQL Server, and structural requirements suggested by pharmacophore design. Anticonvulsant activity was tested against clonic seizures induced by pentylenetetrazole (PTZ, 60 mg/kg, i.p.) and tonic seizures induced by maximal electroshock (MES, 50 mA, 50 Hz, 1 ms duration) by intracerebroventricular (i.c.v.) injection of the screened compounds to mice. RESULTS: Among screened compounds, 4-Phenylbutyric acid, 4-Biphenylacetic acid, phenylacetic acid, and 2-Phenylbutyric acid showed significant protective activity in pentylenetetrazole test with ED50 values of 4, 5, 78, and 70 mM, respectively. In MES test, shikimic acid and 4-tert-Butylcyclo-hexanecarboxylic acid showed significant activity with ED50 values 29 and 637 mM, respectively. Effective compounds had no mortality in mice up to the maximum i.c.v. injectable dose of 1 mM. CONCLUSION: Common electrochemical features and three-dimensional spatial structures of the effective compounds suggest the involvement of the anticonvulsant mechanisms similar to the parent compound DHA.” As taken from Gharibi Loron A et al. 2017. Iran. Biomed. J. 21(1), 32-39. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27592363

“Phenylacetic acid (PAA) is the active moiety in sodium phenylbutyrate (NaPBA) and glycerol phenylbutyrate (GPB, HPN-100). Both are approved for treatment of urea cycle disorders (UCDs) - rare genetic disorders characterized by hyperammonemia. PAA is conjugated with glutamine in the liver to form phenylacetyleglutamine (PAGN), which is excreted in urine. PAA plasma levels > /= 500 ug/dL have been reported to be associated with reversible neurological adverse events (AEs) in cancer patients receiving PAA intravenously. Therefore, we have investigated the relationship between PAA levels and neurological AEs in patients treated with these PAA pro-drugs as well as approaches to identifying patients most likely to experience high PAA levels. The relationship between nervous system AEs, PAA levels and the ratio of plasma PAA to PAGN were examined in 4683 blood samples taken serially from: [1] healthy adults [2], UCD patients of > /= 2 months of age, and [3] patients with cirrhosis and hepatic encephalopathy (HE). The plasma ratio of PAA to PAGN was analyzed with respect to its utility in identifying patients at risk of high PAA values. Only 0.2% (11) of 4683 samples exceeded 500 ug/mL. There was no relationship between neurological AEs and PAA levels in UCD or HE patients, but transient AEs including headache and nausea that correlated with PAA levels were observed in healthy adults. Irrespective of population, a curvilinear relationship was observed between PAA levels and the plasma PAA:PAGN ratio, and a ratio > 2.5 (both in ug/mL) in a random blood draw identified patients at risk for PAA levels > 500 ug/mL. The presence of a relationship between PAA levels and reversible AEs in healthy adults but not in UCD or HE patients may reflect intrinsic differences among the populations and/or metabolic adaptation with continued dosing. The plasma PAA:PAGN ratio is a functional measure of the rate of PAA metabolism and represents a useful dosing biomarker.[Mokhtarani M et al; Mol Genet Metab 110 (4): 446-53 (2013)] **PEER REVIEWED**”

As taken from HSDB, 2018.

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

“During chronic kidney disease (CKD), drug metabolism is affected leading to changes in drug disposition. Furthermore, there is a progressive accumulation of uremic retention solutes due to impaired renal clearance. Here, we investigated whether uremic toxins can influence the metabolic functionality of human conditionally immortalized renal proximal tubule epithelial cells (ciPTEC) with the focus on UDP-glucuronosyltransferases (UGTs) and mitochondrial activity.....a wide variety of

uremic toxins, including .....phenylacetic acid.....reduced 7-OHC glucuronidation with more than 30% as compared with controls ($p<0.05$), whereas UGT1A and UGT2B protein expressions remained unaltered.....". As taken from Mutsaers HA et al. 2013. Biochim Biophys Acta 1832(1):142-50, PubMed 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23017367

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 phenylacetic acid 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 phenylacetic acid. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	96	Carmines, 2002 & Rustemeier et al., 2002
	76	Baker et al., 2004a
	6.5 260 390	JTI KB Study Report(s)
	30	Roemer et al., 2014
In vitro genotoxicity	96	Carmines, 2002 & Röemer et al., 2002
	76	Baker et al., 2004c
	6.5	Renne et al., 2006
	6.5 260	JTI KB Study Report(s)
	325	fGLH Study Report (2010)
	30	Roemer et al., 2014
In vitro cytotoxicity	96	Carmines, 2002 & Roemer et al., 2002
	76	Baker et al., 2004c
	6.5 260	JTI KB Study Report(s)
	325	fGLH Study Report (2010)
	30	Roemer et al., 2014
Inhalation study	22	Gaworski et al., 1998
	96	Carmines, 2002 & Vanscheeuwijck et al., 2002
	76	Baker et al., 2004c

	6.5	Renne et al., 2006
	6.5 260	JTI KB Study Report(s)
	30	Schramke et al., 2014
Skin painting	22	Gaworski et al., 1999
	6.5 260	JTI KB Study Report(s)
In vivo genotoxicity	30	Schramke et al., 2014

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Phenyl Acetic 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 Phenyl Acetic 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	19.5	JTI KB Study Report (s)
In vitro cytotoxicity	19.5	JTI KB Study Report (s)

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

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.0046	Labstat International Inc. (2021a)
In vitro genotoxicity	0.0046	Labstat International Inc. (2021b)
In vitro cytotoxicity	0.0046	Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

Phenylacetic acid's production and use as a starting material in the manufacture of synthetic perfumes, condensation products with aldehydes, in the manufacture of penicillin G, as a perfume, fungicide, plant hormone, flavoring agent, and as a lab reagent may result in its release to the environment through various waste streams. Phenylacetic acid occurs naturally in Japanese peppermint oil, neroli oil, in trace amounts in rose oils, in honey, pine sprouts and pine needles. If released to air, a vapor pressure of 3.8X10-3 mm Hg at 25 deg C indicates phenylacetic acid will exist solely as a vapor in the atmosphere. Vapor-phase phenylacetic acid will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 3.6 days. If released to soil, phenylacetic acid is expected to have very high mobility based upon Koc values of 26, 28 and 31. The pKa of phenylacetic acid is 4.31, indicating that this compound will exist almost entirely in the anion form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Volatilization from moist soil surfaces is not expected to be an important fate process as ions do not volatilize. Total biodegradation of 90% in 3 days using an unadapted soil suspension, indicating that biodegradation may be an important environmental fate process in soil. If released into water, phenylacetic acid is not expected to adsorb to suspended solids and

sediment based upon the Koc values. Total biodegradation was 90% in 3 days using an unadapted soil suspension, suggesting that biodegradation may be an important environmental fate process in water. A pKa of 4.31 indicates phenylacetic acid will exist almost entirely in the anion form at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low. Occupational exposure to phenylacetic acid may occur through inhalation and dermal contact with this compound at workplaces where phenylacetic acid is produced or used. Monitoring and use data indicate that the general population may be exposed to phenylacetic acid via ingestion of food and drinking water. (SRC) **PEER REVIEWED**

Artificial Pollution Sources:

Phenylacetic acid's production and use as a starting material in the manufacture of synthetic perfumes, condensation products with aldehydes(1), in the manufacture of penicillin G, as a perfume, fungicide, plant hormone, flavoring agent, and as a lab reagent(2) may result in its release to the environment through various waste streams(SRC). [(1) O'Neil MJ, ed; The Merck Index. 13th ed. Whitehouse Station, NJ: Merck & Co., p.1305 (2001) (2) Lewis RJ Sr; Hawley's Condensed Chemical Dictionary. 14th ed. NY, NY: John Wiley & Sons, p. 859 (2001)] **PEER REVIEWED**

Environmental Fate:

TERRESTRIAL FATE: Based on a classification scheme(1), Koc values of 31, 26, and 28 from Podzol soil, Alfisol soil, and lake sediment, respectively(2), indicate that phenylacetic acid is expected to have very high mobility in soil(SRC). The pKa of phenylacetic acid is 4.31(3), indicating that this compound will exist almost entirely in the anion form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts(4). Volatilization from moist soil surfaces is not expected to be an important fate process due to its ionic form. Phenylacetic acid is not expected to volatilize from dry soil surfaces(SRC) based upon its vapor pressure(5). Total biodegradation was 90% in 3 days using an unadapted soil suspension(6), indicating that biodegradation may be an important environmental fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 23 (1983) (2) von Oepen B et al; Chemosphere 22: 285-304 (1991) (3) Dean JA; Lange's Handbook of Chemistry 13th ed NY, NY: McGraw-Hill, Inc p. 5-53 (1985) (4) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (5) Perry RH, Green D; Perry's Chemical Engineer's Handbook 6th ed NY, NY: McGraw-Hill, Inc p. 3-59 (1984) (6) Leidner H et al; Xenobiotica 10: 47-56 (1980)] **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), Koc values of 31, 26 and 28, determined from Podzol soil, Alfisol soil, and lake sediment, respectively(2), indicate that phenylacetic acid is not expected to adsorb to suspended solids and sediment(SRC). A pKa of 4.31(3) indicates phenylacetic acid will exist almost entirely in the anion form at pH values of 5 to 9(4) and therefore volatilization from water surfaces is not expected to be an important fate process. According to a classification scheme(5), an estimated BCF of 3(SRC), from a log Kow of 1.41(6) and a regression-derived equation(7), suggests the potential for bioconcentration in aquatic organisms is low(SRC). Total biodegradation was 90% in 3 days using an unadapted soil suspension(8), suggesting that biodegradation may be an important environmental fate process in water(SRC). Under anaerobic conditions, phenylacetic acid biodegraded >90% in 60 days using municipal sewage waste water(9). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) von Oepen B et al; Chemosphere 22: 285-304 (1991) (3) Dean JA; Lange's Handbook of Chemistry 13th ed NY, NY: McGraw-Hill, Inc p. 5-53 (1985) (4) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (5) Franke C et al; Chemosphere 29: 1501-14 (1994) (6) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 41 (1995) (7) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (8) Leidner H et al; Xenobiotica 10: 47-56 (1980) (9) Hofmann K, Hammer E; Chemosphere 38: 2561-8 (1999)] **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), phenylacetic acid, which has a vapor pressure of 3.8X10-3 mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase phenylacetic acid is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 3.6 days(SRC), calculated from its rate constant of 4.5X10-12 cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(3). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Perry RH, Green D; Perry's Chemical Engineer's Handbook 6th ed NY, NY: McGraw-Hill, Inc pg 3-59 (1984) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993)] **PEER REVIEWED**

Environmental Biodegradation:

AEROBIC: Phenylacetic acid, as the source of carbon, was readily biodegraded using an unadapted mixed culture (soil suspension): primary degradation was 100 percent in 2 days (in mixture), primary degradation was 100 percent in three days (by GLC), and total degradation was 90 percent in about 3 days (by dissolved organic carbon measurement)(1). Oxygen consumption was approximately 6 ug/mL after six days when phenylacetic acid was degraded in Hudson Collamer silt loam(2). [(1) Leidner H et al; Xenobiotica 10: 47-56 (1980) (2) Subba-Rao RV, Alexander M; J Agric Food Chem 25: 327-9 (1977)] **PEER REVIEWED**

ANAEROBIC: Anaerobic degradation in two Maxey Flats trench leachate samples by a mixed culture of bacteria decreased the concentration of phenylacetic acid by 7 and 50% after 30 days(1). Phenylacetic acid, at a concentration of 6.5 mg/L, was biodegraded to 0.5 mg/L in municipal sewage waste water in 60 days at 26 deg C in a nitrogen atmosphere(2). [(1) Francis AJ et al; Appl Environ Microbiol 40: 108-113 (1980) (2) Hofmann K, Hammer E; Chemosphere 38: 2561-8 (1999)] **PEER REVIEWED**

Environmental Abiotic Degradation:

.../UV IRRADIATION OF AQ SOLN SHOWED THAT PHENYLACETIC ACID WAS DEGRADED/ TO BENZYL ALCOHOL, BENZALDEHYDES & BENZOIC ACIDS /WHICH/ APPEARED ENTIRELY ANALOGOUS TO PHENOXY ACID PHOTOLYSIS. [Kearney, P.C., and D. D. Kaufman (eds.) Herbicides: Chemistry, Degredation and Mode of Action. Volumes 1 and 2. 2nd ed. New York: Marcel Dekker, Inc., 1975., p. 856] **PEER REVIEWED**

The rate constant for the vapor-phase reaction of phenylacetic acid with photochemically-produced hydroxyl radicals has been estimated as 4.5X10-12 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 3.6 days at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). [(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993)] **PEER REVIEWED**

Environmental Bioconcentration:

An estimated BCF of 3 was calculated in fish for phenylacetic acid(SRC), using a log Kow of 1.41(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 41 (1995) (2) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Soil Adsorption/Mobility:

Koc values of 31, 26, and 28 were experimentally determined for phenylacetic acid in three specific soils: a Podzol (pH = 2.8, sand = 89.2%, silt = 8.2%, and clay = 2.6%), an alfisol (pH = 6.7, sand = 69.7%, silt = 14.4%, and clay = 15.9%) and sediment from Lake Constance (pH = 7.1, sand = 5.5%, silt = 58.8%, and clay = 35.7%), respectively(1). According to a recommended classification

scheme(2), these Koc values suggest that phenylacetic acid is expected to have very high mobility in soil(SRC). The pKa of phenylacetic acid is 4.31(3), indicating that this compound will primarily exist in anion form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts(4). [(1) von Oepen B et al; Chemosphere 22: 285-304 (1991) (2) Swann RL et al; Res Rev 85: 23 (1983) (3) Dean JA; Lange's Handbook of Chemistry 13th ed NY, NY: McGraw-Hill, Inc p. 5-53 (1985) (4) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000)] **PEER REVIEWED**

Volatilization from Water/Soil:

A pKa of 4.31(1) indicates phenylacetic acid will exist almost entirely in the anion form at pH values of 5 to 9(2) and therefore volatilization from water surfaces is not expected to be an important fate process. Phenylacetic acid is not expected to volatilize from dry soil surfaces(SRC) based upon its vapor pressure(3). [(1) Dean JA; Lange's Handbook of Chemistry 13th ed NY, NY: McGraw-Hill, Inc p. 5-53 (1985) (2) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (3) Perry RH, Green D; Perry's Chemical Engineer's Handbook 6th ed NY, NY: McGraw-Hill, Inc p. 3-59 (1984)] **PEER REVIEWED**

Environmental Water Concentrations:

OF 10 WATER SUPPLIES SURVEYED BY EPA (1975), ONLY FINISHED WATER OF SEATTLE CONTAINED PHENYLACETIC ACID, AT 4 UG/L. [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754] **PEER REVIEWED**

DRINKING WATER: Phenylacetic acid was found in the drinking water of Ottumwa, Iowa at a concentration of 4 ug/L(1). Phenylacetic acid was listed as a contaminant found in drinking water for a survey of US cities including Cincinnati, OH, New Orleans, LO, Philadelphia, PA, Ottumwa, IA, and Seattle, WA(2). Phenylacetic acid was identified as a ozone disinfection by-product in drinking water(3). [(1) USEPA; Preliminary Assessment of Suspected Carcinogens in Drinking Water. Interim Report to Congress June 1975 Washington, DC (1975) (2) Lucas SV; GC/MS Anal of Org in Drinking Water Concentrates and Advanced Treatment Concentrates Vol 1 USEPA-600/1-84-020A (NTIS PB85-128239) p. 397 (1984) (3) Richardson SD et al; Environ Sci Tech 33: 3368-77 (1999)] **PEER REVIEWED**

GROUND WATER: At a site in Galloway Township, NJ where an underground storage tank filled with gasoline spilled, phenylacetic acid was found at 5 ug/L in ground water 15.5 feet below land surface(1). Phenylacetic acid has been qualitatively identified in ground water contaminated by a landfill in Florida in 1991(2). At a groundwater site contaminated by wood preserving chemicals in Pensacola FL, phenylacetic acid was detected at the following concentrations and depths: 3.94 mg/L at 6.1 m, 1.3 mg/L at 3.3 m, 1.45 mg/L at 5.8 m, and 0.00 mg/L at 11.0 m(3). In a multiple basalt aquifer polluted by organic wastes dumped into unused quarry holes (Werribee Plains, Australia), phenylacetic acid was qualitatively identified(4). Phenylacetic acid was found in ground water samples at 3.3-3.5 mg/L of total organic carbon from superfund sites(5). Ground water samples taken in 1990 from 7 wells down gradient from an oil spill in Bemidji, MN in 1979 had concentrations ranges from <0.015-0.123 uM(6). [(1) Cozzarelli IM et al; Environ Sci Technol 29: 458-69 (1995) (2) Eckel WP et al; Ground Water 31: 801-4 (1993) (3) Goerlitz DF; in Environ Sci Pollut Control Ser 4(Groundwater Contamination and Analysis at Hazardous Waste Sites): 295-355 (1992) (4) Stepan S et al; Australian Water Resources Council Conf Ser 1: 415-24 (1981) (5) Betowski LD et al; Environ Sci Technol 30: 3558-64 (1996) (6) Cozzarelli IM et al; Geochim Cosmochim Acta 58: 863-77 (1994)] **PEER REVIEWED**

SURFACE WATER: Phenylacetic acid was qualitatively identified in Lake Michigan's St Joseph River(1). Phenylacetic acid was identified in Dokai Bay, Japan(2). [(1) Great Lakes Water Quality Board; An Inventory of Chemical Substances Identified in the Great Lakes Ecosystem. Volume I -

Summary. Report to the Great Lakes Water Quality Board. Windsor Ontario, Canada 195 pp. (1983) (2) Terashi A et al; Bull Environ Contam Toxicol 50: 348-55 (1993)] **PEER REVIEWED**

Effluent Concentrations:

Phenylacetic acid has been detected in the effluents of the following industries: iron and steel manufacturing (114 ng/uL extract), organics and plastics (42766 ng/uL extract), textile mills (7 ng/uL extract), pulp and paper (61 ng/uL extract), pharmaceuticals (62677 ng/uL extract), electronics (152 ng/uL extract), organic chemicals (4568 ng/uL extract), mechanical products (4396 ng/uL extract), and publicly-owned treatment works (110 ng/uL extract)(1). Phenylacetic acid has been reported at Superfund Sites 181 times(2). Phenylacetic acid has been qualitatively detected in the effluent from a publicly-owned treatment works facility in Addison, IL in 1980(3). Water soluble fractions of soil samples taken from a controlled landfill site contained phenylacetic acid: 20.6%, 18.7%, and 41.1% at 1-1.5 m, 3-3.5 m, and 6.5-7 m deep, respectively(4). Phenylacetic acid was qualitatively identified in acid fractions of Iona Island, Vancouver, British Columbia, Canada sewage and sludge(5). [(1) Bursey JT, Pellizzari ED; Analysis of Industrial Wastewater for Organic Pollutants in Consent Decree Survey. Contract No 68- 03-2867 Athens, GA USEPA Env Res Lab pg 167 (1982) (2) Eckel WP; Amer Chem Soc Div Environ Chem Preprint Ext Abstr 208th ACS Nat Meet Vol 34: 67-9 (1994) (3) Ellis DD et al; Arch Environ Contam Toxicol 11: 373-82 (1982) (4) Gonzalez-Vila FJ et al; Chemosphere 31: 2817-25 (1995) (5) Rogers IH et al; Water Poll Res J Canada 21: 187-204 (1986)] **PEER REVIEWED**

Phenylacetic acid has been identified in three leachate samples each from two low-level radioactive waste disposal sites: Maxey Flats, KY Trench Leachate; at 0.56, 1.5 and 3.4 mg/L and West Valley, NY Trench Leachate; 3.8, 7.5 and 6.0 mg/L(1). Phenylacetic acid was detected in only one out of three Swedish municipal landfill leachate samples with a suspected origin of pharmaceuticals, cosmetics, soaps, pesticides, insect repellants, disinfectants, food-, leather, and textile-preservatives, and the degradation of lignin(2). In Woolwich landfill leachate (Waterloo, Ontario), phenylacetic acid was detected at 19.6 and 24.6 meters deep at >10+4 and >10+2 ug/L(3). Phenylacetic acid was detected in primary waste treatment effluent and in the acid fraction of domestic sewage treatment effluents(4). Phenylacetic acid was found in leachate from landfill for municipal wastes in Japan at 49.9 ug/L(5). Phenylacetic acid was listed as a contaminant found in advanced waste treatment water for a survey of US cities including Lake Tahoe, CA, Orange County, CA, Dallas, TX, and Blue Plains, Washington, DC(6). [(1) Francis AJ et al; Nuclear Technology 50: 158-63 (1980) (2) Oman C, Hynning PA; Environmental Pollution 80: 265-71 (1993) (3) Reinhard M, Goodman NL; Environ Sci Technol 18: 953-61 (1984) (4) USEPA; Preliminary Assessment of Suspected Carcinogens in Drinking Water. Interim Report to Congress June 1975 Washington, DC (1975) (5) Yasuhara A et al; Kankyo Kagaku 3: 356-7 (1993) (6) Lucas SV; GC/MS Anal of Org in Drinking Water Concentrates and Advanced Treatment Concentrates Vol 1 USEPA-600/1-84-020A (NTIS PB85-128239) p. 397 (1984)] **PEER REVIEWED**

Burn emission rates of phenylacetic acid in mg/g of organic carbon were found to be 0.055 for red oak(1), 0.286 for paper birch(1), 0.130 for white pine(1), 0.047 for balsam(1), 0.044 for yellow poplar(2), 0.055 for mockernut hickory(2), 0.171 for loblolly pine(2), and 0.228 for slash pine(2). Phenylacetic acid was also detected in emissions from red maple(1), hemlock(1), balsam fir(1), white ash(2), and sweet gum(2). [(1) Fine PM et al; Environ Sci Technol 35: 2665-75 (2001) (2) Fine PM et al; Environ Sci Technol 36: 1442-1451 (2002)] **PEER REVIEWED**

Sediment/Soil Concentrations:

SEDIMENT: Phenylacetic acid was detected, not quantified in 5 of 7 sediment samples collected in 1998 from the German Bight; one sample is directly influenced by discharges from the Elbe River, and one from the Weser and Ems rivers. The remaining three sites were situated farther from the coastal areas(1). [(1) Schwarzbauer J et al; Org Geochem 31: 1713-31 (2000)] **PEER REVIEWED**

As taken from HSDB, 2008

The BIODEG database provides the following additional environmental biodegradation data:

CAS #:	103-82-2 PHENYLACETIC ACID
Parameter Type:	Grab Sample
Study Biodeg Eval:	BFA
Rate:	100; 100; 13
Units:	% DEGRADATION
Oxygen Condition:	AEROBIC
Analysis Method:	GC-MS
Incub Time (days):	21; 30; (NS)
Chem Conc (ppm):	93; 4.9; 13.6
Envir Sample Type:	SEWAGE
Sample Source:	MAXEY FLATS, KY
Temp (deg C):	28
Reference:	FRANCIS,AJ (1982)

CAS #:	103-82-2 PHENYLACETIC ACID
Parameter Type:	Grab Sample
Study Biodeg Eval:	BSA
Rate:	-8; 0
Units:	% DEGRADATION
Oxygen Condition:	ANAEROBIC-WATER
Analysis Method:	GC-MS
Incub Time (days) :	60
Chem Conc (ppm) :	2; 13.6
Envir Sample Type:	SEWAGE
Sample Source:	MAXEY FLATS, KY
Temp (deg C) :	28
pH:	6.7

Reference:	FRANCIS,AJ (1982)

CAS #:	103-82-2 PHENYLACETIC ACID	
Parameter Type:	Grab Sample	
Study Biodeg Eval:	BST	
Rate:	.7; 50	
Units:	% DEGRADATION	
Oxygen Condition:	ANAEROBIC-WATER	
Analysis Method:	GC-MS	
Incub Time (days) :	30	
Chem Conc (ppm)	1.4; 3.8	
Envir Sample Type:	SEWAGE	
Sample Source:	MAXEY FLATS, KY - DISPOSAL SITE	
Microbial Pop:	4.1E2; 1.2E4 (CFU/ML)	
Temp (deg C) :	28	
Remarks: TRENCH LEACHATES, ADDITION OF MINERAL SALTS WITHOUT C SOURCES DID NOT SIGNIFICANTLY INCREASE GROWTH OF BACTERIA		
Reference:	FRANCIS,AJ ET AL. (1980)	
CAS #:	103-82-2 PHENYLACETIC ACID	
Parameter Type:	Screening Test	
Study Biodeg Eval:	BF	
Rate:	100	
Units:	% DEGRADATION	
Test Method:	OECD SCREENING	
Oxygen Condition:	AEROBIC	
Analysis Method:	HPLC, GC	
Incub Time (days) :	3	
Chem Conc (ppm) :	8 (C)	
noculum:		

	SOIL
Temp (deg C) :	20
Remarks CONTROL :	MINERAL SALTS, SOIL SUSPENSION, STERILE
Reference:	LEIDNER,H ET AL. (1980)

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

Bond Method :	4.42E-008 atm-m3/mole (4.48E-003 Pa-m3/mole)
Group Method:	Incomplete
Exper Database:	4.10E-08 atm-m3/mole (4.15E-003 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 5.156E-008 atm-m3/mole (5.225E-003 Pa-m3/mole) VP: 0.00388 mm Hg (source: MPBPVP) WS: 1.35E+004 mg/L (source: WSKOWWIN)

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

Log Kow used:	1.41 (exp database)
Log Kaw used:	-5.776 (exp database)
Log Koa (KOAWIN v1.10 estimate):	7.186
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.9382

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):	0.9836 3.2101 (weeks) 3.9732 (days) 0.4736 0.5513 0.6421
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	1.64 Pa (0.0123 mm Hg)
Log Koa (Koawin est):	7.186
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.83E-006 3.77E-006

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	6.61E-005
Mackay model:	0.000146
Octanol/air (Koa) model:	0.000301

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

OVERALL OH Rate Constant =	4.4546 E-12 cm3/molecule-sec
Half-Life =	2.401 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	28.813 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.000106 (Junge-Pankow, Mackay avg) 0.000301 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	29.56 L/kg (MCI method)
Log Koc:	1.471 (MCI method)

Koc :	8.623 L/kg (Kow method)
Log Koc:	0.936 (Kow method)

Experimental Log Koc: 1.45 (database) **Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:** Rate constants can NOT be estimated for this structure! **Volatilization from Water:** Henry LC: 4.1E-008 atm-m3/mole (Henry experimental database)

Half-Life from Model River:	1.666E+004 hours (694.3 days)
Half-Life from Model Lake:	1.819E+005 hours (7578 days)

Removal In Wastewater Treatment:

Total removal:	1.95 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.86 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	0.654	57.6	1000
Water	26	360	1000
Soil	73.3	720	1000
Sediment	0.0796	3.24e+003	0

Persistence Time: 621 hr

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

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Experimental Biodegradation result (%)	100
Reference (biodegradation result)	FRANCIS,A.J.; MICROBIAL TRANSFORMATION OF LOW-LEVEL RADIOACTIVE WASTE.; IN: IAEA-SM-257/72. ENVIRONMENTAL MIGRATION OF LONG-LIVED RADIONUCLIDES. VIENNA,AUSTRIA: INTER. ATOMIC ENERGY AGENCY.; 1982

Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.5513
TOPKAT probability of biodegradation	1
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	2.401

ROBUST STUDY SUMMARY - Persistence

1.1.1 Item	**Yes**	**No**
Reference: Leidner, H., R. Gloor, D. Wuest and K. Wuhrmann (1980). The influence of the sulphonic group on the biodegradability of n-alkylbenzene sulphonates. Xenobiotica. 10(1):47-56.		
Test Substance (CAS # and name): **1103-82-2; Benzeneacetic acid**		
Chemical composition of the substance (including purity, by-products)		X
Method		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	n/a	
Test design / conditions		
Study type (photodegradation, hydrolysis, biodegradation, other –specify, do **not** assess):**Biodegradation**		
Test type (aerobic or anaerobic - specify, do **not** assess):**Aerobic**		
Test medium (air, water, soil, sediment - specify, do **not** assess):**Water**		
Information on stability of the substance in the media of concern reported? n/a		
Information on controls (positive or negative)		X
Number of replicates (including controls) No replicates or controls mentioned		X
Temperature **20ºC**	X	
Duration of the experiment **28 days**	X	
Analytical method/technique used (Y/N)	n/a	
For photodegradation only		
Light source (specify):		
Light spectrum and relative intensity based on sunlight intensity:		

For hydrolysis only		
Measured concentrations reported?		
Basic water properties (pH, hardness, etc.)		
For biodegradation only		
Ready or inherent biodegradation (specify)		
Substance concentration (Y/N)	X	
Innoculum source	X	
Innoculum concentration **or** number of microorganisms (Y/N) 2 ml of mixed microbial biocenosis (dilution of a suspension of garden soil)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (do **not** assess this item): 100% degradation		
Information on breakdown products (do **not** assess this item): No		
Overall Score: **7/10 = 70%**		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): **Satisfactory**		
Comments:		

Item	**Yes**	**No**
Reference: FRANCIS,A.J.; MICROBIAL TRANSFORMATION OF LOW-LEVEL RADIOACTIVE WASTE.; IN: IAEA-SM-257/72. ENVIRONMENTAL MIGRATION OF LONG-LIVED RADIONUCLIDES. VIENNA,AUSTRIA: INTER. ATOMIC ENERGY AGENCY.; 1982		
Test Substance (CAS # and name): 103822, Phenylacetic acid		
Chemical composition of the substance (including purity, by-products)		x
Method		
References	X	
OECD, EU, national, or other standard method?		X
Justification of the method/protocol if not a standard method was used		X
Test design / conditions		
Study type (photodegradation, hydrolysis, biodegradation, other –specify, do **not** assess):biodeg		
Test type (aerobic or anaerobic - specify, do **not** assess):aerobic		
Test medium (air, water, soil, sediment - specify, do **not** assess):water		
Information on stability of the substance in the media of concern is reported?		X
Controls (positive or negative)	X	
Number of replicates (including controls)	X	
Temperature room temp 28 C	X	

Duration of the experiment **21 days**	X	
For photodegradation only		
Light source (specify):	n/a	
Light spectrum and relative intensity based on sunlight intensity:	n/a	
For hydrolysis only	n/a	
Measured concentrations reported?	n/a	
Basic water properties (pH, hardness, etc.)	n/a	
For biodegradation only		
Ready or inherent biodegradation (specify):inherent	x	
Innoculum (concentration and source)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (do **not** assess this item):100% degradation in aerobic conditions, only 8% degradation under anaerobic conditions		
Information on breakdown products (do **not** assess this item)		x
Overall score: **7/11 = 64%**		
EC Reliability code: 2		
Reliability category (high, satisfactory, low):satisfactory		
Comments: Substance is likely to be persistent under anaerobic conditions, however the paper reported that it may also be persistent under certain aerobic conditions, depending on the leachate.		

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

10.2. Aquatic toxicity

EC50; Species: Xenopus laevis (African clawed frog, embryo); Conditions: freshwater, renewal, pH 7.0-7.8; Concentration: 801900 ug/L for 96 hr (95% confidence interval: 761000-845000 ug/L); Effect: developmental changes (craniofacial defects, abnormal gut coiling) /> or = 98% purity/ [Dawson DA et al; Teratog Carcinog Mutagen 16 (2): 109-24 (1996) Available from, as of June 15, 2007: http://cfpub.epa.gov/ecotox/quick_query.htm] **PEER REVIEWED**

LC50; Species: Xenopus laevis (African clawed frog, embryo); Conditions: freshwater, renewal, pH 7.0-7.8; Concentration: 1273700 ug/L for 96 hr (95% confidence interval: 1252000-1296000 ug/L) /> or = 98% purity/ [Dawson DA et al; Teratog Carcinog Mutagen 16 (2): 109-24 (1996) Available from, as of June 15, 2007: http://cfpub.epa.gov/ecotox/quick_query.htm] **PEER REVIEWED**

EC50; Species: Daphnia magna (Water flea); Conditions: freshwater, static, pH 8; Concentration: 11 mg/L for 24 hr; Effect: behavior, equilibrium [Bringmann G, Kuhn R; Zeitschrift fuer Wasser und Abwasser Forschung 15 (1): 1-6 (1982) Available from, as of June 15, 2007: http://cfpub.epa.gov/ecotox/quick_query.htm] **PEER REVIEWED**

AQUATIC SPECIES/ Lepomis cyanellus (Green sunfish); Conditions of the experiment: 67-168 mm fish exposed for 0.08 to 0.25 hr at 22.8 deg C in a static chamber with dissolved O2 of 4.5 mg/L and ph of 7.2 to 5 mg/L of phenylacetic acid. Effect: No avoidance seen. [European Chemicals Bureau; IUCLID Dataset, Phenylacetic acid (103-82-2) (2000 CD-ROM edition). Available from, as of August 1, 2007: http://ecb.jrc.it/esis/esis.php] **PEER REVIEWED**

As taken from HSDB, 2008

AQUATIC ORGANISMS

Acute/Prolonged Toxicity to Fish

Type:	static
Species:	other: Lepomis cyanellus
Remark:	Effect: No avoidance seen at 5 mg/l test substance.
Source:	ELF ATOCHEM UK Ltd WIDNESS(12)
Test condition:	Age: 108.4 mm, 67–168 mm Exposure regime: 0.08 h to 0.25 h Temperature: 22.8 degree C Dissolved O2: 4.5 mg/l pH: 7.2

As taken from IUCLUD Dataset (2000), Phenylacetic acid (103-82-2).

The acute toxicity of phenylacetic acid to the algae Scenedesmus subspicatus, and the crustaceans Daphnia magna and Thamnocephalus platyurus is 177, 140 and 277 mg/L, respectively (++íepi-+¼ et al., 2003). Based on these data, the PNEC for aquatic species is 140 µg/L applying an assessment factor of 1000 (cited in EFSA, 2012).

Record for phenylacetic acid:

Spec. Sci. Name **Spec. Common Name**	**Exp. Type** **Chem. Anal.**	**Media Type** **Loc**	**Resp. Site** **Obs. Dur. (Days)**	**Endpoint** **BCF**	**Trend** **Eff%**	**Effect** **Effect Meas.**	**Conc (Standardized)** **Appl. Rate**
Xenopus laevis	R	FW	MT/	EC50	INC	DVP	F 801900 (761000-845000) ug/L
African Clawed Frog	U	LAB	4			GDVP/	
Xenopus laevis	R	FW		LC50	INC	MOR	F 1273700 (1252000-1296000) ug/L
African Clawed Frog	U	LAB	4			MORT	

Daphnia magna		FW		EC0		BEH	F 780 ug/L
Water Flea			1			EQUL	
Daphnia magna		FW		EC100		BEH	F 100000 ug/L
Water Flea			1			EQUL	
Daphnia magna		FW		EC50		BEH	F 11000 ug/L
Water Flea			1			EQUL	
Leuciscus idus ssp. melanotus		FW		LC0	INC	MOR	F 11000 ug/L
Carp			2			MORT	
Leuciscus idus ssp. melanotus		FW		LC0	INC	MOR	F 5500 ug/L
Carp			2			MORT	
Leuciscus idus ssp. melanotus		FW		LC100	INC	MOR	F 22000 ug/L
Carp			2			MORT	
Leuciscus idus ssp. melanotus		FW		LC100	INC	MOR	F 27000 ug/L
Carp			2			MORT	
Leuciscus idus ssp. melanotus		FW		LC50	INC	MOR	F 13000 ug/L
Carp			2			MORT	
Leuciscus idus ssp. melanotus		FW		LC50	INC	MOR	F 17000 ug/L
Carp			2			MORT	
Lepomis cyanellus	S	FW			NEF	AVO	F 5000 ug/L
Green Sunfish	U	LAB	(0.003 - 0.01)			CHEM	
Lepomis macrochirus	S	FW			NEF	NOC	F 5000 ug/L
Bluegill	U	LAB	1			MULT/	
Oncorhynchus	S	FW			NEF	NOC	F 5000 ug/L

mykiss							
Rainbow Trout	U	LAB	1			MULT/	
Petromyzon marinus	S	FW			NEF	NOC	F 5000 ug/L
Sea Lamprey	U	LAB	1			MULT/	

As taken from US EPA ECOTOX database

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

Pivotal value for iT (mg/l)	801.9
Experimental result iT (mg/l)	801.9
Test species iT (Latin)	Xenopus laevis
Test species iT (Common)	Clawed toad
Final EndPoint iT	EC50
Exposure duration iT (hours)	96
Reference iT	Teratog.Carcinog.Mutagen.16:109-124; Teratology 51(3):174 (1995) (ABS) (Author Communication Used)
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	3,465.954
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	791.1558
Toxicity to fish (LC50 in mg/l) as predicted by Aster	359.400125
Toxicity to fish (LC50 in mg/l) as predicted by PNN	29.95912
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	23.1
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	573.446
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	142.7
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	3.47E+000

ROBUST STUDY SUMMARY - <u>Inherent Toxicity</u>

Item	**Yes**	**No**
Reference: Dawson, D.A., T.W. Schultz and R.S. Hunter. 1996. Development Toxicity of Carboxylic Acids to Xenopus Embryos: A Quantitative Structure-Activity Relationship and Computer-Automated Structure Evaluation. Teratogenesis, Carcinogenesis and Mutagenesis, 16:109-124.		
<u>Test Substance</u> (CAS # and name): 103-82-2; Phenylacetic acid		
***Chemical composition of the substance (including purity, by-products)**	**X**	
Persistence/stability of test substance in aquatic solution		X
<u>Method</u>		

References	X	
***OECD, EU, national, or other standard method**?	**X**	
Justification of the method/protocol if not a standard method was used		
***GLP (Good Laboratory Practice)**	**n/a**	**n/a**
Test organisms (specify common and Latin names): clawed toad (Xenopus laevis)		
Latin or both Latin and common names reported?	X	
Life cycle age / stage of test organism	X	
Sex	n/a	n/a
Length and weight of test organisms	n/a	n/a
Number of test organisms per replicate	X	
Food type / feeding periods (acclimation/during test)	X	
Test design / conditions		
Test type – acute or chronic (specify, but do **not** assess this item): acute		
Experiment type (laboratory or field) specified?	X	
System type (static, semi-static, flow through)?	X	
Negative or positive controls (specify)? Negative control	X	
Number of replicates (including controls) and concentrations		X
Exposure pathways (food, water, both)	X	
Exposure duration	X	
***Measured concentrations reported?**		**X**
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	X	
Was pH within 6-9 range? (do **not** assess this item)	X	
Was temperature within 5-28 °C range? (do **not** assess this item)	X	
Photoperiod and light intensity		X
Stock and test solution preparation	X	
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances)		X
Analytical monitoring intervals		X

Statistical methods used	X	
Results		
Toxicity values (LC_{50}, EC_{50}, or IC_{50} - specify, do **not** assess this item): EC_{50} = 801.9 mg/L (96hr); LC_{50} = 1273.7 mg/L (96hr)		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, do **not** assess this item): No		
***Was toxicity value below the chemical's water solubility**?	**X**	
Other adverse effects (carcinogenicity, mutagenicity, etc. Do **not** assess this item)		X
Score: major items – 3/**4**; overall score – 16/**22 (73%)**		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): satisfactory		
Comments: One major item reported "no"; overall score 73%. Methods – "Standard guide for conducting the Frog Embryo Teratogenesis Assay: Xenopus (FETAX), ASTM E1439-91 (1991)". Chemical purity – '>= 98%'. Reasonable documentation of organism and test conditions. Test concentration below chemical's water solubility (6422 mg/L). EC_{50} = 'malformation'. Satisfactory confidence; reliability code = 2.		

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

"This study was undertaken to isolate active secondary metabolites from immunostimulatory Alcaligenes faecalis FY-3 and evaluate their activities using grass carp Ctenopharyngodon idella kidney (CIK) cells. By applying chromatography techniques and successive recrystallization, three purified metabolites were obtained and identified by spectral data (mass spectrometry and nuclear magnetic resonance) as: (1) phenylacetic acid, (2) p-hydroxyphenylacetylamide and (3) cyclo-(Gly-(L)-Pro). CIK cells were stimulated by different concentrations (1, 10 and 100 μg/ml) of the isolated compounds, and expression of MyD88, IL-1β, TNF-α, type I-IFN and IL-8 genes at different time points (2, 8 and 24 h) post-stimulation was quantified by real-time PCR. The known immunostimulatory agent lipopolysaccharide (LPS) was used as a positive control. To analyse whether these compounds are toxic to the cells, the methyl tetrazolium assay was employed to measure changes in cell viability. The obtained results revealed that transcribing level of MyD88, an important adaptor molecule in toll-like receptor signalling pathway, was augmented remarkably by all the three isolated compounds and LPS as early as 2-h exposure. These compounds also induced gene expression of cytokines such as IL-1β, TNF-α and type I-IFN. Under the experimental conditions, none of the test compounds is toxic to the CIK cells. These findings demonstrate that the immunostimulatory properties of the three metabolites [phenylacetic acid, p-hydroxyphenylacetylamide and cyclo-(Gly-(L)-Pro)] from A. faecalis FY-3 in CIK cells and highlight the potential of using these metabolites as immunostimulants in fish aquaculture." As taken from Wu ZF et al. 2012. Scand. J. Immunol. 76(2), 131-40. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22606987?dopt=AbstractPlus

"BACKGROUND: P. AERUGINOSA IS KNOWN TO CAUSE ACUTE CYTOTOXICITY AGAINST VARIOUS HUMAN AND ANIMAL CELLS AND TISSUES.....MICROARRAY ANALYSIS REVEALED THAT TREATMENT OF P. AERUGINOSA WITH PAA....(PHENYLACETIC ACID)....DOWN-REGULATED THE TRANSCRIPTIONAL EXPRESSION OF TYPE III SECRETION SYSTEM (T3SS) GENES AND RELATED REGULATORY GENES INCLUDING RSMA AND VFR, WHICH WERE CONFIRMED BY TRANSCRIPTIONAL AND TRANSLATIONAL ANALYSIS. CONCLUSIONS: IDENTIFICATION OF BACTERIAL METABOLITE PAA AS A T3SS-SPECIFIC INHIBITOR EXPLAINS THIS INTRIGUING INVERSE CELL-DENSITY-DEPENDENT-

CYTOTOXICITY PHENOMENON AS T3SS IS KNOWN TO BE A KEY VIRULENCE FACTOR ASSOCIATED WITH CYTOTOXICITY AND ACUTE INFECTION. THE FINDINGS MAY PROVIDE USEFUL CLUES FOR DESIGN AND DEVELOPMENT OF NEW STRATEGIES TO COMBAT THIS FORMIDABLE BACTERIAL PATHOGEN." AS TAKEN FROM WANG J ET AL. 2013. PLOS ONE 8(3), E60187. PUBMED, 2013 AVAILABLE AT http://www.ncbi.nlm.nih.gov/pubmed/23555919

ECOSAR Version 1.11 provides the following aquatic toxicity data for CAS RN 103-82-2:

Values used to Generate ECOSAR Profile Log Kow: 1.428 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.66E+004 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations Not Related to an Existing ECOSAR Class Definition

Estimates provided below use the Neutral Organics QSAR equations which represent baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
--> Acid moeity found: Predicted values multiplied by 10				
Neutral Organics-acid :	Fish	96-hr	LC50	3647.344
Neutral Organics-acid :	Daphnid	48-hr	LC50	1964.516
Neutral Organics-acid :	Green Algae	96-hr	EC50	1176.479
Neutral Organics-acid :	Fish		ChV	334.996
Neutral Organics-acid :	Daphnid		ChV	165.411
Neutral Organics-acid :	Green Algae		ChV	273.930
Neutral Organics-acid :	Fish (SW)	96-hr	LC50	4576.048
Neutral Organics-acid :	Mysid	96-hr	LC50	5009.048
Neutral Organics-acid :	Fish (SW)		ChV	350.350
Neutral Organics-acid :	Mysid (SW)		ChV	512.885

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for phenylacetic acid:

Spec. Sci.	Resp.	Media	Exp.	Dose#	Endpoint	Effect	Signif.	Dose

Name Spec. Common Name	Type Exp. Dur. (Days)	Type Test Loc.	Site Chem. Anal.	Res. Sample Unit	BAF/BCF	Effect Meas.	Sig. Level	Dose Stat. Meth.
Flowers, Trees, Shrubs, Ferns								
Triticum aestivum	COL	AQU	HP	2	LOEL	GRO	ASIG	F 0.26 mg/L
Bread Wheat	1	LAB	U			AREA		
Triticum aestivum	COL	AQU	HP	2	LOEL	GRO	ASIG	F 0.26 mg/L
Bread Wheat	1	LAB	U			AREA		
Watsonia gladioloides	RO	CUL	DA	3	NOEC	DVP	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			FORM	<0.05	
Watsonia lepida	RO	CUL	DA	3	NOEC	DVP	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			FORM	<0.05	
Watsonia vanderspuyae	RO	CUL	DA	3	NOEC	DVP	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			FORM	<0.05	
Watsonia gladioloides		CUL	DA	3	NOEC	MOR	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			SURV	<0.05	
Watsonia lepida		CUL	DA	3	NOEC	MOR	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			SURV	<0.05	
Watsonia laccata		CUL	DA	3	NOEC	MOR	ANOSIG	F 1 mg/L
Waxy Bugle Lily	60	LAB	U			SURV	<0.05	
Watsonia vanderspuyae		CUL	DA	3	NOEC	MOR	ANOSIG	F 1 mg/L
Bugle Lily	60	LAB	U			SURV	<0.05	
Watsonia laccata	RO	CUL	DA	3	NOEC/	DVP	ANOSIG	F 1 mg/L

Waxy Bugle Lily	60	LAB	U			FORM	<0.05	
Triticum aestivum	SS		FS	2	NOEL	CEL	ANOSIG	F 10 mg/L
Bread Wheat	14	FIELDN	U			NCEL		
Triticum aestivum	SS		FS	2	NOEL	GRO	ANOSIG	F 10 mg/L
Bread Wheat	14	FIELDN	U			AREA		
Pisum sativum	SS	CUL	SO			GRO		NC 1 1e-3 M
Pea	1	LAB		SL		SIZE		
Triticum aestivum	COL	CUL	SO			GRO		NC 1 1e-3 M
Bread Wheat	1	LAB		SL		SIZE		
Pisum sativum	SS	CUL	SO			GRO		NC 1 1e-3 M
Pea	1	LAB		SL		SIZE		
Triticum aestivum	COL	CUL	SO			GRO		NC 1 1e-3 M
Bread Wheat	1	LAB		SL		SIZE		
Pisum sativum	SS	CUL	SO			GRO		NC 1 1e-3 M
Pea	1	LAB		SD		SIZE		NC 1 1e-5 M
Avena sp.	COL	CUL	SO			GRO		NC 1 1e-6 M
Oat	1	LAB		SL		SIZE		
Pisum sativum	SS	CUL	SO			INJ		NC 1 1e-3 M
Pea	1	LAB		SL		CURV		
Pisum sativum	SS	CUL	SO			INJ		NC 1 1e-3 M
Pea	1	LAB		SL		CURV		
Helianthus	SS		PT			INJ		NC 0.2

annuus								%
Common Annual Sunflower	12	LAB		VG		TUMR		
Helianthus annuus	LE		PT			PHY		NC 0.2 %
Common Annual Sunflower	1	LAB		VG		NAST		
Helianthus annuus	BD		PT			PHY		NC 1 %
Common Annual Sunflower	0	LAB		SL		DORB		
Rosa sp.	SO		SP			POP		NC (0.01-0.05) M
Rose	21	LAB		MT		ABND		
Flowers, Trees, Shrubs, Ferns; Standard Test Species								
Lactuca sativa		AGR			EC50	REP		NC 0.46 mol/m3
Lettuce	3					GERM		
Lactuca sativa		AGR			EC50	REP		NC 0.46 mmol
Lettuce	3					GERM		
Avena sativa	RO	CUL	SO			GRO		NC 1 1e-4 M
Common Oat	0.83	LAB		SL		SIZE		
Phaseolus vulgaris	RO		SO			POP		NC 1000 ppm
Bean	6.12			SL		ABND		

As taken from US EPA ECOTOX database

ECOSAR Version 1.11 provides the following terrestrial toxicity data for CAS RN 103-82-2:

Values used to Generate ECOSAR Profile Log Kow: 1.428 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.66E+004 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations Not Related to an Existing ECOSAR Class Definition

Estimates provided below use the Neutral Organics QSAR equations which represent baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
--> Acid moeity found: Predicted values multiplied by 10				
Neutral Organics-acid :	Earthworm	14-day	LC50	2713.299

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information:

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):	-0.9300 days (HL = 0.1175 days)
Log BCF Arnot-Gobas method (upper trophic):	0.486 (BCF = 3.062)
Log BAF Arnot-Gobas method (upper trophic):	0.486 (BAF = 3.062)
log Kow used:	1.41 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzeneacetic acid is not bioaccumulative in the environment.

Empirical Log Kow	1.41
Log Kow predicted by KowWin	1.43
Log BAF T2MTL predicted by Gobas	0.408294836552445
Log BCF 5% T2LTL predicted by Gobas	0.354072522767904
Log BCF Max predicted by OASIS	1.41560068912817
Log BCF predicted by BCFWIN	0.5

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

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13. Last audited

April 2022

I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 103–82–2
CAS No.	103–82–2
EINECS Name	phenylacetic acid
EINECS No.	203–148–6
Molecular Weight	136.1
Structural Formula	C6H5CH2COOH
Molecular Formula	C8H8O2

Dataset created by: EUROPEAN COMMISSION – European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non–confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

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European Chemicals Bureau

1.0.1 OECD and Company Information

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1.0.2 Location of Production Site

–

1.0.3 Identity of Recipients

–

1.1 General Substance Information

Substance type: organic
Physical status: solid

1.1.1 Spectra

–

1.2 Synonyms

alpha toluic acid ; phenyl acetic acid ; acetic acid, phenyl ; benzeacetic acid.
Source: Calaire Chimie S.N.C. Calais

Phenylethanoic acid, toluic acid, benzene acetic acid.
Source: ELF ATOCHEM UK Ltd WIDNESS

1.3 Impurities

–

1.4 Additives

–

1.5 Quantity

–

1.6.1 Labelling

–

1.6.2 Classification

–

1.7 Use Pattern

–

1.7.1 Technology Production/Use

–

1.8 Occupational Exposure Limit Values

Type of limit: other: VME
Limit value: 10 mg/m3
Country: France.
Remark: Dust value.
Source: ELF ATOCHEM UK Ltd WIDNESS

(1)

Type of limit:
Limit value:
Remark: Toxicologie : ingestion (oral, rat) = 2250 mg/kg. Occulaire : irritant. Cutanée : irritant. EPA TSCA section 8b. Application : – cosmétologie, fixateur de parfums ; – chimie: fabrication pénicilline.
Source: Calaire Chimie S.N.C. Calais

1.9 Source of Exposure

Remark: Additional information:

– An overview of monitoring data in the environment:
Emission to atmosphere from two point sources associated with production units were measured at 0.5mg/m3 (max) mean 0.3mg/m3 during 1994. This forms part of the ongoing monitoring programme.
Emission to water from the process total 88.4 tonnes/annum approximately.

– Other information that will help to focus the assessment of exposure:

The UK Authorities have so far judged phenylacetic acid as a Class "B" "VOC" within the terms of Integrated Pollution Control.
This is a malodorous substance. Off site odour is the primary environmental concern. The odour threshold is undoubtedly very low but is not quantified. Elf Atochem is obliged at Widnes as part of an Improvement Plan under Integrated Pollution Control, to determine the odour threshold by the end of 1996. Note also that evaluation of the point source dispersion characteristics, abatement stack efficiencies and heights, and fugitive emissions are currently in hand as part of the same programme.

The occupationnal hygiene control regime is targeted mainly at assuring high standards of personal cleanliness to prevent contamination of operators.
Environmental exposure during production and use:

- Distribution pattern:
  from production: 10% to air
  45% to water
  15% to soil
  30% to sediment
  from formulation: not know.

- Primary exposed environment: river and ground water

- Release pattern: point source, diffuse, intermittent.

General use pattern: Consumer use: 0%
Professional use: 100%
Professional occupational exposure during production:

- Process description: reaction of aqueous sodium phenylacetate with hydrochloric acid.
- Number of sites: 1
- Number of workers: 30
- Frequency and duration of such exposure: intermittent during the course of 8 to 12 hour shifts. Primary exposure occurs during flaking and packaging of the product. These activities give rise to exposure to phenylacetic acid dust. Exposure is a low order on a time weighted average basis.

-Protective measures: gloves, goggles, oversuit, helmet, breathing apparatus for emergency use, disposable respirators (HSE approved) worn by operators routinely.

- Industrial hygiene monitoring: limited data available; no personal exposure monitoring; no ongoing monitoring programme, one survey of background dust concentrations; in the working area gave representative results well below 1mg/m3 on a shift weighted average basis.

Source: ELF ATOCHEM UK Ltd WIDNESS

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

Remark: Transport information: not regulated.
Source: ELF ATOCHEM UK Ltd WIDNESS

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2.1 Melting Point

Value: = 76.5 degree C
Method: other
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(2)

Value: 77 – 85 degree C
Method: other
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(3)

2.2 Boiling Point

Value: = 265.5 degree C at 1013 hPa
Method: other
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(4)

2.3 Density

Type: density
Value: = 1.091 g/cm3 at 77 degree C
Method: other
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(2)

2.3.1 Granulometry

–

2.4 Vapour Pressure

Value: ca. 1.33 hPa at 97 degree C
Method: other (measured)
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(5)

2.5 Partition Coefficient

log Pow: = 1.41
Method: other (measured)
Year:
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(6)

log Pow: = 1.41
Method:
Year:
Source: ELF ATOCHEM UK Ltd WIDNESS
(7)

log Pow: = 1.51
Method:
Year:
Source: ELF ATOCHEM UK Ltd WIDNESS
(8)

2.6.1 Water Solubility

Value: = 16.6 mg/l at 20 degree C
Qualitative: slightly soluble
Method: other
GLP: no data
Remark: Freely soluble in hot water
Source: ELF ATOCHEM UK Ltd WIDNESS
(4)

2.6.2 Surface Tension

-

2.7 Flash Point

Value: > 100 degree C
Type:
Method: other
Year:
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(9)

Value: = 132 degree C
Type:
Method: other
Year:
GLP: no data
Source: ELF ATOCHEM UK Ltd WIDNESS
(3)

2.8 Auto Flammability

-

2.9 Flammability

Result:
Remark: Materials that must be preheated before ignition can occur.
Source: ELF ATOCHEM UK Ltd WIDNESS
(9)

2.10 Explosive Properties

-

2.11 Oxidizing Properties

-

2.12 Additional Remarks

Remark: Reactivity: materials wich (in themselves) are normally stable even under fire exposure conditions.
not reactive with water.
Source: ELF ATOCHEM UK Ltd WIDNESS
(9)

Remark: Solubilities: Soluble in alcohol, ether, chloroform, and other chlorinared hydrocarbons.

Dissociation constant: K at 25°C = 5.56 10exp-5
Very soluble in carbon disulfide.
Source: ELF ATOCHEM UK Ltd WIDNESS
(4) (10)

Remark: Refractive index (20°C) = 1.5049

Vapour density: (air=1) = 4

Phenylacetic acid decomposes in a fire releasing toxic fumes of carbon monoxide and carbon dioxide.
Source: ELF ATOCHEM UK Ltd WIDNESS
(3)

3.1.1 Photodegradation

Type:
Method:
Year: **GLP:**
Test substance:
Remark: UV irradiation of aqueous solutions showed that phenylacetic acid was degraded to benzyl alcohol, benzaldehydes and benzoic acids.Analogous to phenoxy acid photolysis.
Source: ELF ATOCHEM UK Ltd WIDNESS

(11)

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2 Monitoring Data (Environment)

Type of measurement:
Medium:
Source: ELF ATOCHEM UK Ltd WIDNESS

3.3.1 Transport between Environmental Compartments

Type:
Media:
Method:
Year:
Source: ELF ATOCHEM UK Ltd WIDNESS

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

-

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

-

3.8 Additional Remarks

–

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: other: Lepomis cyanellus
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Effect: No avoidance seen at 5 mg/l test substance.
Source: ELF ATOCHEM UK Ltd WIDNESS
Test condition: Age: 108.4 mm, 67–168 mm
Exposure regime: 0.08 h to 0.25 h
Temperature: 22.8 degree C
Dissolved O2: 4.5 mg/l
pH: 7.2

(12)

4.2 Acute Toxicity to Aquatic Invertebrates

Species:
Exposure period: 24 hour(s)
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Source: ELF ATOCHEM UK Ltd WIDNESS

4.3 Toxicity to Aquatic Plants e.g. Algae

–

4.4 Toxicity to Microorganisms e.g. Bacteria

–

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

–

4.5.2 Chronic Toxicity to Aquatic Invertebrates

–

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

–

4.6.2 Toxicity to Terrestrial Plants

–

4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

–

4.7 Biological Effects Monitoring

–

4.8 Biotransformation and Kinetics

–

4.9 Additional Remarks

–

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 5000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance:
Remark: A value 2250 mg/kg is given by a Russian author. However he gave exactly the same values for acute orale LD50 in mice and guinea pig (cited by RTECS). Consequently these values are questionable, taking into account the other figures found in scientific papers (5141, 5142, 5143).
Source: ELF ATOCHEM UK Ltd WIDNESS

(13)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: > 5000 mg/kg bw
Method: other
Year: 1975 **GLP:** no data
Test substance:
Source: ELF ATOCHEM UK Ltd WIDNESS

(13)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 1600 mg/kg bw
Method:
Year: 1973 **GLP:** no data
Test substance:
Remark: Calculation by Litchfield and Wilcoxon’s method; 6–8 animals per dose; 8 days observations.
Source: ELF ATOCHEM UK Ltd WIDNESS

(14)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 2270 mg/kg bw
Method:
Year: 1958 **GLP:** no data
Test substance: no data
Source: ELF ATOCHEM UK Ltd WIDNESS

(15)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: = 1500 mg/kg bw
Method:
Year: 1958 **GLP:** no
Test substance: no data
Source: ELF ATOCHEM UK Ltd WIDNESS

(16)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

-

5.2.2 Eye Irritation

-

5.3 Sensitization

-

5.4 Repeated Dose Toxicity

Species:	rat **Sex:** no data
Strain:	Fischer 344
Route of admin.:	other: S.C and I.P
Exposure period:	28 days
Frequency of treatment:	continuous s/c release by osmotic minipump and i.p injection once day
Post. obs. period:	0 day
Doses:	550 mg/kg/d (s/c); 300 mg/kg/d (i.p)
Control Group:	yes, concurrent vehicle
Method:	other
Year:	1994 **GLP:** no data
Test substance:	no data
Remark:	– 50 rats were used. – treatment with phenylacetate extented survival when started on the day of tumor inoculation (P = 0.01) or 7 days after (P =0.03), without adverse effects. Phenylacetate levels in serum after 7 days was in the therapeutic range as determined in vitro: 2.45 mM (ca 333.2 mg/l) and 3.1 mM in cerebrospinal fluid. Electron microscopy demonstrated marked hypertrophy and organization of the rough endoplasmic reticulum, indicating cell differenciation. In addition in vitro studies demonstrated dose-dependent inhibition of the rate of tumor proliferation and restoration of anchorage dependency, a marker of phenotypic reversion. Route of administration: i.p + sc.
Source:	ELF ATOCHEM UK Ltd WIDNESS

(17)

5.5 Genetic Toxicity 'in Vitro'

-

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain:
Route of admin.: oral feed
Exposure period: 21 days
Frequency of treatment: ad libitum or restricted daily consumption of 9 and 6 g
Duration of test: 21 days
Doses: 1 – 1.5 – 2 or 2.5% PA in 20% casein diet
Control Group: other: 20% casein diet
Method: other
Year: **GLP:** no data
Test substance:
Remark: Rats receiving 20% casein diet, were submitted approximately to 0.5% PA, corresponding to 5000 ppm, 500 mg/kg body weight/day.
Result: In experimental groups of rats total food intake during pregnancy decreased, however the decrease was not so large as we had expected. By plotting the fetal brain weight (Y, mg) against maternal food intake (X, g/21 days) in control groups the following hyperbolic regression equation was obtained: $Y = -4243/X + 124.6$ ($n: 18$; $r = 0.08$; $p < 0.001$). Similar plots for excess PA rats fell below this line, indicating that prenatal fetal brain growth was impaired by an excess in PA per se specifically, as well as by decreased food intake nonspecifically. Administering the excess PA diets resulted in decreases in total RNA, total protein and RNA/DNA ratio in the fetal brain, whereas total DNA was unchanged, showing the impairment of protein synthesis, not proliferation. No remarkable changes in concentration and pattern of free amino acids in maternal plasma in excess PA groups were observed.
Source: ELF ATOCHEM UK Ltd WIDNESS

(18)

Species: other: mouse embryo **Sex:**
Strain:
Route of admin.: other: whole embryo culture
Exposure period: 24 hours
Frequency of treatment:
Duration of test: 24 hours
Doses: 0.01 mM to 10 mM
Control Group: no
Method:
Year: 1990 **GLP:**
Test substance:
Remark: The embryotoxic concentration 10 mM (1.5 mg/ml), toxic concentration for man following IV perfusion. In rat, after 800 mg/kg/day, the serie concentration was only 0.33 mg/ml.
Result: The aim of this study was to det. the teratogenicity of phenylalanine (Phe) and Phe metabolites in neurulating mouse embryos. Therefore, the system of whole embryo culture was employed and D9 (neurulating) mouse embryos were exposed to Phe, phenylethylamine (PEA), phenylpyruvic acid (PPA), phenylacetic acid (PAA), 2-OH phenylacetic acid (2-OHPAA), and phenyllactic acid (PLA) at concns. ranging from 0.01 mM to 10 mM for 24 h. After 24 h, embryos were examined for morphology abnormalities and protein content. Phe at 1 and 6 mM concns. was not teratogenic; however, 10 mM inhibited cranial neural tube closure in 82% of the embryos. PEA was the most toxic factor and concns. of 1 and 10 mM were embryo-lethal, whereas neural tube closure defects (NTDs) were obsd. in 67% of the embryos at 0.1 mM. 2-OH PAA was the second most toxic metabolite with concns of 1 and 10 mM producing NTDs in 10 and 100% of the embryos, resp. PLA and PAA produced no NTDs at concns of 1 mM 60% at 5 mM, and 100% at 10 mM. Finally, PPA produced approx. 50% NTDs at both 1 mM and 10mM concns. PLA, PAA, 2-OH PAA and PPA produced a significant redn. in embryonic protein, and PEA and 2-OH PAA reduced yolk sac protein values. PEA, 2-OH PAA, PAA, and PLA also produced craniofacial abnormalities, i.e., incomplete expansion of the forebrain, collapse of the optic vesicle, and hypoplasia of the mandible and/or the maxilla. The results showed that the relative teratogenicity of Phe and its metabolites differs and suggested that a metabolite of Phe and/or a combinaison of Phe and one or more of its metabolites play a role in the phenylketonuric embryopathy.
Source: ELF ATOCHEM UK Ltd WIDNESS

(19)

Species: other: rat embryo **Sex:**
Strain:
Route of admin.: other: whole embryo culture
Exposure period: 26 hours
Frequency of treatment:
Duration of test: 26 hours
Doses:
Control Group:
Method:
Year: 1991 **GLP:**
Test substance:
Remark: The toxic effects at the considerable concentration 1 mg/ml are confirmed.
Result: Mothers with untreated PKU can have offspring whose prenatal development is affected by the mothers' disease. These congenital malformations are associated with accumulation of PHE and its metabolites in maternal blood. We have studied the possible embryotoxicity of PHE and four PKU- related metabolites, phenylpyruvic acid (phepyr.), phenyllactic acid (phelac), o-hydroxyphenylacetic acid (hypheac) and phenylacetic acid (pheac) in the postimplantation whole embryo culture systeme. Day 10 rat embryos (4 or 8 somites) were cultured in rat serum to which tested compounds were added. After 26 h of culture, embryos were scored morphologically. PHE was not embryotoxic in the system, even in the presence of maternal hepatocytes to facilitate metabolism of PHE. HPLC - analysis of medium after coculture revealed the formation of tyrosine, but no PKU - related metabolites were detected. Phepyr induced some growth retardation at 1 mg/ml, whereas phelac had no effect. Hypheac and pheac induced dose-related embryotoxicity above 0.3 mg/ml. These results suggest a possible role for PKU-related phe metabolites in the induction of congenital malformations.
Source: ELF ATOCHEM UK Ltd WIDNESS

(20)

Species: rat **Sex:** female
Strain:
Route of admin.: unspecified
Exposure period: during pregnancy
Frequency of treatment:
Duration of test:
Doses: 0,2 the DL50
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: Russian publication.
Result: Tests were set up in 75 albino rats from which 737 embryos had been obtained. Subject to determination were: the numbers of live and dead embryos, their weight, lenght, anatomic changes in the internal organs, skeletal structure and also the RNA and DNA content in their liver. Investigations showed the phenylacetic acid, phenylethyl and cinnamic alcohols administered to pregnant rats in a dose of 0.2 LD50 during critical periods of embryogenesis to produce an embryotoxic effect of differing intensity. Most pronounced proved the action of phenylacetic acid, which manifested itself in that the test embryos, as contrasted to the control ones, demonstrated on the 20th day of embryogenesis a lagging body weight gain, retarded skeletal ossification, a reduced RNA content in the liver, the number of resorbed embryos being twice as great.
Source: ELF ATOCHEM UK Ltd WIDNESS

(21)

5.10 Other Relevant Information

Type: Biochemical or cellular interactions
Remark: The in vivo formation of (1–14C)acetyl–coenzyme A from D–(3–14C)3–hydroxybutyrate in the brain of the suckling rat was not affected by postnatal exposure to phenyl acetate. However, utilization of the generated acetyl–coenzyme A was significantly inhibited in certain metabolic reactions, namely synthesis of fatty acids and of sterols, but not in others as the Krebs cycle reactions that lead to the production of dicarboxylic amino acids. The incorporation of D–(U–14C)glucosamine into N–acetylneuraminic acid bound to glycoproteins was appreciably diminished in the rat pup previously exposed to maternal phenylketonuria induced by phenyl acetate. During the period of very rapid development of the brain, interference by phenyl acetate and/or its metabolites with certain critical biosynthetic pathways that require acetyl–coenzyme A would significantly contribute to retarded maturation of the brain that occurs in phenylketonuria.
Source: ELF ATOCHEM UK Ltd WIDNESS

(22)

Type: Biochemical or cellular interactions
Remark: The main interst of this paper is to confirm the safety of sodium phenylacetate in man, even if these experimentals are not sustained by animal experiments. In both sickle cell (SS) anemia and beta-thalassemia (beta-thal), an increase in fetal hemoglobin (HbF) ameliorates the clinical symptoms of the underlying disease. Several pharmocologic agents have been used to elevate HbF levels in adults; however, concerns regarding adverse effects of the prevailing drugs raise an urgent need for other agents capable of stimulating HbF production.We show here that sodium phenylacetate (NaPA) and its precursor, sodium 4-phenylbutyrate (NaPB), can enhance HbF production in cultured erythroid progenitor derived from normal donors and patients with SS anemia or beta-thal, when used at pharmacologic concentrations. Treatment resulted in (1) reduced cell proliferation, (2) elevated hemoglobin (Hb) content per cell (mean cellular Hb (MCH)), and (3) an increased proportion of HbF produced, associated with elevated levels of gamma-globin mRNA. Moreover, the active phenyl-fatty acids, with NaPA as a prototype, potentiated HbF induction by other drugs of clinical interest, including hydroxyurea (HU), sodium butyrate, and 5-azacytine (5AzaC). Efficacy could be further enhanced by introducing chlorine substituents at the phenyl ring to increase drug lipophilicity. Our findings indicate that NaPA and NaPB, both already proven safe and effective in treatment of children with urea cycle disorders, might benefit also patients with severe hemoglobinopathies. The two-phase liquid culture procedure used in this study should prove valuable in further studies exploring the mechanisms of HbF induction by these agents, and might provide an essay to predict patient response in the clinical setting.
Source: ELF ATOCHEM UK Ltd WIDNESS

(23)

Type: Biochemical or cellular interactions
Remark: Sodium phenylacetate (NaPA) at concentrations ranging from 2 to 10 mM (ca 300 to 600 mg/l) promoted myogenic differenciation of the human alveolar rhabdomyosarcoma cell line KFR. These concentrations inhibited DNA synthesis of the cells in a dose-dependent manner without significant effect on cell viability. The morphological differenciation of small mononuclear elements to terminal, elongated multinuclear structures resembling myotubes was accompanied by the expression of skeletal muscle myosin. The proportion of differentiated myosin-positive cells which was around 0.8-1.7% in control cultures, 12 days after seeding was increased by NaPA treatment up to 47%. In the cytoplasm of differentiated cells, features of sarcomerogenesis were observed. These results suggest that NaPA is an effective inducer of rhabdomyosarcoma cell differenciation at concentrations that have been achieved in humans with no significant adverse effects.
These results are of special interest, obtained by an other team than Thibault, Samid , and Ram confirming the lack of toxicity and the range of efficient concentration.
Source: ELF ATOCHEM UK Ltd WIDNESS

(24)

Type: Cytotoxicity
Remark: The effect of PA were tested on the proliferation of neuroectodermal tumor-derived cell lines. Human astrocytoma (U-251), medulloblastoma (DAOY, D-283), and rat malignant glioma (RG-2) cell lines were placed in 96 well microtiter plates with 2500 cell/100µl of MEM + 15% fetal calf serum/well and incubated in concentrations of PA ranging from 0 to 20 mM. Cell proliferation was mesured using a 4 hr. (3H)thymidine uptake assay at 24 hr intervals for 7 days. All 4 cell lines demonstrated dose-dependent inhibition of proliferation. The ID 50 for the human cell lines was in the 8 mM range; RG-2 cells responded only at the 20 mM concentration. Growth inhibition was not accompanied by changes in cell morphology. Autoradiographs of D-283 cell Western immunoblots demonstrated transforming growth factor beta (TGFB) 1 and 2 immunoreactivity, both before and after treatment with 10 mM PA. Affinity labeling with 125I-TGFB showed D-283 cells express TGFB-receptor subtypes 1, 2, and 3. PA inhibits proliferation of human malignant astrocytoma and medulloblastoma-derived cell lines at concentrations potentially achievable in patients.
Source: ELF ATOCHEM UK Ltd WIDNESS

(25)

Type: Cytotoxicity
Remark: Phenylacetate, a deaminated metabolite of phenylalanine, has been implicated in damage to immature brain in phenylketonuria. Because primary brain tumors are highly reminiscent of the immature central nervous system, these neoplasms should be equally vulnerable. We show here that sodium phenylacetate can induce cytostasis and reversal of malignant properties of cultured human glioblastoma cells, when used at pharmacological concentrations that are well tolerated by children and adults. Treated tumor cells exhibited biochemical alterations similar to those observed in phenylketonuria-like conditions, including selective decline in de novo cholesterol synthesis from mevalonate. Systemic treatment of rats bearing intracranial gliomas resulted in signifiant tumor suppression with no apparent toxicity to the host. The data indicate that phenylacetate, acting through inhibition of protein prenylation and other mechanisms, may offer a safe and effective novel approach to treatment of malignant gliomas and perhaps other neoplasms as well.
Source: ELF ATOCHEM UK Ltd WIDNESS

(26)

Type: Metabolism
Remark: A. Man and anthropoïd species fail to congugate phenylacetic acid with glycine and utilize glutamine instead.

B. The main detoxification pathway in man is glutamine conjugaison. This activity occur both in liver and kidney. The proposed steps are:

1. Phenylacetate + ATP ----> Phenacetyl-AMP + PPI

2. Phenacetyl–AMP + CoASH –––> Phenacetyl SCoA + AMP
3. Phenacetyl SCoA + L glutamine –––>
Phenacetylglutamine + CoASH

There does not appear to be an impairement of the glutamine conjugaison of phenylacetic acid in either phenyl ketonuria or in case of premature ageing.

C. Taurine conjugaison is a minor route of metabolisation.

Source: ELF ATOCHEM UK Ltd WIDNESS

(27)

5.11 Experience with Human Exposure

Remark: In public use before the 1920's.
Use in fragrance in states amounts approximately 7000lb/year

Concentration in final product (%):

	Soap	Detergent	Cream lotions	perfume
Usual	0.01	0.001	0.005	0.1
Maximum	0.1	0.01	0.03	0.2

Phenylacetic acid was given GRAS status by FEMA (1965) and is approved by the FDA for food use (21 CFR 121.1164). The council of Europe (1974) included phenyl acetic acid at a level of 25 ppm in the list of artificial flavouring substances that may be added to foodstuffs without hazard to public health. The Food Chemicals Codex (1972) has a monograph on phenylacetic acid.

Source: ELF ATOCHEM UK Ltd WIDNESS

(28)

Remark: Human skin irritation:
At 2% in petrolatum produced no irritation after a 48 hours closed patch test.

Source: ELF ATOCHEM UK Ltd WIDNESS

(28)

Remark: Human sensitization.

A maximisation test was carried out on 25 volunteers. The material was tested at 2% in petrolatum and produced no sensitization.

Source: ELF ATOCHEM UK Ltd WIDNESS

(28)

Remark: Phenylacetate (PA) induces terminal differenciation of HL60 cells and has preclinical activity against prostate cancer (PC) and glioblastoma multiforme (GBM). A phase I trial of PA was conducted in 17 patients (pts) with advanced solid tumors (PC:9, primary CNS:7, mesothelioma:1). Each pt received a single i.v. bolus followed by a 14–day continuous i.v. infusion (CIVI). 21 cycles of therapy were administered at 4 CIVI dose levels. PA displayed nonlinear pharmacokinetics (parameters, mean +/– SD: Km = 105 + 45 g/ml, Vmax = 24 +/– 5 mg/kg/hr and Vd = 19 +/– 3L)

Conversion to phenylacetylglutamine, the major urinary metabolite, accounted for 99% of PA elimination.
Dose–limiting toxicity, seen only during CIVI complicated by drug accumulation (PA concentration, in the serum of patients, mean +/– SD: 950+/– 300 µg/ml), consisted of emesis and reversible CNS depression. Three pts with PC had stable PSA levels for greater than 2 months. One had improved bone pain. Two pts with GBM have sustained functional improvement for more than 6 months. In summary, PA given by CIVI has antineoplastic activity against PC and CNS tumors.

Source: ELF ATOCHEM UK Ltd WIDNESS

(29)

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7.1 Risk Assessment

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

PHENYLACETIC ACID

COE No.:	672
FEMA No.:	2878
JECFA No.:	1007
Chemical names:	PHENYLACETIC ACID
Synonyms:	BENZYLCARBOXYLIC ACID; alpha-TOLUIC ACID
Functional class:	FLAVOURING AGENT
Latest evaluation:	2002
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 913-JECFA 59/53
Specifications:	COMPENDIUM ADDENDUM 10/FNP 52 Add.10/56
Tox monograph:	FAS 50-JECFA 59/215

8 Mar 03

See Also:
Toxicological Abbreviations
Phenylacetic acid (ICSC)

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations

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Joint FAO/WHO Expert Committee on Food Additives

Geneva, 4–13 June 2002

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

Toxicological evaluation of certain food additives. WHO Food Additives Series No. 50, in press

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 10. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International programme on Chemical Safety (IPCS).

The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 4 to 13 June 2002. The meeting was opened by Mr D.G. Aitken, Chef de Cabinet, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Aitken noted that a number of general issues were on the agenda, including consideration of papers on risk analysis and intake that had been referred by the Codex Committee on Food Additives and Contaminants (*1*) to the present Committee for comment. These two papers outline the specific roles of the Expert and Codex Committees in the development of Codex standards. The iterative process by which the two committees consider these and other issues highlights the importance of communication between them and for ensuring that the Expert Committee adequately responds to the needs of the Codex and that the roles of the two committees are properly defined.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*2*), there have been fifty-eight previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the fifty-seventh meeting (Annex 1, reference *154*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);

— to evaluate certain food additives and flavouring agents (sections 3 and 4 and Annex 2); and

— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

Annatto extracts, ethyl carbamate, methylmercury and sodium dichloroisocyanurate were removed from the agenda because data necessary for their evaluation were not available. The evaluation of curcumin was deferred until 2003 because the Committee its fifty-seventh meeting extended the temporary acceptable daily intake (ADI) to that time. Gum arabic was removed from the agenda because new information was not provided on differences in the

origin, manufacture, quality and use patterns of gum arabic from *Acacia senegal* and from *Acacia seyal*.

Nitrite was added to the agenda because the pivotal observed toxic effects of nitrate, which was evaluated at the present meeting, are consequent on its conversion to nitrite in vivo.

The Committee also considered the safety of the secondary components of a large number of flavouring agents for which the minimum assay values were below 95% and confirmation of use as flavours of a number of flavouring agents.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of the food additives on the agenda, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as the principles elaborated at subsequent meetings of the Committee (Annex 1, references *77, 83, 88, 94, 101, 107, 116, 122, 131, 137, 143, 149, 152* and *154*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) embraces the major observations, comments and recommendations contained, up to the time of its publication, in the reports of the Committee and other associated bodies. The Committee noted that the publication reaffirms the validity of recommendations that are still appropriate and points out the problems associated with those that are no longer valid in the light of technical advances.

2.2.1 *Safety evaluation of flavouring agents*

Flavouring agents with other functional uses

The Committee noted that a small number of the chemicals submitted for evaluation as flavouring agents have other uses in foods, for example as solvents, acidity regulators or preservatives, and that the Committee has previously evaluated them for these uses under the normal procedures for food additives. The Committee re-affirmed its position that the safety evaluation of these chemicals should include consideration of existing safety evaluations for other uses and that their evaluation as flavouring agents should make reference to the previous evaluations.

Flavouring agents that undergo chemical change in food

The Committee noted that some flavouring agents are added to food in the expectation that they will undergo chemical conversion during food processing or storage to form the compounds that provide the flavouring

effect in the food as consumed. Such agents can be evaluated by the Procedure for the Safety Evaluation of Flavouring Agents only if the breakdown and reaction products can be chemically identified. The Committee therefore stressed that information on breakdown and transformation products should be made available in submissions of data on flavouring agents for evaluation.

2.3 Project to update principles and methods of risk assessment of chemicals in food

The Committee was informed of progress being made on the Project, which was initiated by FAO and WHO. General principles and methods for the assessment of food additives, contaminants, residues of veterinary drugs and pesticides and food ingredients that have been developed over the years and published in Environmental Health Criteria, Nos 70 (Annex 1, reference *76*) and 104 (*3*) and in reports of JECFA and the Joint FAO/WHO Meeting on Pesticide Residues will be updated and consolidated, and the utility of new assessment procedures will be considered.

The final product will contain a historical background, and the activity will be placed in the context of risk analysis. A framework for incorporation of new principles and methods will be developed. The Project will focus on:

— chemical characterization (including contaminants and natural constituents) and development of specifications for food additives, pesticides and veterinary drugs;

— maximum residue levels for pesticides and veterinary drugs;

— exposure assessment (including short- and long-term intake, deterministic and probabilistic approaches and cumulative and aggregate exposure);

— toxicological test procedures and evaluation (including general issues and specific toxicological end-points);

— human data (clinical studies, epidemiological studies including biomarkers, potentially susceptible populations, allergenicity, intolerance and interactions with the diet);

— evaluations (including such issues as thresholds of toxicological concern, potency estimates, margins of safety, benchmark doses, acute reference doses, special considerations for contaminants, uncertainty and variability, scientific criteria for re-evaluation);

— principles related to specific substances (e.g. flavouring agents and substances consumed in large amounts); and

— a glossary (nomenclature and terminology).

The Committee emphasized the importance of this activity to its work, and encouraged FAO and WHO to proceed with it in a timely manner. It recommended that:

— the approach not be prescriptive, in order to maintain maximum flexibility in evaluating chemicals and in incorporating new methods of assessment;

— the intended audience, including risk managers and potential submitters of data, be clearly defined and kept in mind during all stages of the Project;

— new testing procedures and methods of assessment be validated before they are used; and

— harmonization and cooperation with risk assessment activities of other international and national organizations be considered, as appropriate.

2.3.1 *Specifications of identity and purity of food additives*

The *Project to Update Principles and Methods for Risk Assessment of Chemical in Foods* will include the definition of substances considered for safety by JECFA and the Joint FAO/WHO Meeting on Pesticide Residues. The Committee has a defined process for drafting specifications for food additives; as part of its input to the Project, the Committee considered a paper on the development of food additive specifications that had been prepared for the Project.

In general, the guidance given in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*) for drafting food additive specifications remains valid. However, this document was written before the role of the Committee in risk analysis had been defined, and it does not discuss how formulation of specifications fits into the Committee's work on risk assessment.

In considering this issue, the Committee recommended that:

— The updated principles should explicitly recognize that development of specifications is an integral part of the risk assessment of food additives.

— Risk assessment requires data on the manufacture and composition of an additive at all steps in its development and safety testing. In addition, information is needed on technological function and current and intended uses.

— Risk assessments should include specifications for the material evaluated and the product to be marketed.

— The updated principles should require continuous review of specifications in the light of possible changes in the manufacture, use and consumption of the additive.

2.4 Procedure for evaluating flavouring agents that are members of groups that have been evaluated previously by the Committee

The Committee has evaluated a large number of flavouring agents with the Procedure for the Safety Evaluation of Flavouring Agents. To facilitate the evaluations, the substances have been grouped according to their chemical structure. A number of flavouring agents in development and additional ones in commerce fit into the groups of flavouring agents that have already been evaluated, and the Committee concluded that these flavouring agents should be evaluated and the evaluations documented in a manner consistent with the previous practices and procedures of the Committee. Past evaluations should serve as the basis for evaluating new or additional flavouring agents and, if a substantial body of new data became available, the previous evaluation might need to be reconsidered. The Committee recommended that the *Guidelines for the preparation of working papers (monographs) on flavouring agents*[1] be updated accordingly.

2.5 Risk analysis principles and exposure assessment

The Codex Committee on Food Additives and Contaminants at its Thirty-fourth Session (*1*) asked JECFA to review and comment on papers that it had prepared, entitled *Application of risk analysis principles for food additives and contaminants* and *Draft principles for exposure assessment of contaminants and toxins in food.* The Expert Committee reviewed these papers and provided comments to the Codex Committee in the form of letters from the Chairman and Vice-Chairman of the present Committee to the Chairman of the Codex Committee.

2.6 Risk analysis terms related to food safety

In considering the definitions of 'hazard' and 'risk' adopted by the Codex Alimentarius Commission for risk assessment, the Committee noted that different definitions have been used by other bodies involved in chemical risk assessment. This could cause problems in communication and interpretation of risk assessments made by different organizations. For example, in the Codex definition, 'hazard' is considered to be *a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect,* whereas some other bodies (e.g. the

[1] Available at http://www.who.int/pcs

International Union of Pure and Applied Chemistry) consider hazards to be intrinsic chemical and biological properties (potentially adverse effects) of a chemical. JECFA has implicitly tended to the latter interpretation of 'hazard'. Similarly, 'risk' is defined in purely probabilistic terms by some organizations, whereas the Codex definition of 'risk' includes not only the probability of an adverse effect but also its 'severity'. The word 'severity' has been used variously to describe either the magnitude of response or the qualitative nature of the effect of a substance; this difference could also lead to confusion. The term 'severity' is used by the Committee to refer to the magnitude of a response and not to its qualitative nature.

The Committee noted the ongoing Joint IPCS/OECD project to harmonize the terminology used in chemical hazard or risk assessment[1], in which these issues are being addressed, and recommended that the harmonized terminology agreed upon in that project be adopted by the Committee and by the Codex Alimentarius Commission.

2.7 Consideration of guidelines

The Committee confirmed that detailed guidelines for the preparation of working papers were required and should be revised regularly on the basis of comments provided by members of the Committee. At its present meeting, the Committee considered guidelines that had been prepared by the Joint Secretariat and made comments and suggestions for improvement.

Comprehensive assessments of the intake of food additives are a recent task of the Committee. Therefore, at the present meeting, specific attention was paid to the *Guidelines for the preparation of working papers on the intake of food additives*. The Committee suggested that intake from dietary sources other than food additives and exposure to non-dietary sources might also be of relevance. The terms for intake assessment used in working papers should be standardized within the Committee and harmonized with those developed by OECD/IPCS (as summarized in the *Glossary of exposure assessment-related terms*[2]).

A draft guideline for working procedures to be followed by the Committee in elaborating specifications for food additives was considered, and substantial changes and amendments were suggested to the Secretariat.

2.8 Specifications for flavouring agents

The Committee concluded that it was not desirable to develop separate specifications for flavouring agents (e.g. benzoic acid) that it has evaluated for other uses. In such cases, the material used for flavouring purposes should comply with the specifications for its use for other technological purposes,

[1] Available at http://www.ipcsharmonize.org/index.html

and the list of specifications for flavouring agents should simply make reference to the full specifications monograph.

The Committee recognized that information on secondary components of flavouring agents should be incorporated into specifications when relevant to the safety evaluation. As necessary, this information would include upper limits on the content of such components. The Committee agreed that this information should appear as a distinct item under the heading 'Other requirements'. At the same time, the Committee decided to adjust the format for other items included under 'Other requirements' in the specifications tables to distinguish more clearly between genuine requirements and items included for information. The preamble to the table of specifications published in Food and Nutrition Paper 52 was expanded to explain how the information listed under 'Other requirements' is to be used.

3. Specific food additives (other than flavouring agents)

The Committee evaluated several food additives for the first time and re-evaluated others. The evaluations and specifications are summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Safety evaluations

3.1.1 *Alitame*

Alitame is an intense sweetener, with a sweetness potency 2000 times greater than that of sucrose. It is a dipeptide of L-aspartic acid and D-alanine, with a terminal *N*-substituted tetramethylthietanylamine moiety. Alitame is prepared by a multistep synthesis involving the reaction between two intermediates, (*S*)-[2,5-dioxo-(4-thiazolidine)] acetic acid and (*R*)-2-amino-*N*-(2,2,4,4-tetramethyl-3-thietanyl)propanamide. The final product is isolated and purified by crystallization of an alitame-4-methylbenzenesulfonic acid adduct, followed by additional purification steps, and finally recrystallization from water.

Alitame was considered by the Committee at its forty-fourth and forty-sixth meetings (Annex 1, references *116* and *122*). Most of the toxicological studies were examined at the forty-fourth meeting, and specifications and a toxicological monograph were prepared; however, an ADI could not be allocated because of concern about deficiencies in the studies of carcinogenicity. At its forty-sixth meeting, the Committee reconsidered this issue in the light of a general discussion of the survival rates of animals in contemporary long-term studies and a further statistical analysis of the data from the two available

studies. At that meeting, the Committee concluded that there was no evidence that alitame is carcinogenic. An ADI of 0–1 mg/kg bw was allocated on the basis of the NOEL of 100 mg/kg bw per day in an 18-month study in dogs. The Committee noted that, although not specifically requested, a further study of tolerance to repeated doses of alitame in persons with diabetes was under way and that, as is customary, the results should be forwarded to the Committee when available.

While consideration of the 90-day study of tolerance in diabetic subjects was on the agenda of the present meeting, it was postponed pending receipt of a final report. The ADI of 0–1 mg/kg bw allocated by the Committee at its forty-sixth meeting was retained.

No data on the dietary intake of alitame were available at the previous meetings of the Committee. At its present meeting, the Committee compared the maximum levels of alitame listed in the Codex draft General Standard on Food Additives (GSFA) with the theoretical maximum level calculated by the budget method (see Annex 1, reference *137*). On the assumption that alitame is used in all foods, the theoretical maximum level of alitame, based on the current ADI, was calculated to be 40 mg/kg. The maximum levels in the GSFA are up to 300 mg/kg in a wide range of foods and beverages, with no limit in 'other sugars and syrups' or in table-top sweeteners. Detailed assessments of the intake of alitame when used in foods were therefore required.

National assessments of intake based on recent individual dietary records and GSFA maximum levels were provided by Australia and by New Zealand. These data showed that the ADI might be exceeded by some consumers, at levels representing 148% of the ADI in Australia and 140% of the ADI in New Zealand for consumption at the 95th percentile. These are likely to be overestimates, as the surveys were of short duration (24 h) and because it was assumed that alitame was systematically present in some major food categories. The intake of alitame from 'other sugars and syrups' was not assessed since no manufacturer's use levels were available. In both Australia and New Zealand, the main contributor to the mean estimated intake was sweet bakery wares, which was responsible for 89% of the overall estimated intake from all food categories in Australia and 88% in New Zealand. These estimates are based on data from only two countries, and further work is required to refine the intake estimates with recent data from other countries.

The Committee identified GSFA food category 7 (bakery wares, including bread) as a food group that might contribute to a high intake of alitame, as this group includes foods that are staples in many countries. The Committee therefore suggested that the Codex Committee on Food Additives and Contaminants consider reviewing the standards for alitame.

An addendum to the monograph summarizing the available data on intake was prepared. The specifications for alitame were revised. The addresses of suppliers of some of the chemicals needed for the purity tests were updated. In addition, in line with the decision made by the Committee at its fifty-seventh meeting (Annex 1, reference *154*), a brief description of the method of manufacture of alitame was included. The revised specifications also incorporate the heavy metals limits established by the Committee at its fifty-seventh meeting.

3.1.2 *Cross-linked sodium carboxymethyl cellulose*

Cross-linked sodium carboxymethyl cellulose is manufactured by acidifying an aqueous suspension of sodium carboxymethyl cellulose and heating the suspension to achieve cross-linking. The product is then washed and dried. It is also produced during the manufacture of sodium carboxymethyl cellulose by lowering the pH and heating to cause cross-linking. Cross-linked sodium carboxymethyl cellulose is used in tablets of table-top sweeteners and dietary food supplements, as it facilitates disintegration in aqueous solutions, with a maximum level of use of 30 g/kg. It is also widely used as an excipient in pharmaceutical applications.

Cross-linked sodium carboxymethyl cellulose has not been evaluated previously by the Committee. The parent compound, sodium carboxymethyl cellulose, was considered by the Committee in its evaluations of modified celluloses at its fifth, seventh, tenth, thirteenth, seventeenth, twenty-sixth, twenty-seventh, thirtieth and thirty-fifth meetings (Annex 1, references *5*, *7*, *13*, *19*, *32*, *59*, *62*, *73* and *88*) and of ethyl cellulose and ethylhydroxyethyl cellulose at the twenty-sixth and twenty-seventh meetings (Annex 1, references *59* and *62*). At its thirty-fifth meeting, the Committee reviewed new data on seven modified celluloses (Annex 1, reference *89*), including sodium carboxy-methyl cellulose. The data consisted of the results of studies in rats on caecal enlargement, effects on caecal flora and developmental toxicity and studies of mutagenicity in vitro. New data were also available from studies in humans, indicating that some individuals may experience laxative effects at a dose as low as 5 g per person per day. The Committee concluded that modified celluloses are of low toxicity and allocated a group ADI 'not specified'[1] to seven modified celluloses: methyl cellulose, ethyl

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

cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, sodium carboxy-methyl cellulose, ethyl cellulose and ethylhydroxyethyl cellulose. The Committee pointed out that the laxative properties of these celluloses should be taken into account when they are used as food additives.

Enzymatically hydrolysed sodium carboxymethyl cellulose was evaluated by the Committee at its fifty-first meeting (Annex 1, reference *137*). The Committee compared the toxicity and absorption, distribution and metabolism of enzymatically hydrolysed carboxymethyl cellulose with that of the parent compound and concluded that the similarities in the results were consistent with the absence of any toxicologically significant difference between the two. Therefore, the enzymatically hydrolysed form was included in the group ADI 'not specified' with the other seven modified celluloses.

At its present meeting, the Committee reviewed two studies on cross-linked sodium carboxymethyl cellulose and considered its properties in the light of previous evaluations of modified celluloses. Like other modified celluloses, cross-linked sodium carboxymethyl cellulose is considered to be of low toxicity. Because cross-linking renders it insoluble in water, it is less likely to be absorbed than the parent compound.

In a 90-day dietary study in rats, the only treatment-related observations were increased food consumption, decreased body-weight gain and effects on the kidneys of animals receiving cross-linked sodium carboxymethyl cellulose at 50 000 mg/kg of diet, equivalent to 3900–4700 mg/kg bw per day averaged over the study period. The changes in food consumption or body-weight gain were consistent with a 5% reduction in the nutritional content of the diet, as compared with the basal diet fed to control animals, due to addition of the test substance. In contrast to the effects of some other non-digestible substances evaluated previously by the Committee, caecal enlargement was not observed. Mineralization at the corticomedullary junction was observed in the kidneys of some female rats, with a higher incidence in treated groups than in controls. The report equated the renal mineralization to nephrocalcinosis and noted that, although this is a spontaneous change in control female rats, it was related to treatment in female rats fed cross-linked sodium carboxymethyl cellulose at 50 000 mg/kg of diet, but not at 10 000 mg/kg of diet.

Pelvic nephrocalcinosis and corticomedullary nephrocalcinosis were observed in rats given sodium carboxymethyl cellulose or enzymatically hydrolysed carboxymethyl cellulose in a study evaluated by the Committee at its fifty-first meeting (Annex 1, reference *137*). In that study, it was also reported that corticomedullary nephrocalcinosis was observed more frequently in females than in males. Because of the inconsistency of the dose–response relationship, however, the Committee at its fifty-first meeting

had not been convinced that these changes were of toxicological concern. The changes observed in the study evaluated at the current meeting appeared to represent a minimal increase in the incidence and severity of a spontaneous lesion, which may be within normal variation. As discussed in the report of the twenty-sixth meeting (Annex 1, reference *59*), nephrocalcinosis is a common finding in laboratory rodents. No implications for human hazard have been established. Therefore, the NOEL for cross-linked sodium carboxymethyl cellulose in the above study was 3900 mg/kg bw per day.

A study of developmental toxicity in rats showed no evidence of maternal toxicity or teratogenicity at the highest possible dose of cross-linked sodium carboxymethyl cellulose of 50 000 mg/kg of diet. The NOEL was 4500 mg/kg bw per day.

The Committee noted that evaluation of the results of the 90-day toxicity study was not straightforward, because of nutritional differences between the basal diet given to control animals and the diets supplemented with cross-linked sodium carboxymethyl cellulose. However, taking into account evaluations of other modified celluloses, the Committee concluded that the effects of cross-linked sodium carboxymethyl cellulose were consistent with dietary overloading with a poorly absorbed substance of low toxicity. The Committee included cross-linked sodium carboxymethyl cellulose in the group ADI 'not specified' with the other modified celluloses.

A toxicological monograph and new specifications were prepared.

3.1.3 *Mineral oils (low- and medium-viscosity)*

Explanation

Mineral oils (medium- and low-viscosity) comprise a subgroup of the family of mineral oils and waxes. They are manufactured from crude mineral oils in various refining steps, such as distillation, extraction and crystallization, and are subsequently purified by acid treatment (oleum method) and/or hydrotreatment (catalytic hydrogenation). Mineral oils (medium- and low-viscosity) are mixtures of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling-points greater than 200 °C.

In order to define the materials more clearly, the Committee at its forty-fourth meeting (Annex 1, reference *116*) prepared individual specifications for microcrystalline wax and for two groups of mineral oils: mineral oils (high-viscosity) and mineral oils (medium- and low-viscosity). The latter group was furthermore divided into three classes. For the purpose of characterization of the different types of oils and waxes, the criteria viscosity, average relative molecular mass and carbon number at 5% distillation-point were used in the specifications (see Table 1).

Food-grade mineral oils were last evaluated by the Committee at its forty-fourth meeting (Annex 1, reference *116*), when four 90-day studies conducted in Fischer 344 rats with a range of mineral oils and waxes representative of materials in commercial use were reviewed. The materials tested included low-, medium- and high-viscosity mineral oils (N10(A), N15(H), P15(H), N70(A), N70(H), P70(H) and P100(H)); paraffin waxes (low-melting-point wax and intermediate-melting-point wax); and microcrystalline waxes (high-melting-point wax and high-sulfur wax). All the substances, with the exception of the microcrystalline waxes, appeared to accumulate in the tissues of the animals to varying degrees, depending on the material and dose. Except for P70(H) and P100(H) oils, there was evidence of accumulation of the mineral hydrocarbons and effects indicative of a reaction to a foreign body at one or more doses. The types of effects seen were similar and included

Table 1

Classification of mineral hydrocarbons

Name	ADI (mg/kg bw)[a]	Viscosity at 100 °C (mm^2/s)	Average relative relative molecular mass	Carbon number at 5% distillation-point
Microcrystalline wax	0–20[a,b]	≥ 11	≥ 500	≥ 25
High-melting-point wax		Not included in the present studies		
Low-melting-point wax	Withdrawn[c]	No specification		
Low-melting-point wax		3.3	380	22
Mineral oil (high viscosity)	0–20[a]	> 11	> 500	≥ 28
P100		11	520	29
Mineral oil (medium and low viscosity) class I	0–10[d]	8.5–11	480–500	≥ 25
P70		9.0	480	27
Medium-viscosity liquid petroleum		8.7	480	25
P70(H)		8.6	480	27
Mineral oil (medium and low viscosity) class II	0–0.01[e,f]	7.0–8.5	400–480	≥ 22
N70(H)		7.7	420	23
Mineral oil (medium and low viscosity) class III	0–0.01[e,f]	3.0–7.0	300–400	≥ 17
P15(H)		3.5	350	17
N15(H)		3.5	330	17

P100 oil, crude: paraffinic, viscosity (40 °C): 100 mm^2/s; P70 oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s; P70(H) oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation); N70(H) oil, crude: naphthenic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation; P15(H) oil, crude: paraffinic, viscosity (40 °C): 15 mm^2/s, hydrotreated (catalytic hydrogenation; N15(H) oil, crude: naphthenic, viscosity (40 °C): 15 mm^2/s, hydrotreated (catalytic hydrogenation)

[a] Established by the Committee at its forty-fourth meeting
[b] Group ADI
[c] Previous ADI 'not specified'
[d] Established at the present meeting
[e] Temporary group ADI
[f] Extended at the present meeting

focal histiocytosis, increased weights of liver, lymph nodes, spleen and kidneys, granulomas or microgranulomas of the liver, haematological changes typical of a mild, chronic inflammatory reaction and biochemical changes indicative of mild hepatic damage.

The ADI of 0–20 mg/kg bw established at the forty-fourth meeting (Annex 1, reference *116*) for mineral oils with the specifications of high-viscosity oils and of high-melting-point and high-sulfur waxes was based on NOELs at the highest dose tested (2% in the diet) in 90-day studies in Fischer 344 rats. The NOELs for all the other materials except P70(H) oil (i.e. class II and III medium- and low-viscosity oils and low-melting-point wax) were based on an increased incidence of histiocytosis in the lymph nodes at the next highest dose. For P70(H) oil, the NOEL was based on an increased incidence of pigmented macrophages in male rats at a dietary concentration of 2%, a minor effect considered of doubtful biological significance. Accordingly, because effects were observed at all doses, the ADIs for low-melting-point and intermediate-melting-point waxes were withdrawn. A group temporary ADI of 0–0.01mg/kg bw was allocated for class II and III medium- and low-viscosity mineral oils, the temporary nature of the ADI being due to uncertainty about the long-term significance of the inflammatory response to accumulated dietary mineral hydrocarbons. A temporary ADI of 0–1 mg/kg bw was allocated for P70(H) oil. These temporary ADIs were extended at the fifty-first meeting (Annex 1, reference *137*).

At its forty-fourth meeting, the Committee considered that, although the types of effects seen were essentially reactions to a foreign body, it was possible that a prolonged inflammatory response of the type observed could result in functional changes in the immune system and that this aspect required further investigation. It also noted that the oils and waxes for which high NOELs were observed contained a greater proportion of hydrocarbon components of high relative molecular mass (high carbon number) and had higher viscosities than those materials with a low NOEL, which contained a greater proportion of hydrocarbon components of lower relative molecular mass (low carbon number). The Committee required information about the compositional factors in mineral oils that influence their absorption and toxicity. It also required a study of at least 1 year's duration on one of these materials in Fischer 344 rats, including an assessment of immune function at appropriate times and an investigation of the kinetics of accumulation of the material, to determine whether a plateau is reached. It recommended that a reversal period of 1 year be included, in order to determine whether the granulomatous hepatic lesions observed in rats in the 90-day studies were fully reversible.

At its present meeting, the Committee reviewed a number of new studies, including the results of a combined 2-year study of toxicity and carcino-

genicity and a 1-year study of toxicity with a 1-year recovery period with P70(H) and P100(H) oils (with the specifications of class-I medium- and low-viscosity mineral oils and of high-viscosity mineral oil, respectively) conducted in parallel. In addition, the Committee received and reviewed a number of studies conducted with low- and medium-viscosity mineral oils, including: a 2-year study of the carcinogenicity of a medium-viscosity liquid petroleum (class I medium- and low-viscosity mineral oil); studies of pharmacokinetics and studies of humoral immune function after administration to P15(H) mineral oil (class III medium- and low-viscosity mineral oil) in Fischer 344 and Sprague-Dawley rats; and a 90-day study of histopathological responses and compositional analysis of absorbed hydrocarbons with N15(H), N70(H) and P70(H) oils (classes III, II and I medium- and low-viscosity mineral oils, respectively). Several studies of low-melting-point paraffin wax were also reviewed by the Committee, as they were considered to provide information relevant for the evaluation of low- and medium-viscosity mineral oils relating to the difference in response to mineral hydrocarbons in Fischer 344 and Sprague-Dawley rats.

Toxicological data

In the long-term studies of toxicity, Fischer 344 rats were given a diet containing P70(H) or P100(H) oil, at concentrations that were varied to provide constant doses ranging from 60 to 1200 mg/kg bw per day, for up to 24 months. At 24 months, treatment with either test material was associated with mild treatment-related effects, which included: dose-related increases in the absolute and relative weights of the mesenteric lymph nodes; an increase in the severity of reticuloendothelial-cell hyperplasia (previously described as focal histiocytosis) of the mesenteric lymph nodes; increases in the incidence and/or severity of vacuolation of periportal hepatocytes; and an increased incidence of sinusoidal dilatation in the liver of male rats only, which was not dose-related. These effects were observed even at the lowest dose tested, 60 mg/kg bw per day. There were no treatment-related effects on survival with either material. Neither P70(H) nor P100(H)was carcinogenic in this study.

The effects observed at 12 months were marginal and included a slight increase in the severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes in male and female rats treated with P70(H) at the highest dose, the only dose for which histopathological data were available, as well as statistically significantly increased absolute and relative weights of mesenteric lymph nodes in males at this dose. Because of the marginal nature of these changes at 12 months, the reversibility of the lesions at the end of the recovery period could not be assessed; however, the results did indicate lack of progression of tissue alterations after dietary exposure to mineral hydrocarbons for 12 months.

No special indicators of immune function were measured in the long-term studies, although standard end-points of haematology, clinical chemistry, organ weight and histopathology, indicative of immune function, were evaluated. The only alteration in these end-points was exacerbation of mild cellular infiltration of the mesenteric lymph nodes, which had not been observed previously after administration for 90 days.

Aside from the earlier onset and increased severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes and the greater increase in the weights of mesenteric lymph nodes in response to dietary administration of P70(H), there was little difference in response to the two oils. None of the effects at 24 months was reported in 90-day studies at concentrations up to 2% of the diet (equivalent or equal to 2000 mg/kg bw per day) of either material. The pigmented macrophages observed with P70(H) oil at 2% in the diet (equivalent to 2000 mg/kg bw per day) in the 90-day study reviewed at the forty-fourth meeting, on which the ADI was based, were not observed after 24 months' intake at doses up to 1200 mg/kg bw per day.

Accumulated hydrocarbon was detected in the liver, mesenteric lymph nodes and spleen; the amounts of hydrocarbon could be quantified only in the liver. In the liver, a steady-state level of hydrocarbon accumulation appeared to have been reached in rats treated with the highest dose (1200 mg/kg bw per day) by 3 months, the only dose for which data on the complete time course were available. The concentration of P70(H) reached in the liver at any given time was about twice that of P100(H). The concentrations of mineral hydrocarbons in the liver returned to control levels within 12 months of cessation of treatment.

Medium-viscosity liquid petroleum (corresponding to class I medium- and low-viscosity mineral oils, including P70(H) oil) was not carcinogenic when fed to Fischer 344 rats at a concentration of 2.5% or 5% in the diet (equivalent to 1250 and 2500 mg/kg bw per day). Increased incidence or severity of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes was also observed in rats of each sex in this study at both dietary concentrations.

The pharmacokinetics of single doses of P15(H) oil given by gavage at a dose of 34 or 340 mg/kg bw over 96 h was studied in female Fischer 344 and Sprague-Dawley rats, with [^{14}C]1-eicosanylcyclohexane as the tracer. In one of the studies, the animals received a 2-week dietary pretreatment with low-viscosity mineral oil. The faeces were the major route of elimination, and urinary excretion was a minor route. Elimination of radiolabel by either route was more rapid in Sprague-Dawley than in Fischer 344 rats. Estimates of the area under the curve for serum concentrations of mineral hydrocarbons indicated that the bioavailability of mineral hydrocarbon was greater in Fischer 344 rats. Rats of this strain also accumulated and retained a larger

proportion of the administered dose in the liver (2% vs 0.1–0.5%) and, to a much lesser extent (about 100-fold), in the mesenteric lymph nodes. While a longer delay in faecal excretion of radiolabel was seen in Fischer 344 rats than in Sprague-Dawley rats in one study, suggesting a strain-related difference in intestinal absorption of mineral hydrocarbons, this difference was not observed in two similar studies. Rather, the results suggested that Sprague-Dawley rats metabolized and excreted radiolabelled mineral hydrocarbon tracer more efficiently than Fischer 344 rats, which exhibited deposition and retention in the liver. Dietary pretreatment with mineral oil did not affect the extent to which hydrocarbon accumulated in the liver of either strain of rats. In another study, the rate of metabolism of *n*-heptadecane by liver microsomes in vitro differed by rat strain, but not sex, in the order Wistar > Sprague-Dawley > Fischer 344. Metabolism of *n*-octadecane was negligible in all strains, consistent with the findings for hydrocarbons with more than 17 carbons in microsomal systems in vitro.

New short-term studies in which the responses of female Fischer 344 and Sprague-Dawley rats to dietary administration of P15(H) oil (for 120 and 90 days, respectively) at concentrations of up to 2% were compared indicated a more severe response in the Fischer 344 strain. The effects seen in these rats were: increased weights of the liver, mesenteric lymph nodes and spleen; granulomatous inflammation of the liver; and reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes. In Sprague-Dawley rats, only statistically significant increases in liver weight and reticuloendothelial-cell hyperplasia of the lymph nodes, mostly of minimal severity, were observed. The increased liver weights in Fischer 344 rats receded during a 30-day recovery period. Reversibility was not assessed in Sprague-Dawley rats.

The results of the new 90-day study in female Fischer 344 rats with low-melting-point wax and with N15(H), N70(H) and P70(H) oils at a dietary concentration of 2% (equal to approximately 2000 mg/kg bw per day at 90 days) confirmed the previous findings in lymph nodes, liver and spleen, with an additional finding of inflammation of the cardiac mitral valve, which had not been seen previously with this material. This effect occurred at a lower incidence than with low-melting-point wax in this study.

The Committee reviewed the results of a study of T-dependent humoral immune function in response to 90 days' administration of P15(H) oil in female Fischer 344 and Sprague-Dawley rats. No effects were observed in the Sprague-Dawley rats. Interpretation of the results in the Fischer 344 rats was complicated by the increase in spleen weight that resulted from treatment. No data were provided to determine whether the increased spleen weight was associated with a change in the proportion of spleen cell types, which could alter interpretation of the results.

The responses of female Fischer 344 and Sprague-Dawley rats to mineral hydrocarbons were also compared in a 90-day toxicity study with low-melting-point wax at a dietary concentration of 0.2% or 2.0%. This substance had induced the most severe effects in similar studies in Fischer 344 rats with a range of food-grade mineral hydrocarbons, in which, in general, female rats had a stronger response to these materials. The results for Fischer 344 rats in the new study confirmed the findings of the previously evaluated 90-day studies with low-melting-point wax: altered haematological and hepatic clinical chemical parameters, increased weights of the liver, mesenteric lymph nodes and spleen; hepatic vacuolation (affecting both hepatocytes and Kupffer cells) and granulomatous inflammation; reticuloendothelial-cell hyperplasia of mesenteric and cervical lymph nodes; and focal inflammation of the cardiac mitral valve. In addition, increased severity of extramedullary haematopoiesis of the spleen, differential effects on serum immunoglobin concentrations and lipid-like material in crystalline form in hepatocytes and Kupffer cells were observed in this strain. In contrast, the only effects observed in female Sprague-Dawley rats were increased liver weights and slightly increased reticulo-endothelial-cell hyperplasia of the mesenteric lymph nodes and severity of hepatocellular and Kupffer-cell vacuolation. The crystalloid structures and inflammatory response in the liver were absent in this strain.

Tissue accumulation of mineral hydrocarbon after administration of low-melting-point wax also showed a clear strain difference in terms of degree, onset and the tissues affected. Quantifiable amounts of hydrocarbon were detected in the liver and mesenteric lymph nodes of Fischer 344 rats in a time- and dose-related manner, starting at 30 days in the livers of animals at the higher dose and occurring in both tissues in animals at both doses by 90 days. However, only the mesenteric lymph nodes of Sprague-Dawley rats had quantifiable amounts of hydrocarbon, and only at 90 days in animals at the higher dose. This observation was confirmed in a qualitative investigation of the accumulation of low-melting-point wax in the liver and Kupffer cells of female Fischer 344 and Sprague-Dawley rats after administration of a diet containing 2% wax for 14, 30 or 60 days. Detectable amounts were found in liver cells of Fischer 344 rats by 14 days, but none was found at any time in Sprague-Dawley rats.

In a separate study of low-melting-point wax, feeding a diet containing 2% resulted in alterations in Kupffer-cell function and morphology after 60 days in female Fischer 344, but not Sprague-Dawley, rats. Effects were also seen on selected parameters (i.e. leukocytes, serum liver enzyme activities and hepatic histopathology) that have been associated with administration of low-melting-point wax in this strain, and these were accompanied by accumulation of mineral hydrocarbon in the liver.

No new information was available on the compositional elements of mineral oils that affect their absorption or toxicity.

As the materials tested in the long-term studies reviewed at the present meeting, P70(H) and P100(H) oils, were not associated with induction of liver granulomas in Fischer 344 rats, the studies did not help the Committee to determine the long-term consequences or reversibility of the liver granulomas that had been seen in previous studies in response to consumption of low- and medium-viscosity mineral oils and low-melting-point waxes by Fischer 344 rats. In addition, the Committee was unable to interpret the effects in the study of humoral immune function in response to dietary administration of P15(H) oil.

The results of the studies on the effects of P15(H) oil and low-melting-point wax in Fischer 344 and Sprague-Dawley strains indicated that the more extensive response of Fischer 344 rats, in particular that of females, is associated with greatly enhanced retention of mineral hydrocarbons in the tissues, which is probably due to a reduced ability to metabolize absorbed hydrocarbons. The Committee concluded that additional studies are needed in order to determine whether the Fischer 344 rat is an appropriate model of human response to dietary intake of food-grade mineral hydrocarbons. In particular, elucidation of the metabolic differences between Fischer 344 rats and other strains and species, including humans, would be useful.

Neither P70(H) nor P100(H) oil was carcinogenic in the combined study of toxicity and carcinogenicity reviewed by the Committee at its present meeting. The effects observed even at the lowest dose, i.e. enhanced reticuloendothelial-cell hyperplasia, increased weights of mesenteric lymph nodes and increased incidence and grade of vacuolation of hepatocytes, were shown not to progress to more severe effects, and there was no indication that accumulated test material contributed to suppression or activation of an inflammatory response. Consequently, these effects were considered to be indicators of exposure to mineral hydrocarbon rather than adverse effects. At the present meeting, the NOEL for P70(H) oil was identified as the highest dose tested in the combined study of toxicity and carcinogenicity in rats, 1200 mg/kg bw per day, to which a safety factor of 100 was applied. An ADI of 0-10 mg/kg bw was allocated for class I medium- and low-viscosity mineral oils, which include P70(H) oil. An ADI of 0–20 mg/kg bw for P100(H) oil already exists (see Table 1).

No data were available that would permit allocation of a full ADI for medium- and low-viscosity mineral oils in classes II and III. The Committee noted that the new information reviewed at the present meeting indicated that the observed effects of these mineral oils, on which the temporary ADI is based, may be strain- and sex-specific. The Committee therefore extended the

temporary group ADI of 0–0.01 mg/kg bw for class II and III medium- and low-viscosity mineral oils until 2006, pending information on the relevance to humans of the response of Fischer 344 and Sprague-Dawley rats to these materials. In order for the data to be applicable to as wide a range of mineral oils as possible, the Committee suggested that commercial mineral oils of the lowest viscosity be used in such studies. Additional studies might be required, depending on the outcome of these studies.

Intake

The major food uses of mineral oils are in vegetable and fruit coatings and in packaging, bakery and confectionery manufacture and grain and seed dust control, and these uses are also the major sources of dietary intake of mineral oils in some countries. Dietary intake was assessed from data on the levels of use in foods and on migration from coatings and packaging materials into foods, combined with national data on food consumption in the 1990s. In both the United Kingdom and the USA, the average total intake of mineral hydrocarbons (excluding petroleum jelly) from food use was estimated to be 0.47 mg/kg bw per day; the intake at the 90th percentile of consumption by the population of the United Kingdom was 0.80 mg/kg bw per day. Class III medium- and low-viscosity mineral oils (including P15(H) oil) accounted for 0.21 mg/kg bw per day in the United Kingdom and 0.25 mg/kg bw per day in the USA; these values are 21 and 25 times the temporary ADI of 0–0.01 mg/kg bw, respectively, whereas class I and II medium- and low-viscosity mineral oils and high-viscosity mineral oil (including P70(H) and N70(H) oils, respectively) accounted for 0.18 and 0.19 mg/kg bw per day, respectively. As these different categories of mineral oil have different ADIs, but data on intake are not available for separate categories, the intake of each category cannot be compared with the corresponding ADI. Use of solid hydrocarbons (e.g. microcrystalline wax (high-melting-point wax) and paraffin wax (low-melting-point wax)) accounted for the remainder of the total intake.

The intake of high-viscosity and class I, II and III medium- and low-viscosity mineral oils that have migrated into food from coating and packaging materials was estimated to be 0.001 mg/kg bw per day in both the United Kingdom and the USA, while the combined intake of paraffin wax and microcrystalline wax from this source was estimated to be 0.005 and 0.006 mg/kg bw per day, respectively.

Naturally occurring hydrocarbons are widely distributed in many edible plants and animals, and they contribute significantly to the overall dietary intake of hydrocarbons. For example, the dietary intake of naturally occurring hydrocarbons was estimated to be 0.47, 0.25 and 0.19 mg/kg bw per day in the populations of the European Union, the United Kingdom and the USA,

respectively. It is clear, therefore, that account should be taken of intakes from naturally occurring hydrocarbons when evaluating the safety of mineral oils.

Mineral oil is included in the Codex draft GSFA in numerous food categories, with maximum use levels of 200–3000 mg/kg and, in some categories, in accordance with good manufacturing practice. The Committee suggested that the Codex Committee on Food Additives and Contaminants consider reviewing the standards for mineral oil, particularly in view of the fact that the intake of class III medium- and low-viscosity mineral oil is more than 20 times higher than its temporary ADI of 0–0.01 mg/kg bw.

An addendum to the toxicological monograph, including data on intake, was prepared. The existing specifications for mineral oils (medium- and low-viscosity) were revised, with minor editorial changes.

3.1.4 *Nitrate and nitrite*

Nitrite

Explanation

Nitrite occurs in the environment, in food and water, and is produced endogenously. It is also used as a food additive, mainly as a preservative, antimicrobial agent and colour fixative. It is used in foods such as cheese and cheese products, raw and processed meats, edible casings, processed fish and fish products and roe.

Nitrite was reviewed by the Committee at its sixth, eighth, seventeenth, twentieth and forty-fourth meetings (Annex 1, references *6, 8, 32, 41* and *116*). At its sixth meeting, the Committee allocated an ADI of 0–0.4 mg/kg bw to this substance, expressed as sodium nitrite. This ADI was based on a marginal reduction in body-weight gain at a dose of 100 mg/kg bw per day in a long-term study in rats. At its seventeenth meeting, the Committee lowered the ADI for sodium nitrite to 0–0.2 mg/kg bw and made it temporary At that time, the Committee used a safety factor higher than normal (500) because the ADI was based on a marginal effect and there is a possibility of endogenous formation of *N*-nitroso compounds from nitrite and *N*-nitrosatable compounds present in food and the gastrointestinal tract. At its twentieth meeting, the Committee considered the reports of a WHO task group and of a working group of the International Agency for Research on Cancer on *N*-nitroso compounds but concluded that they did not provide sufficient evidence to revise the temporary status of the ADI.

After that meeting, numerous toxicological and epidemiological data became available, which the Committee considered at its forty-fourth meeting. Nitrite both with and without nitrosatable precursors was found to be genotoxic in vitro in several tests, but, with the exception of a test in *Drosophila*

melanogaster, the results obtained in vivo were negative. The results of carcinogenicity studies with nitrite were negative, except those in which extremely high doses of both nitrite and nitrosatable precursors were administered simultaneously or premixed in the diet. In addition, the epidemiological data provided no evidence for an association between exposure of humans to nitrite and nitrate and the risk for cancer.

At its forty-fourth meeting, the Committee noted that several controlled laboratory studies had shown that *N*-nitroso compounds are formed endogenously when both nitrite and *N*-nitrosatable compounds are present at high concentrations; it observed, however, that quantitative data were available only on those *N*-nitroso compounds that are readily formed endogenously, such as *N*-nitrosoproline, which is not carcinogenic. As there was no quantitative evidence of the endogenous formation of carcinogenic *N*-nitroso compounds at the levels of intake of nitrite and nitrosatable precursors achievable in the diet, a quantitative risk assessment of nitrite on the basis of endogenously formed *N*-nitroso compounds was considered to be inappropriate. The Committee at its forty-fourth meeting therefore based its safety evaluation on studies of toxicity with nitrite. The NOELs in these studies were 5.4 mg/kg bw per day (expressed as nitrite ion) in a 90-day toxicity study in rats, in which hypertrophy of the zona glomerulosa was observed, and 6.7 mg/kg bw per day (expressed as nitrite ion) in a 2-year study in rats in which effects on the heart and lungs were observed. On this basis, the Committee allocated an ADI of 0–0.06 mg/kg bw, expressed as nitrite ion, by applying a safety factor of 100.

At its present meeting, the Committee reviewed the data that had become available on nitrite since its forty-fourth meeting, including data on the relevance and adverse nature of certain effects, with new information on the mode of action of effects on the adrenals and on toxicokinetics.

Toxicological and epidemiological data

The Committee considered a new study of toxicokinetics in human volunteers given sodium nitrite in their drinking-water. The maximum plasma nitrite concentrations were observed 15–30 min after dosing, and nitrite disappeared rapidly from plasma, with a half-life of approximately 30 min. The bioavailability of sodium nitrite was similar and virtually total after low and high oral doses. An intravenous dose of sodium nitrite of 290–380 mg per person induced methaemoglobinaemia, with maximum percentages of 8.4–12%. After oral administration, the maximum concentration of methaemoglobin was reached 0.70 h after dosing.

Several new studies were available on the acute and short-term toxicity of nitrite in humans, in which the severity of methaemoglobinaemia was reported after accidental, high intake of nitrite. Most were case reports of poisoning

incidents. None of these studies added data that could be used for the safety evaluation.

The Committee considered studies recently completed within the National Toxicology Program in the USA. In 14-week studies of the toxicity of sodium nitrite administered in drinking-water to mice at doses equal to 0, 90, 190, 340, 750 or 990 mg/kg bw per day and to rats at doses equal to 0, 30, 55, 120, 200 or 310 mg/kg bw per day, elevated levels of methaemoglobinaemia were observed in rats in all treatment groups, but not in mice. The Committee considered a level of methaemoglobin formation of up to 5% not to be adverse. The lowest NOEL value was observed in a study in male rats and was equal to 55 mg/kg bw per day (37 mg/kg bw per day expressed as nitrite ion) on the basis of reduced sperm motility at higher doses. Reduced sperm motility was also seen in mice at higher doses. The results of 2-year carcinogenicity studies with sodium nitrite added to drinking-water of mice and rats at a concentration of 750, 1500 or 3000 mg/l (equal to 45, 90 and 160 mg/kg bw per day in mice and to 35, 70 and 130 mg/kg bw per day in rats) led to the conclusion that nitrite is not carcinogenic in rats or male mice. The authors noted that there was equivocal evidence of carcinogenic activity of sodium nitrite in female mice, in view of the positive trend in the incidence of squamous-cell papilloma and carcinoma (combined) in the forestomach. As no evidence of genotoxicity in vivo or of cytotoxicity in the forestomach was found, the mode of action of sodium nitrite in inducing forestomach neoplasia is unclear.

A study of reproductive toxicity in mice given drinking-water containing sodium nitrite at concentrations up to 0.24% w/w (equivalent to 240 mg/kg bw per day) did not reveal adverse effects on reproductive performance or on end-points examined at necropsy.

New studies on genotoxicity were available. Sodium nitrite was mutagenic in a test for reverse mutation but did not induce micronucleus formation in the bone marrow or peripheral erythrocytes of mice treated in vivo, consistent with previous results.

A few studies on the endogenous formation of *N*-nitroso compounds after intake of nitrite or nitrate and of nitrosatable compounds (amines and amides) and the possible association with cancer were available. In addition, one long-term study of toxicity and carcinogenicity was reviewed, in which rats were fed fishmeal at various concentrations concomitantly with sodium nitrite. Dose-dependent increases in the incidences and multiplicity of atypical renal tubules, adenomas and renal-cell carcinomas were found. The concentration of *N*-nitrosodimethylamine in the stomach contents after 4 weeks of treatment with 64% fishmeal plus 0.12% sodium nitrite was twice that measured after administration of 8% fishmeal plus 0.12% sodium nitrite.

However, the diets used in this study were considered to be nutritionally inappropriate for the rat: they had a high protein content, resulting in abnormally high renal nitrogen clearance. These studies did not provide additional insight for the safety evaluation of nitrite.

A number of epidemiological studies of the relationship between the intake of nitrite and cancer risk have been published since the forty-fourth meeting. At its present meeting, the Committee ranked the study designs according to their capacity to provide evidence of a relationship. In the descriptions below, relative risk estimates are given for those studies in which levels of intake of nitrite were provided. Four of the studies were cross-sectional, involving measurement of nitrite in, e.g., salivary or gastric juice in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore, these studies cannot contribute to a causal interpretation of the results of studies of nitrite and cancer risk.

Nine (longitudinal) case–control studies on previous nitrite intake and various cancer types were reviewed. For oral and laryngeal cancer, no association was found with nitrite intake. One study conducted in the USA reported a positive association with oesophageal cancer, with odds ratios of 1.0 (reference category), 1.2 and 1.6 for persons with a daily nitrite intake of < 1.1 mg, 1.1–1.6 mg and > 1.6 mg, respectively. The odds ratios and the trend across odds ratios were not statistically significant, however. The association between nitrite intake and oesophageal cancer was stronger, and it was significant for persons with a history of canker sores. Another study in the USA, however, found no association between nitrite intake and oesophageal cancer, nor with the subtypes adenocarcinoma and squamous-cell carcinoma; a positive association was found only with gastric cancer other than of the cardia. A positive association with gastric cancer was also reported in an Italian case–control study (average consumption, 2.4 mg/day), while no association was found in a French study (average consumption, 1.9 mg/day).

An association of borderline significance was found between nitrite intake and urinary bladder cancer in men but not women of Japanese descent, nor in whites of either sex, in Hawaii, USA. Although a positive association was reported from a study in the USA between brain tumours in children and their mothers' consumption of processed meat, no association was found with nitrite intake during gestation or in childhood in a recent case–control study from Israel. One study on nasopharyngeal cancer among Taiwanese reported no association with nitrite intake in adulthood (as reported by cases and controls), but a positive association was found with childhood nitrite

intake as recalled by the mothers of the cases and controls. The validity of recall of remote dietary intake is questionable, however.

Two prospective cohort studies have been conducted on nitrite intake and cancer risk. A cohort study from the Netherlands, with 6 years of follow-up, on dietary nitrite and gastric cancer risk reported relative risks of 1.0 (reference category), 1.2, 1.2, 0.9 and 1.4 for increasing mean quintiles of nitrite intake of 0.01, 0.04, 0.09, 0.16 and 0.35 mg/day, respectively. Neither the relative risks nor the trend was significant. A Finnish cohort study, with 24 years of follow-up, reported no association with the incidence of stomach, colorectal, or head-and-neck tumours. The average nitrite intake by this cohort was reported to be 5.3 mg/day.

Thus, some studies indicated increased risks for oesophageal and gastric cancer; however, other studies — particularly prospective cohort studies — revealed no such association. The results for brain tumours in children and for urinary bladder cancer in adults were equivocal. Wide variation between the studied populations in the recorded intake of nitrite was noted. In none of these studies was a possible interaction between nitrite and nitrosatable amines evaluated in respect of cancer risk. The results of these studies and those of the epidemiological studies considered by the Committee at its forty-fourth meeting do not provide evidence that nitrite is carcinogenic to humans. In addition, studies on nitrate intake and cancer risk (of relevance because of the conversion of nitrate to nitrite) also did not provide evidence of a positive association.

A 6-week study was performed in young adult and old rats to determine whether older animals are more sensitive to the toxic effects of nitrite, particularly with regard to the known effects on the kidney and on the functions of some hormones (insulin, thyroxine). No age-related differences were found. The hypertrophy of the zona glomerulosa in the adrenals of rats of this strain was seen only at an intake equivalent to 50 mg/kg bw per day, expressed as nitrite ion.

Several studies were available on the mechanism of action of nitrite on the vascular system, blood pressure and the adrenals in rats. Doses of 50 mg/kg bw or higher, expressed as nitrite ion, are known to lower blood pressure by causing vasodilatation. The Committee calculated that an oral dose equivalent to 160 mg/kg bw of nitrate ion induced a reduction in blood pressure of 15–20 mm Hg.

Administration of nitrite to rats resulted in minimal hypertrophy of the zona glomerulosa of the adrenal gland and secretion of aldosterone, which was reversible after 60 days. Inhibition of angiotensin-converting enzyme indicated that the effect was produced indirectly via stimulation of the renin–angiotensin axis.

The Committee concluded that the minimal hypertrophy reflected physiological adaptation to small fluctuations in blood pressure and should not be considered a direct toxic action on the adrenals. This conclusion implies that the safety evaluation should not be based on the NOEL for minimal hypertrophy of the adrenal zona glomerulosa, used by the Committee at its forty-fourth meeting, but on NOELs for other end-points. The NOEL of 5.4 mg/kg bw per day (expressed as nitrite ion) was therefore considered to be no longer relevant, as it was based on the indirect effect on the adrenals described above. The Committee established an ADI of 0–0.07 mg/kg bw, expressed as nitrite ion, on the basis of the NOEL of 6.7 mg/kg bw per day for effects on the heart and lung in a 2-year study in rats and a safety factor of 100.

Nitrite causes methaemoglobinaemia. As this might occur after a single dose, it would be appropriate to establish an acute reference dose for nitrite. The data available for review at the present meeting related primarily to its long-term toxicity and were not relevant for establishing an acute reference dose. The Committee recommended that the acute toxicity of nitrite be reviewed at a future meeting.

Intake

Ideally, all sources of nitrite should be included in an intake assessment; however, only food and water were included in the assessments made at this meeting. The intake of nitrite from non-food additive sources, on the basis of the concentrations in food derived from European studies and the GEMS/Food regional diets and with an addition for water was below the ADI (40–50% of the ADI for the various diets). Major contributors to the estimated intake from sources other than food additives are cereals (35–60% of the estimated intake) and water (20–40% of the estimated intake).

The estimated intakes from use of nitrite as a food additive derived from national assessments were above the ADI, assuming maximum levels in the Codex draft GSFA. The intake of nitrite by consumers at the mean represented 460–500% of the ADI, while that of consumers at the 95th percentile represented 1300–1400% of the ADI. The intakes of nitrite from food additive use based on maximum permitted levels in national standards also exceeded the ADI (consumers at the mean, 100–130% of the ADI; consumers at the 95th percentile, 320–360% of the ADI). The estimated intakes based on the GSFA do not reflect reality, as the calculations include the GSFA maximum levels, which often are significantly higher than the typical levels of addition to foods by manufacturers. The calculations also do not take into consideration degradation of nitrite over time and do not reflect the concentrations in foods as consumed. The intakes are based on 24-h food consumption data, which tend to result in overestimates of intake by consumers at high percentiles.

More realistic estimates of the intake of nitrite from all dietary sources, submitted in total diet and duplicate diet studies, showed mean intakes representing 5–15% of the ADI, although the intakes by consumers at higher percentiles approached the ADI and exceeded it in one study, representing 55–330% of the ADI. A major contributor to the total intake estimated from these studies was beverages (specific types not stated), which accounted for 30–35% of the total estimated intake, owing more to the high volumes consumed than to high concentrations of nitrite. Analysed concentrations in foods as consumed were used in these studies. These results confirm that the estimates of intake from use of nitrite as a food additive at the listed maximum levels in the GSFA or national maximum permitted levels are overestimates.

The Committee recommended that assessments of the intake of nitrite should also include sources other than food additives, such as vegetables and drinking-water. Studies with more accurate methods of assessment should be conducted, based on analysed concentrations of nitrite in foods. Furthermore, foods that are 'ready to consume' should be included to account for losses of the chemicals over time and during food storage, preparation and cooking, as occurs for nitrite.

The Committee recommended that the Codex Committee on Food Additives and Contaminants reconsider the list of maximum levels of nitrite in the GSFA, as the estimated intakes might exceed the ADI.

An addendum to the toxicological monograph, including an assessment of intake, was prepared.

Nitrate

Explanation

Nitrate occurs in the environment, in air, food (particularly in vegetables and fruits) and water, and is produced endogenously. It is also used as a food additive, mainly as a preservative and anti-microbial agent. It is used in foods such as cheese and cheese products, raw and processed meats, edible casings, processed fish and fish products and spirits and liqueurs.

Nitrate was reviewed by the Committee at its sixth, eighth, seventeenth and forty-fourth meetings (Annex 1, references *6, 8, 32* and *116*). At its sixth meeting, the Committee allocated an ADI of 0–5 mg/kg bw, expressed as sodium nitrate. This ADI was based on a NOEL of 500 mg/kg bw per day for body-weight gain at a higher dose in a long-term study in rats and a short-term study of toxicity in dogs, with a safety factor of 100. This ADI was retained by the Committee at its eighth and seventeenth meetings. After the latter meeting, numerous toxicological and epidemiological data became

available, which were considered by the Committee at its forty-fourth meeting.

At that meeting, the Committee concluded that nitrite should also be considered, because nitrate is readily converted in humans to nitrite. The rat was considered an unsuitable animal model for assessing the toxicity of nitrate, as it does not convert nitrate to nitrite in a quantitatively similar way to humans. Nevertheless, as the data on the toxicity of nitrate in other animal species were considered limited, the toxicity of nitrite in rats and the conversion rate of nitrate to nitrite were also evaluated.

The Committee at its forty-fourth meeting concluded that nitrate itself was not genotoxic, and the results of studies of carcinogenicity with nitrate were negative except when extremely high doses of both nitrate and nitrosatable precursors were administered. The available epidemiological data were considered to provide no evidence for an association between exposure of humans to nitrite and the risk for cancer. On the basis of this information, the NOEL of 370 mg/kg bw per day, expressed as nitrate ion, in a long-term study in rats was considered to be the most appropriate for the safety evaluation. When the proportion of nitrate converted to nitrite in humans was taken as 5% for the average individual and 20% for those with a high level of conversion, and when the NOEL for nitrite (6 mg/kg bw per day, expressed as nitrite ion) was used to calculate the 'transposed' NOEL for nitrate, expressed as nitrate ion, these values were estimated to be 160 and 40 mg/kg bw per day for average and high responders, respectively. As these figures were derived in part from data on human pharmacokinetics, use of a safety factor of less than 100 was considered to be justified.

On the basis of the NOEL of 370 mg/kg bw per day, expressed as nitrate ion, and a safety factor of 100, an ADI of 0–5 mg/kg bw expressed as sodium nitrate or 0–3.7 mg/kg bw expressed as nitrate ion was allocated. On the basis of the 'transposed' NOEL for nitrate of 160 mg/kg bw per day for normally responding persons (5% rate of conversion) and a safety factor of 50, an ADI of 0–3.2 mg/kg bw, expressed as nitrate ion, could be allocated. These two methods of deriving the ADI for nitrate thus resulted in similar figures, and the Committee at its forty-fourth meeting therefore retained the previously established ADI of 0–3.7 mg/kg bw, expressed as nitrate ion.

The Committee at its present meeting reviewed data relevant to the evaluation of nitrate which had become available since its forty-fourth meeting. Studies illustrating the relevance and severity of certain effects and other supporting data were also considered. New information on metabolism and toxicokinetics were included. Data on the putative health benefits of nitrate were not assessed, as this is not a safety issue and is therefore not within the purview of the Committee.

The few new studies on the toxicokinetics and metabolism of nitrate in animals that have become available since the forty-fourth meeting of the Committee confirm that the rat is not a good surrogate species for humans in this respect, as it does not show salivary transport of nitrate and therefore has limited conversion of nitrate to nitrite .

In a study of the conversion of nitrate to nitrite in humans, in which sodium nitrate was administered in drinking-water at a single dose of 7.3 mg/kg bw, expressed as nitrate ion, neither blood pressure nor methaemoglobin concentration was affected. The nitrite concentration of the gastric juice was approximately six times higher after administration of nitrate in combination with pretreatment with omeprazole at 40 mg/day (which increased the gastric pH) than after nitrate alone. Nitrate was absorbed rapidly, the concentration in plasma increasing within 10 min, and the half-life of nitrate in plasma was about 6.5 h; about 70% of the dose was excreted in urine within 10 h of dosing. The plasma concentration of nitrite did not change after nitrate administration. About 8% of the total nitrate administered was converted to nitrite in saliva, as found in other studies. The Committee at its forty-fourth meeting concluded that the range of nitrate conversion is 5–7% for normal individuals and 20% for individuals with a high rate of conversion.

The results of studies in humans that considered the potential of high intake of nitrate to cause methaemoglobinaemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinaemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinaemia. No increase in methaemoglobin concentration was seen in volunteers after a single administration of sodium nitrate in drinking-water providing a dose of 7.3 mg/kg bw, expressed as nitrate ion.

A study in humans showed that nitrate in vegetable matrices and from other sources, such as drinking-water, is almost totally bioavailable.

As nitrate shares a common transport mechanism with iodide, studies were conducted to determine whether nitrate affects thyroid function. A 28-day study with volunteers given sodium nitrate in drinking-water at a concentration equivalent to 15 mg/kg bw per day (11 mg/kg bw per day expressed as nitrate ion) showed no effects on thyroid function and no increase in the per cent of methaemoglobinaemia. A 90-day study of toxicity in rats showed that sodium nitrate at a dose of 50 mg/kg bw per day did not affect the thyroid or the zona glomerulosa of the adrenals.

In studies in humans, consumption of drinking-water containing sodium nitrate at a concentration of 2800 mg/l concomitantly with volatile *N*-nitro-

satable amines in the diet (in cod, salmon or shrimp) led to a two- to threefold increase in urinary excretion of *N*-nitrosodimethylamine and *N*-nitrosopiperidine.

Several studies were reviewed on the effect of administration of nitrate on the release of nitric oxide at the junction of the oesophagus and the stomach in humans, which, it was speculated, might be associated with an increased incidence of cancer at this site. However, no such association has been observed in epidemiological studies.

A number of epidemiological studies have been published since the forty-fourth meeting of the Committee on the relationship between nitrate intake and cancer risk. At its present meeting, the Committee ranked the study designs according to their capacity to provide evidence of a relationship. In the descriptions below, relative risk estimates are given for those studies in which levels of intake of nitrate were provided.

Six ecological (correlation) studies were reported on nitrate in drinking-water and mortality from or incidence of cancer. Elevated risks were found for prostate cancer and for brain tumours (each in one study), but the results of six studies on gastric cancer were conflicting. The results of ecological studies (in which populations are the units of measurement) cannot be extrapolated to the individual level. Furthermore, most of the ecological studies were based on limited data on nitrate concentrations and on cancer mortality rates (rather than incidence rates), and none took an induction period for cancer into account.

Three of the studies were cross-sectional, involving measurement of, e.g., salivary nitrate in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore these studies could not contribute to a causal interpretation of the results of studies of nitrate intake and cancer risk.

Seven case–control studies on nitrate in drinking-water and/or food and cancers at various sites were reviewed. In the studies on nitrate in drinking-water, conflicting results were reported with regard to an association with non-Hodgkin lymphoma, and no association was found with brain tumours. In the studies on dietary nitrate, no association was found with oral, oesophageal, gastric or testicular cancer. No other cancer sites have been studied.

Three prospective cohort studies have been conducted on nitrate intake and cancer risk. A cohort study in the Netherlands, with 6 years of follow-up, found no significant association between the incidence of gastric cancer and

intake of nitrate from food or drinking-water, with relative risks for increasing quintiles of total nitrate intake of 1.0 (reference quintile), 1.2, 0.7, 0.9 and 0.9 for mean intakes of 60, 85, 100, 120 and 180 mg/day, respectively. Neither the relative risks nor the trend across relative risks was significant. A further analysis of the effect of nitrate within tertiles of vitamin C intake also did not reveal a positive association between nitrate intake and gastric cancer. A Finnish cohort study on dietary nitrate, with 24 years of follow-up, reported no association with the risks for tumours of the stomach, colorectum or head and neck. The average nitrate intake in this cohort was reported to be 77 mg/day. A cohort study in Iowa, USA, with 11 years of follow-up, revealed no consistent association between intake of nitrate from drinking-water and the risks for cancers at many sites, and an inverse association was reported with cancers of the uterus and rectum. Positive associations with nitrate intake were observed only for cancers of the ovary and urinary bladder, although it was not possible to determine whether other factors in drinking-water were responsible for these associations. In addition, no evidence of a dose–response relationship was found for any of the cancer sites addressed in the study in Iowa. The cohort studies included control for various potential confounders, such as intake of vegetables, age and smoking.

Overall, the epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. These data, combined with the results of the epidemiological studies considered by the Committee at its forty-fourth meeting, did not provide evidence that nitrate is carcinogenic to humans.

A number of studies were performed to determine whether there are associations between nitrate intake in drinking-water and insulin-dependent diabetes mellitus, neural tube defects or sudden infant death syndrome. In none of these studies was a hypothesis proposed for the mechanism of an association. Two studies were conducted on the incidence of insulin-dependent diabetes mellitus and nitrate intake via drinking-water. One study in Yorkshire, United Kingdom, suggested a positive association, but the authors considered that the finding required confirmation. A study in the Netherlands with a larger number of subjects did not show a positive association. The two studies on nitrate intake and neural tube defects also showed no association. In a recent ecological study in Sweden, a correlation was reported between nitrate concentration in drinking-water and the occurrence of sudden infant death syndrome; however, no confounding factors were taken into account. The Committee concluded that it would be premature to include these observations in its safety assessment.

The Committee concluded that the pivotal observed toxic effects of nitrate are consequent on its conversion to nitrite in vivo. The Committee at its present meeting established an ADI of 0–0.07 mg/kg bw for nitrite (see earlier

part of this section). As the new data on nitrite would not provide a basis for a significant change in the previous ADI for nitrate, the Committee retained the ADI of 0–5 mg/kg bw expressed as sodium nitrate, or 0–3.7 mg/kg bw, expressed as nitrate ion, established at its forty-fourth meeting.

Intake

Ideally, all sources of nitrate should be included in an intake assessment; however, contributions from air were not included here. Nitrate intake from sources other than food additives, based on concentrations in food derived from European studies and the GEMS/Food regional diets and with an addition for water, was below the ADI (10–70% of the ADI for the various diets). Major contributors to intake were vegetables (30–65% of the estimated intake) and water (5–45% of the estimated intake). The nitrate content of both groundwater and surface water is highly variable, and wide variations are seen between countries.

The estimated intakes from use of nitrate as a food additive derived from national assessments were below the ADI, assuming the maximum levels in the Codex draft GSFA. The intake by consumers at the mean was about 10% of the ADI, while the intake of those at the 95th percentile was 30–35% of the ADI, on the basis of national data on food consumption. The intakes of nitrate from food additive use based on maximum permitted levels in national standards were also below the ADI (consumers at the mean, 10% of the ADI; consumers at the 95th percentile, 35% of the ADI). Major contributors from its use as a food additive were processed comminuted meats in national assessments (50–55% of estimated intake) and raw meats on the basis of GSFA maximum levels (50–55% of the estimated intake) from national food consumption data. These intakes were based on 24-h food consumption data, which tend to result in overestimates of the intake of consumers at high percentiles.

Assessments of intake of nitrate from all dietary sources, including drinking-water, showed that they were below the ADI. In total diet studies in the United Kingdom, the mean intakes were between 40% and 45% of the ADI and those by consumers at the 95th percentile were 60–75% of the ADI. Duplicate diet studies showed mean intakes representing 30–35% of the ADI and intakes by consumers at the 95th percentile representing 95–125% of the ADI. These studies included one of vegetarians, who are likely to have higher intakes of nitrate owing to their greater consumption of vegetables; however, their intakes were not considerably different from those of the general population. Major contributors to total dietary intake of nitrate were vegetables, which accounted for 70–75% of the total estimated intake. The Committee noted that the food additive use of nitate is not a major source of intake.

The Committee recommended that the Codex Committee on Food Additives and Contaminants reconsider the maximum levels of nitrate in the GSFA, as the estimated intakes of nitrate might exceed the ADI.

The Committee recommended that assessments of the intake of nitrate also include sources other than food additives, such as vegetables and drinking-water. Studies should be conducted in which more accurate methods of assessment are used, which provide estimates of intake based on analysed concentrations of chemicals in foods. Studies should be conducted in different seasons to account for natural variations in concentrations in foods such as vegetables.

An addendum to the toxicological monograph, including an assessment of intake, was prepared.

3.1.5 *Salatrim*

Explanation

Salatrim is the acronym for **s**hort- **a**nd **l**ong-chain **acyltri**glyceride **m**olecules. Salatrim preparations are tailored mixtures of triglycerides containing at least one long-chain fatty acid moiety (principally stearic acid) and one or two short-chain fatty acid moieties (acetic, propionic and/or butyric acid). Salatrim is synthesized by non-enzymatic inter-esterification of triacetin, tripropionin, tributyrin or their mixtures with hydrogenated canola, soya bean, cottonseed or sunflower oils. The physical properties of salatrim may be fitted to its food applications by selecting specific short-chain fatty acids and hydrogenated oils for synthesis. Salatrim is intended for use as a low-calorie fat in soft sweets, coatings (e.g., wafers and confectionery), dairy products (including spreads), margarines and bakery products.

Salatrim of various compositions was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*), when it concluded that the available studies did not provide an adequate basis for evaluating the safety and the nutritional effects of salatrim. At that meeting, the Committee recommended that additional, appropriately designed studies be performed to assess fully both the toxicological and nutritional consequences of ingestion of salatrim.

The Committee noted at its forty-ninth meeting that the specifications for salatrim permit compositions that include a triglyceride mixture with up to 0.87 g of stearate per gram of fat, but that the available biological data did not provide information on materials of such a composition. Since that time, the sponsor has proposed that the specifications for salatrim be limited to preparations with ≤ 70% by weight of total saturated long-chain fatty acids. Studies were available on the biological effects in humans of salatrim containing up to 0.61 g of stearate per gram of fat.

The Committee previously evaluated studies on the caloric value of salatrim, taking into account the fact that short-chain fatty acids supply fewer kilocalories per gram than long-chain fatty acids. Moreover, it concluded that the claim of reduced absorption of stearic acid has not been proven for humans. In addition, as there is no specific composition for salatrim, a single caloric value could not be assigned.

In evaluating the safety of salatrim at its forty-ninth meeting, the Committee considered studies in which salatrim of various compositions was administered to rats or minipigs at concentrations of up to 10% in the diet. These studies showed no toxicologically significant effects, but the study in minipigs was considered to have been of insufficient duration. The Committee also discussed one home-based trial and four clinic-based studies with volunteers who ingested up to 60 g salatrim per person per day. The studies indicated that the consumption of diets containing salatrim, particularly at 60 g per day, was associated with an increased incidence of mild gastrointestinal symptoms and significantly elevated serum enzyme activities.

Thus, the Committee concluded at its forty-ninth meeting that the available studies in experimental animals and humans did not provide an adequate basis for a toxicological evaluation. In addition, as the studies were not optimized to detect potential nutritional effects, the Committee concluded that they did not provide an adequate basis for a nutritional evaluation.

In response to the Committee's earlier request, four additional home-based trials in humans and one study of nutrition in rats were submitted for consideration at the present meeting.

Toxicological data

The Committee concluded that the newly submitted study of nutrition in rats did not resolve the issue of the extent to which salatrim might reduce energy intake since, as stated previously, the rat is not a good model for humans with respect to stearic acid absorption.

The aim of the home-based trials was to investigate plasma lipid profiles in small groups of adults ingesting salatrim at 30 g/day for up to 5 weeks. Diets containing salatrim did not affect the blood concentration of total high-density or low-density lipoprotein cholesterol. The post-prandial increase in serum triglyceride concentration was lower after ingestion of a meal containing 30 g of salatrim than after ingestion of a meal containing 30 g of oleate or cocoa butter. In one study, it was reported that some of the volunteers complained of gastric discomfort, nausea or feeling 'bloated' or 'satiated and averse to eating until the early evening' after a breakfast containing 30 g of salatrim. However, adequate controls were not included in this study, and it was not clear if these subjective symptoms were related to ingestion of

salatrim. The Committee was informed that, in one of these trials, serum liver enzyme activities were found to be within the normal range; however, these results were not included in the published manuscript.

Overall, the Committee concluded that the new studies did not respond to the questions raised at its forty-ninth meeting and the available information was inadequate for a toxicological evaluation. The Committee noted that, in general, toxicological studies in experimental animals should be conducted at doses that allow an adequate margin of safety in relation to anticipated human intake. Such safety margins cannot feasibly be attained with substances, such as salatrim, that are intended for use as major components of food. Additionally the rat is not a good model for humans with respect to stearic acid absorption, and further studies in experimental animals would not contribute to the safety evaluation of salatrim. The Committee therefore paid particular attention to the results of the studies in humans. These studies indicated that adverse effects (gastrointestinal and hepatic effects) might be experienced by adults consuming diets containing salatrim at 60 g/day (equivalent to 1 g/kg bw per day). The available information was not adequate to evaluate whether effects might occur at intakes of less than 60 g/day, nor if the effects differ with the composition of salatrim.

Intake

Data on the projected intake of salatrim were assessed by the Committee at its forty-ninth meeting. These data, with small revisions, and with projections of intake based on data on consumption in the United Kingdom, were submitted to the present meeting. Manufacturers' use levels for salatrim vary from 1–2 g/100 g in some dairy products and crackers to more than 80 g/100 g in margarine.

The mean intake of salatrim after hypothetical substitution of added fats by this substance in all food categories would be 45 g/day, but this value is unrealistic. The mean intake of salatrim based on hypothetical substitution of fats in specified food categories would vary from 11 to 13 g/day. Intake by adolescent consumers at the 90th percentile would reach 35 g/day. On a gram per kilogram body-weight basis, the highest intake would be that of children aged less than 2 years, as those at the 90th percentile of consumption would have an intake of 1.5 g/kg bw per day. Children aged 1–6 years who are consumers of the category 'chocolates and confectionery' at the 90th percentile would have an intake of salatrim from this food category alone of 1.1 g/kg bw per day, which is higher than the intake reported to have effects in adults in clinical studies.

The Committee concluded that the available data did not provide an adequate basis for evaluating the safety of salatrim and recommended that additional studies be performed in humans to assess fully the safety of salatrim covering

the range of compositions intended for use. The studies should be designed appropriately for safety evaluation and should include investigation of the gastrointestinal and hepatic effects observed in previous studies.

A monograph reproducing the previous monograph and incorporating the information that had become available since the previous evaluation was prepared. The updated monograph includes corrections to the information provided to the Committee at its forty-ninth meeting.

Specifications

The existing specifications for salatrim were revised. The assay was amended by introducing a maximum upper limit of 70% by weight of saturated long-chain fatty acids. Calculation of the weight per cent of saturated long-chain fatty acids in salatrim requires that the molar ratio of short-chain to long-chain fatty acids be determined with analysis for fatty acid butyl esters by gas chromatography (method I in the original specifications). Therefore, the alternative method II, with identification of fatty acid residues by proton nuclear magnetic resonance spectrometry, was considered to have no further value and was deleted.

3.2 Revision of specifications

3.2.1 *Amyloglucosidase from* Aspergillus niger, *var.*

Amyloglucosidase from *Aspergillus niger,* var. is a hydrolytic enzyme produced by controlled fermentation of this organism. The enzyme is isolated from the growth medium and is used to hydrolyse α-glucosidic linkages in starch.

The existing specifications for amyloglucosidase from *A. niger,* var. were revised, and a new procedure was incorporated for assaying enzyme activity in products, including those containing glucose. The tentative designation to the specifications was removed.

3.2.2 *Limits for heavy metals in colours and acidity regulators*

At its fifty-fifth meeting (Annex 1, reference *149*), the Committee began implementation of a systematic 5-year programme to replace the outdated test for heavy metals (as lead) in all existing food additive specifications with appropriate limits for individual metals of concern. At the present meeting, the heavy metals and arsenic limits of 52 colours and 44 acidity regulators were reviewed

Colours

In response to the call for data, some proposed limits and supporting data were received for a number of colours from biological sources ('natural colours'). No data were received for synthetic or inorganic colours.

The proposed changes to the current limits are:

For natural colours

- The limits for arsenic were maintained because information was received that the plant sources for certain natural colours grow in areas where the level of arsenic in groundwater is high. The Committee agreed to review these limits at a future meeting when arsenic is on the agenda for evaluation.
- The proposed limit for lead was set at 2 mg/kg, except in certain natural colours containing higher levels or levels set at meetings following the forty-ninth meeting of the Committee. The limit of 2 mg/kg is consistent with the previously agreed policy.
- No limits were proposed for cadmium.
- Limits for mercury were proposed for two natural colours.
- Limits for heavy metals (as lead) were deleted.

For synthetic colours in accordance with the Committee's policy:

- Limits for arsenic were deleted.
- The limit for lead was set at 2 mg/kg.
- No limits were proposed for cadmium.
- Limits for mercury were deleted.
- Limits for heavy metals (as lead) were deleted.

For inorganic colours

The Committee concluded that, because of the high levels of heavy metals in the present specifications for aluminium powder, iron oxides and titanium dioxide, the specifications should be reconsidered. Therefore the Committee maintained the existing limits and decided to call for data on the raw materials, manufacturing methods and analytical data on impurities for review at a future meeting.

Acidity regulators

No data were received. The Committee followed its past practice of including:

- a limit of 3 mg/kg of arsenic for phosphates and calcium compounds;
- a limit of 4 mg/kg of lead for phosphates, but 2 mg/kg for other substances.
- Levels of cadmium and mercury were not considered to be of concern and were not specified.
- Limits for heavy metals (as lead) were deleted.

The conclusions of the Committee on limits for heavy metals in colours and acidity regulators are summarized in Table 2.

Table 2
Limits for heavy metals in 52 colours and 44 acidity regulators

Category	Food additive	INS		As	Pb	Cd	Hg
Colour	Allura red AC	0129		-	2	-	-
Colour	Aluminum powder	0173		3	20	-	-
Colour	Amaranth	0123		-	2	-	-
Colour	Annatto extracts(oil & alkali extracted)	0160	b	3	2	-	1
Colour	Annatto extracts(solvent extracted)	0160	b	3	2	-	1
Colour	β-Apo-8'-carotenal	0160	e	-	2	-	-
Colour	β-Apo-8'-carotenic acid ethyl ester	0160	f	-	2	-	-
Colour	Azorubine	0122		-	2	-	-
Colour	Beet red	0162		3	2	-	-
Colour	Blackcurrant extract	0163	ii	-	2	-	-
Colour	Brillant Black BN	0151		-	2	-	-
Colour	Brillant Blue FCF	0133		-	2	-	-
Colour	Brown FK	0154		-	2	-	-
Colour	Brown HT	0155		-	2	-	-
Colour	Canthaxanthin	0161	g	-	2	-	-
Colour	Caramel colours	0150	a,b,c,d	1	2	-	-
Colour	Carmines	0120		-	5	-	-
Colour	β-Carotene, synthetic	0160	a(i)	-	2	-	-
Colour	β-Carotene from *Blakeslea trispora*	0160	a	-	2	-	-
Colour	Carotenes (algae)	0160	a(ii)	-	5	-	-
Colour	Carotenes (vegetable)	0160	a(ii)	-	5	-	-
Colour	Carthamus red	-		-	5	-	-
Colour	Carthamus yellow	-		-	5	-	-
Colour	Chlorophyllins, copper complexes	0141	ii	3	5	-	-
Colour	Chlorophylls	0140		3	5	-	-
Colour	Chlorophylls, copper complexes	0141	i	3	5	-	-
Colour	Cochineal extract	0120		-	5	-	-
Colour	Curcumin	0100		-	2	-	-
Colour	Erythrosine	0127		-	2	-	-
Colour	Fast Green FCF	0143		-	2	-	-
Colour	Fast Red E	-		-	2	-	-
Colour	Grape skin extract	0163	ii	3	2	-	-
Colour	Green S	0142		-	2	-	-
Colour	Indigotine	0132		-	2	-	-
Colour	Iron oxides	0172		3	10	10	1
Colour	Lithol rubine BK	0180		-	2	-	-
Colour	Mixed carotenoids			-	5	-	-
Colour	Paprika oleoresin	0160	c	3	2	-	-
Colour	Patent Blue V	0131		-	2	-	-
Colour	Ponceau 4R	0124		-	2	-	-
Colour	Quinoline Yellow	0104		-	2	-	-
Colour	Red 2G	0128		-	2	-	-
Colour	Riboflavin	0101	i	-	2	-	-
Colour	Riboflavin 5'-phosphate sodium	0101	ii	-	2	-	-
Colour	Riboflavin from *Bacillus subtilis*	0101	I	-	1	-	-
Colour	Saffron	-		3	2	-	-
Colour	Sunset Yellow FCF	0110		-	2	-	-
Colour	Tagetes extract	-		-	2	-	-
Colour	Tartrazine	0102		-	2	-	-
Colour	Titanium dioxide	0171		3	10	-	1
Colour	Turmeric oleoresin	0100	ii	3	2	-	-
Colour	Vegetable carbon	0153		3	2	-	-

Table 2 (continued)

Category	Food additive	INS		As	Pb	Cd	Hg
Acidity regulator	Acetic acid, glacial	0260		-	2	-	-
Acidity regulator	Ammonia solution	0527		-	2	-	-
Acidity regulator	Ammonium carbonate	0503	i	-	2	-	-
Acidity regulator	Ammonium dihydrogen phosphate	0342	i	3	4	-	-
Acidity regulator	Calcium citrates	0333		-	2	-	-
Acidity regulator	Calcium dihydrogen phosphate	0341	i	3	4	-	-
Acidity regulator	Calcium DL-malate	0352	ii	-	2	-	-
Acidity regulator	Calcium hydroxide	0526		-	2	-	-
Acidity regulator	Calcium lactate	0327		-	2	-	-
Acidity regulator	Calcium oxide	0529		-	2	-	-
Acidity regulator	Diammonium hydrogen phosphate	0342	ii	3	4	-	-
Acidity regulator	Dicalcium pyrophosphate	0450	vi	3	4	-	-
Acidity regulator	Dipotassium hydrogen phosphate	0340	ii	3	4	-	-
Acidity regulator	Disodium hydrogen phosphate	0339	ii	3	4	-	-
Acidity regulator	Disodium pyrophosphate	0450	i	3	4	-	-
Acidity regulator	Hydrochloric acid	0507		-	1	-	-
Acidity regulator	Magnesium hydroxide carbonate	0504	ii	-	2	-	-
Acidity regulator	Magnesium hydroxide	0528		-	2	-	-
Acidity regulator	Magnesium DL-lactate	0329		-	2	-	-
Acidity regulator	Phosphoric acid	0338		3	4	-	-
Acidity regulator	Potassium carbonate	0501	i	-	2	-	-
Acidity regulator	Potassium dihydrogen citrate	0332		-	2	-	-
Acidity regulator	Potassium hydrogen carbonate	0501	ii	-	2	-	-
Acidity regulator	Potassium hydroxide	0525		-	2	-	-
Acidity regulator	Sodium acetate	0262	I	-	2	-	-
Acidity regulator	Sodium carbonate	0500	I	-	2	-	-
Acidity regulator	Sodium dihydrogen citrate	0331	i	-	2	-	-
Acidity regulator	Sodium dihydrogen phosphate	0339	i	3	4	-	-
Acidity regulator	Sodium DL-malate	0350	ii	-	2	-	-
Acidity regulator	Sodium fumarate	0365		-	2	-	-
Acidity regulator	Sodium hydrogen carbonate	0500	ii	-	2	-	-
Acidity regulator	Sodium hydrogen DL-malate	0350	i	-	2	-	-
Acidity regulator	Sodium hydroxide	0524		-	2	-	-
Acidity regulator	Sodium sesquicarbonate	0500	iii	-	2	-	-
Acidity regulator	Sulfuric acid	0513		-	2	-	-
Acidity regulator	Triammonium citrate	0380		-	2	-	-
Acidity regulator	Tripotassium citrate	0332	ii	-	2	-	-
Acidity regulator	Tripotassium phosphate	0340	iii	3	4	-	-
Acidity regulator	Trisodium citrate	0331	iii	-	2	-	-
Acidity regulator	Trisodium phosphate	0339	iii	3	4	-	-

INS, International Numbering System

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

Six groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116, 122, 131, 137, 143, 149* and *154*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

- Class I. Flavouring agents that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity by the oral route.
- Class II. Flavouring agents that have structural features that are less innocuous than those of substances in Class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Flavouring agents that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting:

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

Endogenous substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Intake data

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in the European Union during the previous year.

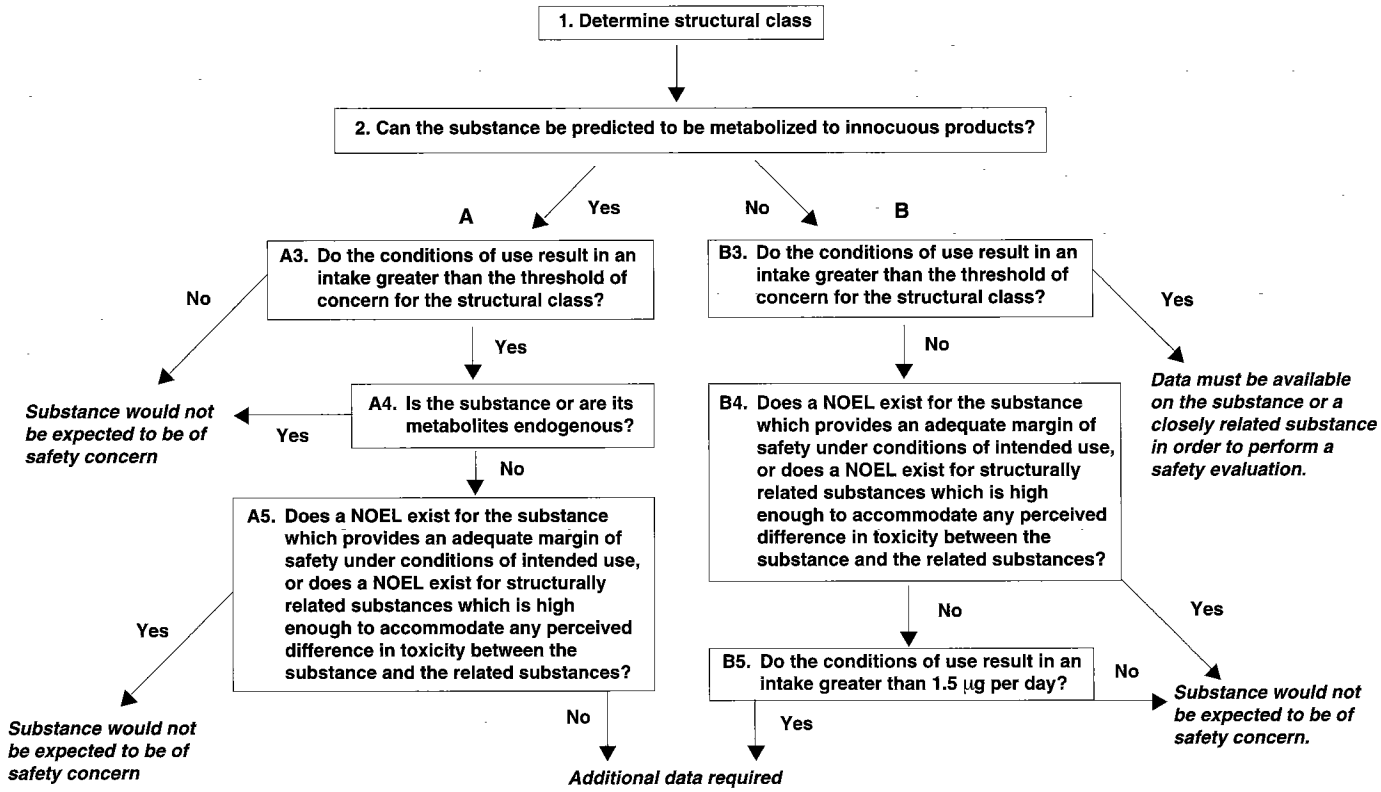


Figure 1
Procedure for the safety evaluation of flavouring agents

Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the National Academy of Sciences National Research Council (under contract to the Food and Drug Administration) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added in a number of broad food categories.

In using the data from these surveys to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported in Europe and 80% of the amount used is reported in the USA and that the total amount used in food is consumed by only 10% of the population.

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

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

Several of the flavouring agents that were evaluated at the present meeting were not included in the above surveys or were placed on the market after the surveys were conducted. Intakes of these flavouring agents were estimated on the basis of anticipated use by the manufacturer in the USA, and the standard formula was applied.

4.1.1 *Alicyclic primary alcohols, aldehydes, acids and related esters*

The Committee evaluated a group of 26 flavouring agents that included alicyclic primary alcohols, aldehydes, acids and related esters (see Table 3), using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). The Committee had not evaluated any of these agents previously.

Twenty of the 26 flavouring agents in this group are terpenoid alcohols, aldehydes or carboxylic acids or their related esters. Thirteen are common components of food and have been detected in vanilla, bourbon, rum, mango, rosemary, grapefruit juice, mandarin peel oil and blackberries.

Estimated daily per capita *intake*

The total annual volume of the 26 flavouring agents in this group that is produced is approximately 170 kg in Europe and 130 kg in the USA. None of the agents is produced in an annual volume greater than 55 kg. Most of the total annual volume in Europe is accounted for by (2,2,3-trimethylcyclopent-3-en-1-yl)acetaldehyde (No. 967; campholenic aldehyde), *p*-mentha-

Table 3

Summary of results of safety evaluation of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents

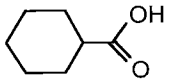
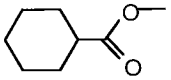
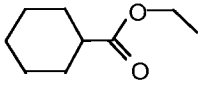
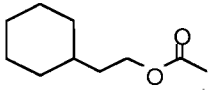
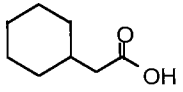
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Structural class I						
Cyclohexanecarboxylic acid	961	98-89-5 	A3 – No Europe: 0.07 USA: 4	N/R	See note 1	No safety concern
Methyl cyclohexanecarboxylate	962	4630-82-4 	A3 – No Europe: 0.09 USA: 0.01	N/R	See notes 1,2	No safety concern
Ethyl cyclohexanecarboxylate	963	3289-28-9 	A3 – No Europe: ND USA: 0.1	N/R	See notes 1,2	No safety concern
Cyclohexaneethyl acetate	964	21722-83-8 	A3 – No Europe: 1 USA: ND	N/R	See notes 2,3	No safety concern
Cyclohexaneacetic acid	965	5292-21-7 	A3 – No Europe: 0.1 USA: 0.4	N/R	See note 3	No safety concern

Table 3 (continued)

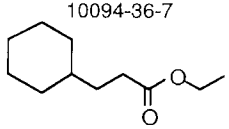
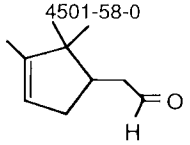
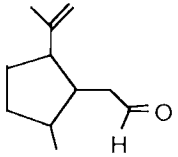
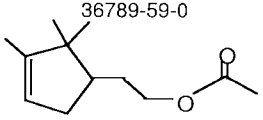
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Ethyl cyclohexanepropionate	966	10094-36-7 	A3 – No Europe: ND USA: 0.1	N/R	See notes 1,2	No safety concern
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	4501-58-0 	A3 – No Europe: 6 USA: ND	N/R	See note 4	No safety concern
cis-5-Isopropenyl-*cis*-2-methylcyclopentan-1-carboxaldehyde	968	55253-28-6 	A3 – No Europe: 0.01 USA: ND	N/R	See note 4	No safety concern
Campholene acetate	969	36789-59-0 	A3 – No Europe: 0.07 USA: ND	N/R	See notes 2,4	No safety concern

Table 3 (continued)

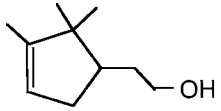
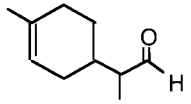
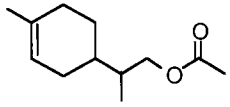
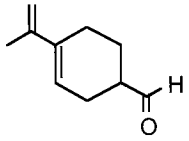
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
α-Campholenic alcohol	970	1901-38-8 	A3 – No Europe: 0.01 USA: ND	N/R	See note 4	No safety concern
p-Menth-1-ene-9-al	971	29548-14-9 	A3 – No Europe: 0.1 USA: ND	N/R	See note 4	No safety concern
1-*p*-Menthen-9-yl acetate	972	17916-91-5 	A3 – No Europe: 1 USA: ND	N/R	See notes 2,4	No safety concern
p-Mentha-1,8-dien-7-al	973	2111-75-3 	A3 – No Europe: 2 USA: 2	N/R	See note 4	No safety concern

Table 3 (continued)

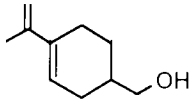
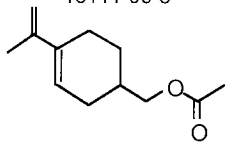
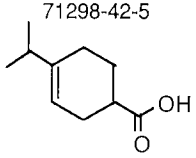
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
p-Mentha-1,8-dien-7-ol	974	536-59-4 	A3 – No Europe: 2 USA: 1	N/R	See note 4	No safety concern
p-Mentha-1,8-dien-7-yl acetate	975	15111-96-3 	A3 – No Europe: 0.4 USA: 0.07	N/R	See notes 2,4	No safety concern
1,2,5,6-Tetrahydrocuminic acid	976	71298-42-5 	A3 – No Europe: 0.01 USA: ND	N/R	See note 1	No safety concern

Table 3 (continued)

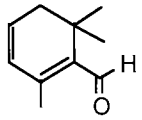
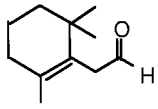
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	977	116-26-7 	B3 – No Europe: 4 USA: 0.07	Yes. The NOELs of 120 mg/kg bw per day for perillyl alcohol in 90-day studies in rats and dogs and NOELs of 10 mg/kg bw per day for α-ionone and β-ionone in a 90-day study in rats are > 2 million and > 200 000 times, respectively, the estimated intake of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal when used as a flavouring agent.		No safety concern
2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	978	472-66-2 	A3 – No Europe: 0.3 USA: 2	N/R	See note 3.	No safety concern

Table 3 (continued)

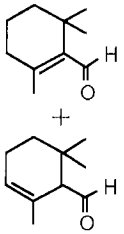
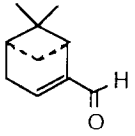
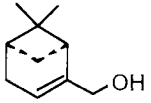
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
2,6,6-Trimethyl-1-cyclohexen-1-carboxaldehyde	979	432-25-7 	A3 – No Europe: 0.4 USA: ND	N/R	See note 1	No safety concern
2-Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	564-94-3 	A3 – No Europe: 4 USA: 7	N/R	See note 4	No safety concern
Myrtenol	981	515-00-4 	A3 – No Europe: 0.4 USA: 0.03	N/R	See note 4	No safety concern

Table 3 (continued)

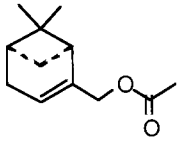
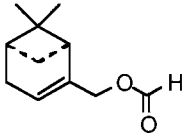
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Myrtenyl acetate	982	1079-01-2 	A3 – No Europe: 0.3 USA: 0.04	N/R	See note 4	No safety concern
Myrtenyl formate	983	72928-52-0 	A3 – No Europe: 0.4 USA: ND	N/R	See note 4	No safety concern

Table 3 (continued)

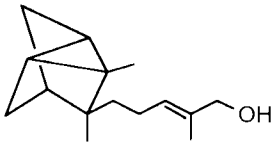
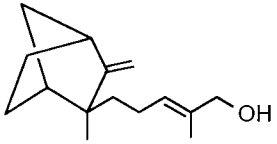
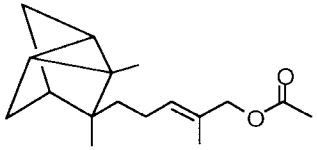
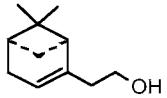
Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Santalol (α and β)	984	115-71-9  α	A3 – No Europe: 0.04 USA: ND	N/R	See note 4	No safety concern
		77-42-9  β				
Santalyl acetate	985	1323-00-8 	A3 – No Europe: ND USA: 0.01	N/R	See note 4	No safety concern

Table 3 (continued)

Flavouring agent	No.	CAS No. and structure	Steps A3 and B3[a,b] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
10-Hydroxymethylene-2-pinene	986	128-50-7 	A3 – No Europe: ND USA: 0.01	N/R	See note 4	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation

[a] Step 2: Except for No. 977, all the flavouring agents in this group were predicted to be metabolized to innocuous products.

[b] The threshold for human intake for structural class I is 1800 µg/day. All intake values are expressed in µg/day. The combined intake of all flavouring agents in this group is 22 and 18 µg/person per day in Europe and the USA, respectively.

Notes:

1. Metabolized by β-oxidation, aromatization and conjugation with glycine and glucuronic acid
2. Metabolized by hydrolysis followed by oxidation and/or conjugation
3. Metabolized by β-oxidation and eliminated largely by metabolism via the citric acid cycle
4. Metabolized largely by oxidation of the side-chain to the corresponding carboxylic acid, which is excreted unchanged and as conjugates

1,8-dien-7-al (No. 973; perillaldehyde), *para*-mentha-1,8-dien-7-ol (No. 974; perillyl alcohol), 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980; myrtenal). In the USA, approximately 91% of the total annual volume is accounted for by cyclohexanecarboxylic acid (No. 961), *para*-mentha-1,8-dien-7-al (No. 973; perillaldehyde), 2,6,6-trimethyl-1-cyclohexen-1-acetaldehyde (No. 978; β-cyclocitral) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980; myrtenal). The estimated daily *per capita* intake of 11 of the substances in this group is ≤ 10 μg.

Absorption, distribution, metabolism and elimination

The number of potential pathways of metabolism of alicyclic substances increases as the number and types of functional groups and ring substituents in the molecule increase. The cyclohexane derivatives (carboxylic acids and their related esters) in this group (Nos 961–966) can undergo β-oxidation and subsequent ring cleavage and/or aromatization of the cyclohexane ring and/or can be excreted in urine as cyclohexane carboxylic acid and its glycine and glucuronide conjugates. If there is an even number of carbons in the side-chain, β-oxidative cleavage leads to ring-opened oxygenated metabolites, primarily dicarboxylic acids, which are metabolized via the citric acid cycle. If there is an odd number of carbons in the side-chain, β-oxidative cleavage leads to a cyclohexane carboxylic acid derivative that may subsequently undergo dehydrogenation to yield unsaturated derivatives. The two non-terpenoid substances containing a cyclohexene ring (Nos 978 and 979) would be metabolized and eliminated by similar pathways. The substance with two endocyclic double bonds, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), is a structural analogue of dehydrodihydroionol (No. 397), which was evaluated by the Committee at its fifty-first meeting (Annex 1, reference *137*), and, like that agent, cannot be predicted to be metabolized to innocuous products.

The flavouring agents with a cyclopentane or cyclopentene ring (Nos 967–970) also have an oxygenated side-chain, and this is predicted to be the main site of oxidative metabolism prior to excretion of more polar acid metabolites. The processes of side-chain and alicyclic ring oxidation were considered by the Committee at its fifty-first meeting (Annex 1, reference *137*).

Monocyclic terpenoid primary alcohols (e.g., No. 974, perillyl alcohol) and aldehydes (e.g., Nos 971 and 973) would be oxidized to yield the corresponding carboxylic acid, while the esters (Nos 972 and 975) would undergo hydrolysis prior to oxidation. The acid metabolites of the above agents and 1,2,5,6-tetrahydrocuminic acid (No. 976) would be conjugated with glucuronic acid and excreted mainly in the urine. In a minor pathway,

the aldehyde can be reduced to the alcohol and excreted as the glucuronic acid conjugate. If an endocyclic double-bond is present, the flavouring agent or its metabolite could be reduced by the action of gut microflora. The acid metabolite may also undergo aromatization of the ring to yield a hippuric acid derivative. Bicyclic and tricyclic compounds (e.g., Nos 980–986) would undergo similar metabolism to the monocyclic terpenoids and, in addition, may undergo ring oxidation.

Four agents in this group contain an α,β-unsaturated carbonyl group (Nos 973, 977, 979 and 980), which is a structural alert for toxicity. The Committee, at previous meetings, devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference *154*) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with the use of compounds such as flavouring agents. These protective processes include conjugation with glutathione and reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned all 26 agents to structural class I.

Step 2. The simple cyclohexane and cyclohexene derivatives (Nos 961–966, 978 and 979), the cyclopentane and cyclopentene compounds (Nos 967–970) and the simple mono-, bi- and tricyclic terpenoid derivatives (Nos 971–976 and 980–986) were predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded *via* the A (left-hand) side of the decision tree (see Figure 1). The remaining substance, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), cannot be predicted to be metabolized to innocuous products and was therefore evaluated *via* the B (right-hand) side of the decision tree.

Step A3. The daily *per capita* intakes of each of the 25 flavouring agents evaluated at this step were below the daily human intake thresholds for structural class I (1800 μg per person), indicating that they present no safety concern when consumed as flavouring agents at current estimated levels.

Step B3. The daily *per capita* intake of the monocyclic substance with two endocyclic double-bonds evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), was below the threshold for daily human intake of compounds of structural class I, and its evaluation therefore proceeded to step B4.

Step B4. As the agent evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal), is structurally related to perillyl alcohol

(No. 974), data on the toxicity of perillyl alcohol were used to evaluate its safety. Perillyl alcohol given by intragastric gavage changed the weights of several organs in female rats when given at 400 mg/kg bw per day, but not at 120 mg/kg bw per day, in a 90-day study; changes in organ weights were not reported in male rats. Doses of 40, 120 and 400 mg/kg bw per day produced hyperexcitability and salivation, which the authors considered may have been due to its irritating properties. A daily dose of 120 mg/kg bw was well tolerated by dogs in a 90-day study. The daily intake of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) is 0.052 μg/kg bw in Europe and 0.001 μg/kg bw in the USA. The margin of safety between these intakes and 120 mg/kg bw per day is > 2 000 000. The compound also shares structural similarities with α-ionone and β-ionone (Nos 388 and 389), which were evaluated by the Committee at its fifty-first meeting (Annex 1, reference *137*). The NOELs for these compounds were 10 mg/kg bw per day in a 90-day study in rats, providing a margin of safety of about 200 000. Therefore, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (No. 977; safranal) would not be a safety concern.

Consideration of combined intakes

In the unlikely event that all 26 flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for structural class I. Therefore, the combined intake of the agents in this group would not represent a safety concern.

Conclusions

The Committee concluded that none of the 26 flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters would present a safety concern at the current estimated levels of intake.

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

4.1.2 *Phenethyl alcohol, aldehyde, acid and related acetals and esters*

The Committee evaluated 43 flavouring agents that are derivatives of phenethyl alcohol (No. 987) and phenoxyethyl alcohol (see Table 4). The group includes 39 phenethyl derivatives, comprising phenylacetaldehyde (No. 1002), phenylacetic acid (No. 1007) and structurally related esters and acetals. The group also includes four phenoxyethyl alcohol derivatives: phenoxyacetic acid (No. 1026), the sodium salt of a structurally related phenoxyacetic acid (No. 1029), a phenoxyethyl ester (No. 1027) and a phenoxyacetic acid ester (No. 1028). The evaluations were conducted with the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). None of these agents has previously been evaluated by the Committee.

Table 4

Summary of results of safety evaluations of phenethyl alcohol, aldehyde, acid, and related acetals and esters [a]

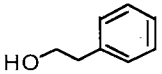
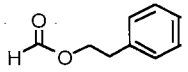
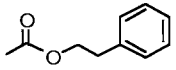
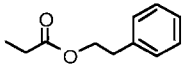
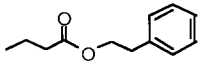
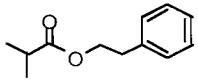
Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Structural class I							
Phenethyl alcohol	987	60-12-8 	No Europe: 1400 USA: 330	N/R	N/R	See note 1.	No safety concern
Phenethyl formate	988	104-62-1 	No Europe: 2 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl acetate	989	103-45-7 	No Europe: 100 USA: 60	N/R	N/R	See note 2.	No safety concern
Phenethyl propionate	990	122-70-3 	No Europe: 1 USA: 3	N/R	N/R	See note 2.	No safety concern
Phenethyl butyrate	991	103-52-6 	No Europe: 30 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl isobutyrate	992	103-48-0 	No Europe: 20 USA: 60	N/R	N/R	See note 2.	No safety concern

Table 4 (contd)

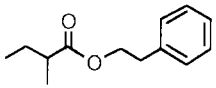
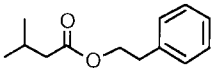
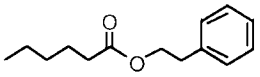
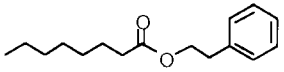
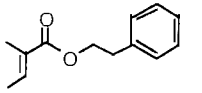
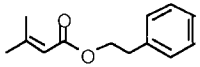
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenethyl 2-methylbutyrate	993	24817-51-4 	No Europe: 0.4 USA: ND	N/R	N/R	See note 2.	No safety concern
Phenethyl isovalerate	994	140-26-1 	No Europe: 100 USA: 30	N/R	N/R	See note 2.	No safety concern
Phenethyl hexanoate	995	6290-37-5 	No Europe: 10 USA: 2	N/R	N/R	See note 2.	No safety concern
Phenethyl octanoate	996	5457-70-5 	No Europe: 30 USA: 0.1	N/R	N/R	See note 2.	No safety concern
Phenethyl tiglate	997	55719-85-2 	No Europe: 0.3 USA: 1	N/R	N/R	See note 2.	No safety concern
Phenethyl senecioate	998	42078-65-9 	No Europe: 2 USA: ND	N/R	N/R	See note 2.	No safety concern

Table 4 (contd)

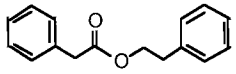
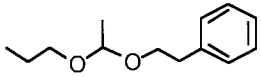
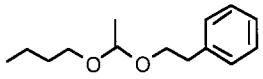
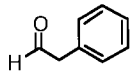
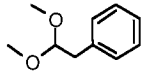
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenethyl phenylacetate	999	102-20-5 	No Europe: 40 USA: 80	N/R	N/R	See note 3.	No safety concern
Acetaldehyde phenethyl propyl acetal	1000	7493-57-4 	No Europe: 0.1 USA: 6	N/R	N/R	See note 4.	No safety concern
Acetaldehyde butyl phenethyl acetal	1001	64577-91-9 	No Europe: 0.01 USA: ND	N/R	N/R	See note 4.	No safety concern
Phenylacetaldehyde	1002	122-78-1 	No Europe: 40 USA: 60	N/R	N/R	See note 5.	No safety concern
Phenylacetaldehyde dimethyl acetal	1003	101-48-4 	No Europe: 20 USA: 40	N/R	N/R	See note 6.	No safety concern

Table 4 (contd)

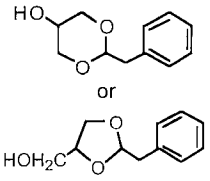
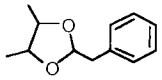
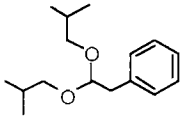
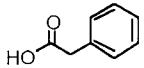
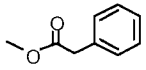
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Phenylacetaldehyde glyceryl acetal	1004	29895-73-6 	No Europe: 0.1 USA: 1	N/R	N/R	See note 6.	No safety concern
Phenylacetaldehyde 2,3-butylene glycol acetal	1005	5468-06-4 	No Europe: ND USA: 1	N/R	N/R	See note 6.	No safety concern
Phenylacetaldehyde diisobutylacetal	1006	68345-22-2 	No Europe: 30 USA: 0.4	N/R	N/R	See note 6.	No safety concern
Phenylacetic acid	1007	103-82-2 	No Europe: 290 USA: 60	N/R	N/R	See note 7.	No safety concern
Methyl phenylacetate	1008	101-41-7 	No Europe: ND USA: 20	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

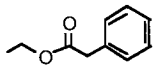
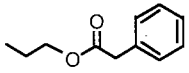
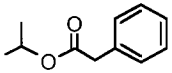
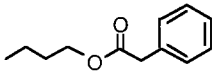
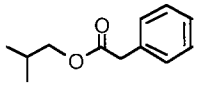
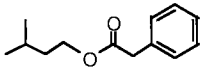
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Ethyl phenylacetate	1009	101-97-3 	No Europe: 130 USA: 20	N/R	N/R	See note 8.	No safety concern
Propyl phenylacetate	1010	4606-15-9 	No Europe: ND USA: 0.3	N/R	N/R	See note 8.	No safety concern
Isopropyl phenylacetate	1011	4861-85-2 	No Europe: 0.07 USA: ND	N/R	N/R	See note 8.	No safety concern
Butyl phenylacetate	1012	122-43-0 	No Europe: 3 USA: 3	N/R	N/R	See note 8.	No safety concern
Isobutyl phenylacetate	1013	102-13-6 	No Europe: 20 USA: 20	N/R	N/R	See note 8.	No safety concern
Isoamyl phenylacetate	1014	102-19-2 	No Europe: 30 USA: 30	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

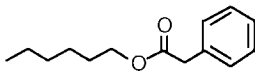
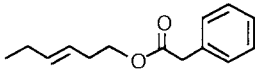
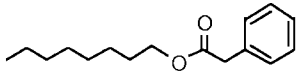
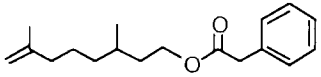
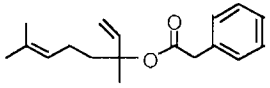
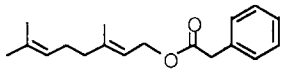
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Hexyl phenylacetate	1015	5421-17-0 	No Europe: 8 USA: ND	N/R	N/R	See note 8.	No safety concern
3-Hexenyl phenylacetate	1016	42436-07-7 	No Europe: 1 USA: 0.05	N/R	N/R	See note 8.	No safety concern
Octyl phenylacetate	1017	122-45-2 	No Europe: 0.004 USA: 0.006	N/R	N/R	See note 8.	No safety concern
Rhodinyl phenylacetate	1018	10486-14-3 	No Europe: 0.001 USA: ND	N/R	N/R	See note 9.	No safety concern
Linalyl phenylacetate	1019	7143-69-3 	No Europe: 0.09 USA: ND	N/R	N/R	See note 9.	No safety concern
Geranyl phenylacetate	1020	102-22-7 	No Europe: 2 USA: 2	N/R	N/R	See note 9.	No safety concern

Table 4 (contd)

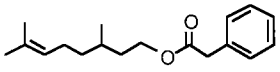
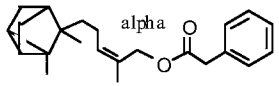
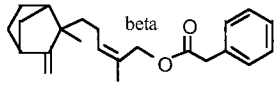
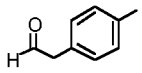
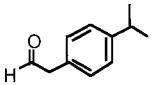
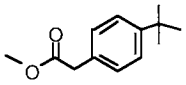
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Citronellyl phenylacetate	1021	139-70-8 	No Europe: 1 USA: 2	N/R	N/R	See note 9.	No safety concern
Santalyl phenylacetate	1022 α	1323-75-7 	No Europe: ND USA: 1	N/R	N/R	See note 9.	No safety concern
	β						
p-Tolyacetaldehyde	1023	104-09-6 	No Europe: 6 USA: 3	N/R	N/R	See note 5.	No safety concern
p-Isopropylphenyl-acetaldehyde	1024	4395-92-0 	No Europe: 0.1 USA: 0.01	N/R	N/R	See note 5.	No safety concern
Methyl *para-tert*-butylphenylacetate	1025	3549-23-3 	No Europe: 20 USA: 20	N/R	N/R	See note 8.	No safety concern

Table 4 (contd)

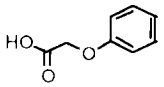
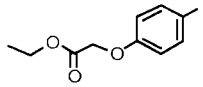
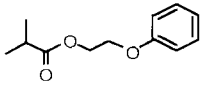
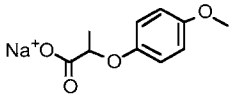
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Structural class III							
Phenoxyacetic acid	1026	122-59-8 	No Europe: 40 USA: 0.1	N/R	N/R	See note 10.	No safety concern
Ethyl (*p*-tolyl-oxy)acetate	1027	67028-40-4 	No Europe: 0.1 USA: ND	N/R	N/R	See note 11.	No safety concern
2-Phenoxyethyl isobutyrate	1028	103-60-6 	Yes Europe: 2 USA: 110	No	Yes. The NOEL of 15 mg/kg bw per day for the related chemical No. 1027 and the NOEL of 250 mg/kg bw per day for the related chemical No. 1029 are >1000 times the estimated intake of 2-phenoxyethyl isobutyrate when used as a flavouring agent.	See note 12.	No safety concern

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Sodium 2-(4-methoxy-phenoxy)propanoate	1029	13794-15-5 	No Europe: ND USA: 6	N/R	N/R	See note 13.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation.

[a] Step 2: All the flavouring agents in this group were predicted to be metabolized to innocuous products.

[b] The thresholds for human intake for classes I and III are 1800 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/person per day. The combined intakes of flavouring agents in class I are 2300 and 920 µg/person per day in Europe and the USA, respectively. The combined intakes of flavouring agents in class III are 42 and 120 µg/person per day in Europe and the USA, respectively.

Notes:

1. Oxidized to phenylacetic acid and either completely oxidized or conjugated and excreted primarily in the urine
2. Hydrolysed to phenethyl alcohol (see note 1) and the corresponding acid, which is further oxidized to carbon dioxide and water
3. Hydrolysed to phenethyl alcohol (see note 1) and phenylacetic acid (see note 7)
4. Hydrolysed to phenethyl alcohol (see note 1) and the corresponding aldehyde, which is further oxidized to carbon dioxide and water
5. Phenylacetaldehyde derivative is oxidized to phenylacetic acid derivative (see note 7).
6. Hydrolysed to phenyacetaldehyde (see note 5) and the corresponding alcohol, which is further oxidized to carbon dioxide and water
7. Phenylacetic acid is excreted as the glutamine acid conjugate.
8. Hydrolysed to phenylacetic acid derivative (see note 7) and the corresponding alcohol, which is completely oxidized.
9. Hydrolysed to phenylacetic acid (see note 7) and terpene alcohol, which is further oxidized to a polar, excretable metabolite.
10. Excreted unchanged in the urine
11. Hydrolysed to *para*-tolyloxy acetic acid and ethanol; the acid is excreted unchanged.
12. Hydrolysed to isobutyric acid and 2-phenoxyethanol, which is oxidized to phenoxyacetic acid and excreted unchanged in the urine.
13. Excreted mainly unchanged and as the *O*-demethylated metabolite, (±)2-(4-hydroxyphenoxy) propionic acid.

Twenty of the 39 flavouring agents in this group are natural components of foods. Phenethyl alcohol has a mild rose aroma. The agents have been detected in a wide range of products; for example, the parent alcohol and its derivatives have been detected in beans, fruits, vegetables, cheeses, milk, oils and alcoholic and non-alcoholic beverages. Only one phenoxyacetic acid derivative is a natural component of food: sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) has been detected in coffee.

Estimated daily per capita *intake*

The total annual volume of production of the 43 phenethyl alcohol and phenoxyethyl alcohol derivatives in this group is approximately 17 000 kg in Europe and 7800 kg in the USA. About 75% and 45% of the total annual volume in Europe and the USA, respectively, is accounted for by use of phenethyl alcohol (No. 987), its corresponding acetate ester (No. 989) and phenylacetic acid (No. 1007). The total annual volume of production of the four derivatives of phenoxyethyl alcohol (Nos 1026–1029) is approximately 260 kg in Europe and 870 kg in the USA, accounting for about 2% and 11% of the total annual volumes in Europe and the USA, respectively.

Five flavouring agents in the group account for the highest estimated intakes. These are 1400 mg/day for phenethyl alcohol (No. 987), 290 μg/day for phenylacetic acid (No. 1007), 130 μg/day for ethyl phenylacetate (No. 1009) and 100 μg/day for phenethyl acetate (No. 989) and for phenethyl isovalerate (No. 994) in Europe and 330 μg/day for phenethyl alcohol (No. 987) and 110 μg/day for phenoxyethyl isobutyrate (No. 1028) in the USA. The intakes of all the other flavouring agents in the group ranged from 0.001 to 80 μg/day, the intake of about 70% of them being less than 25 μg/day.

Absorption, distribution, metabolism and elimination

Phenethyl and phenylacetate esters and phenyacetaldehyde acetals are rapidly hydrolysed in vivo to yield phenethyl alcohol (No. 987), phenylacetic acid (No. 1007) and phenylacetaldehyde (No. 1002), respectively. Phenethyl alcohol and phenylacetaldehyde are both oxidized to phenylacetic acid, which is conjugated and excreted primarily in the urine. Similarly, the phenoxyethyl and phenoxyacetate esters, 2-phenoxyethyl isobutyrate (No. 1028) and ethyl (*para*-tolyloxy)acetate (No. 1027), respectively, are anticipated to be hydrolysed to their component acids and alcohols. Phenoxyethyl alcohol is rapidly oxidized to phenoxyacetic acid, which in turn is rapidly absorbed and excreted primarily unchanged in the urine. Sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) is also rapidly absorbed and excreted primarily unchanged, with a small amount of the *O*-demethylated metabolite, (±)2-(4-hydroxyphenoxy) propionic acid. Therefore, all the flavouring agents in this group were predicted to hydrolyse and/or oxidize

to yield phenylacetic acid or a phenoxyacetic acid derivative that is excreted either free or in conjugated form.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned phenylacetic acid (No. 1007) to structural class I because it is a normal component of human urine. The other 38 phenethyl alcohol derivatives were also assigned to structural class I because they are simple aromatic compounds with a primary oxygenated functional group.

The Committee assigned all four phenoxyacetic acid derivatives in this group to structural class III. These are aromatic substances with an ether linkage between the benzene ring and an unsaturated side-chain with a primary oxygenated functional group.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. Their evaluation therefore proceeded via the A (left-hand) side of the decision tree.

Step A3. The estimated daily intakes of each of the 39 flavouring agents in structural class I are below the threshold for daily human intake for that class (1800 μg per person). According to the Procedure, the safety of these 39 flavouring agents raises no concern when they are consumed at currently estimated levels.

The estimated daily intakes of three of the four flavouring agents in structural class III are below the threshold for daily human intake for that class (90 μg per person). According to the Procedure, the safety of these three flavouring agents (Nos 1026, 1027 and 1029) raises no concern when they are consumed at their currently estimated levels. The daily intake of 2-phenoxyethyl isobutyrate (No. 1028) in the USA (110 mg /person) exceeds the threshold for daily human intake for compounds in structural class III (90 mg per person). Accordingly, the evaluation of this substance proceeded to step A4.

Step A4. 2-Phenoxyethyl isobutyrate (No. 1028) is not endogenous. Therefore, its evaluation proceeded to step A5.

Step A5. Although no NOEL was available for 2-phenoxyethyl isobutyrate (No. 1028), data were available on the toxicity of two structurally related flavouring agents in this group (Nos 1027 and 1029). The NOEL of 15 mg/kg bw per day for ethyl (*para*-tolyloxy)acetate (No. 1027) in a 90-day dietary study in rats and the NOEL of 250 mg/kg bw per day for sodium 2-(4-methoxyphenoxy)propanoate (No. 1029) in another 90-day dietary study in rats provide adequate margins of safety (> 1000 times) in relation to the estimated daily per capita intake of 2-phenoxyethyl isobutyrate (No. 1028) in Europe (0.03 μg/kg bw) and the USA (2 μg/kg bw). The Committee therefore concluded that the safety of this agent would not be a concern.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 39 of the phenethyl alcohol derivatives (Nos 987–1025) were consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for daily human intake of compounds in structural class I (1800 mg per person). At the levels of intake associated with their use as flavouring agents, all 39 agents are expected to be metabolized efficiently, without saturating metabolic pathways. The same holds true for two of the four flavouring agents in structural class III: the estimated combined intakes of phenoxyacetic acid (No. 1026) and 2-phenoxyethyl isobutyrate (No. 1028) would exceed the threshold for daily human intake of compounds in structural class III (90 mg per person). At the levels of intake associated with their use as flavouring agents, both agents are expected to be metabolized efficiently, without saturating metabolic pathways. The other two agents, ethyl (*para*-tolyloxy)acetate (No. 1027) and sodium 2-(4-methoxyphenoxy)propanoate (No. 1029), are not converted to a common metabolite, and there is no need to consider their combined intake from use as flavouring agents. On the basis of the evaluation of all the data, combined intake would raise no safety concern.

Conclusions

The Committee concluded that none of the 43 flavouring agents in this group would present a safety concern at current estimated levels of intake. No data on toxicity were required for application of the Procedure to 42 of the 43 flavouring agents, as they were predicted to be metabolized to innocuous agents and the estimated intakes were below the human intake threshold associated with the relevant structural class. Data on the toxicity of related agents were used to evaluate 2-phenoxyethyl isobutyrate (No. 1028). Data on the toxicity and metabolism of phenethyl alcohol, phenylacetaldehyde, phenylacetic acid, phenoxyethanol and phenoxyacetic acid and related agents were consistent with the results of the safety evaluation.

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

4.1.3 *Sulfur-containing heterocyclic compounds*

The Committee evaluated a group of 30 flavouring agents comprising sulfur-containing heterocyclic compounds (see Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1). The group is composed of both five- and six-member S-containing aromatic and non-aromatic heterocyclic compounds, including thiazole itself and derivatives of thiazole, dithiazine, thiazoline and thiophene. Thiazole is a five-membered aromatic heterocyclic compound containing sulfur and nitrogen atoms in the 1- and 3-ring positions, respectively (Nos 1030–1044 and 1054–1057).

Table 5

Summary of the results of safety evaluations of sulfur-containing heterocyclic compounds[a]

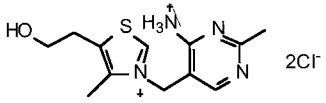
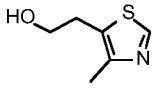
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Structural class II							
Thiamine hydrochloride	1030	67-03-8 	Yes[c] A side	Yes Europe: 2900 USA: 1200	No	A5 Yes. The NOEL of 36 mg/kg bw per day is > 500 times the estimated intake of thiamine hydrochloride when used as a flavouring agent.	No safety concern
4-Methyl-5-thiazoleethanol	1031	137-00-8 	Yes[d] A side	No Europe: 170 USA: 380	N/R	N/R	No safety concern
Thiazole	1032	288-47-1 	No B side	No Europe: 0.01 USA: 0.07	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 900 000 times the estimated intake of thiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

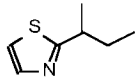
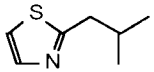
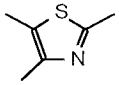
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-(1-Methylpropyl)-thiazole	1033	18277-27-5 	No B side	No Europe: 0.03 USA: 0.01	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 1 million times the estimated intake of 2-(1-methyl-propyl)-thiazole when used as a flavouring agent.	No safety concern
2-Isobutylthiazole	1034	18640-74-9 	No B side	No Europe: 3 USA: 0.4	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is 10 000 times the estimated intake of 2-isobutylthiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl-thiazole	1035	3581-91-7 	No B side	No Europe: 0.2 USA: 0.4	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 200 000 times the estimated intake of 4,5-dimethyl-thiazole when used as a flavouring agent.	No safety concern
2,4,5-Trimethyl-thiazole	1036	13623-11-5 	No B side	No Europe: 1 USA: 0.3	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 90 000 times the estimated intake of 2,4,5-trimethyl-thiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

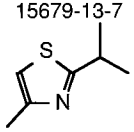
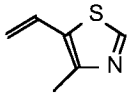
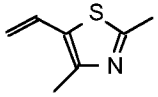
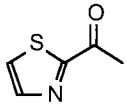
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Isopropyl-4-methylthiazole	1037	15679-13-7 	No B side	No Europe: 22 USA: 10	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 2000 times the estimated intake of 2-isopropyl-4-methylthiazole when used as a flavouring agent.	No safety concern
4-Methyl-5-vinylthiazole	1038	1759-28-0 	No B side	No Europe: 2 USA: 0.2	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 20 000 times the estimated intake of 4-methyl-5-vinylthiazole when used as a flavouring agent.	No safety concern
2,4-Dimethyl-5-vinylthiazole	1039	65505-18-2 	No B side	No Europe: ND USA: 0.007	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day is > 9 million times the estimated intake of 2,4-dimethyl-5-vinylthiazole when used as a flavouring agent.	No safety concern
2-Acetylthiazole	1041	24295-03-2 	No B side	No Europe: 11 USA: 10	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day is > 200 000 times the estimated intake of 2-acetylthiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

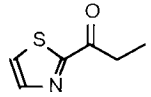
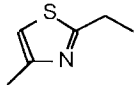
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Propionylthiazole	1042	43039-98-1 	No B side	No Europe: ND USA: 0.2	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day for the related flavouring agent No. 1041 is > 10 million times the estimated intake of 2-propionylthiazole when used as a flavouring agent.	No safety concern
4-Methylthiazole	1043	693-95-8 	No B side	No Europe: 0.1 USA: 0.05	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 400 000 times the estimated intake of 4-methylthiazole when used as a flavouring agent.	No safety concern
2-Ethyl-4-methylthiazole	1044	15679-12-6 	No B side	No Europe: 4 USA: 1	N/R	B5 Yes. The NOEL of 0.92 mg/kg bw per day for the related flavouring agent No. 1039 is > 10 000 times the estimated intake of 2-ethyl-4-methylthiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

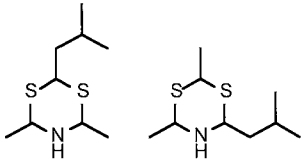
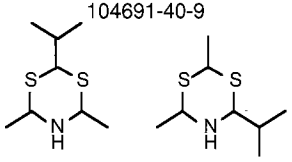
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyl-dihydro-1,3,5-dithiazine (mixture)	1046	101517-87-7 101517-86-6  18% 62%	No B side	No Europe: 0.1 USA: 0.05	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day is > 5 million times the estimated intake of a mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine when used as a flavouring agent.	No safety concern
2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyl-dihydro-1,3,5-dithiazine (mixture)	1047	104691-41-0 104691-40-9  27% 44%	No B side	No Europe: ND USA: 0.07	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day is > 10 million times the estimated intake of a mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

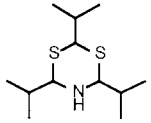
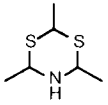
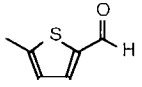
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2,4,6-Triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine	1048	74595-94-1 	No B side	No Europe: ND USA: 2.6	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day for the related flavouring agent No. 1046 is > 200 000 times the estimated intake of 2,4,6-triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine when used as a flavouring agent.	No safety concern
2,4,6-Trimethyl-dihydro-4*H*-1,3,5-dithiazine	1049	638-17-5 	No B side	No Europe: ND USA: 3.3	N/R	B5 Yes. The NOEL of 11 mg/kg bw per day for the related flavouring agent No. 1046 is > 5 million times the estimated intake of 2,4,6-trimethyldihydro-4*H*-1,3,5-dithiazine when used as a flavouring agent.	No safety concern
5-Methyl-2-thio-phenecarboxy-aldehyde	1050	13679-70-4 	No B side	No Europe: 1 USA: 0.01	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 20 million times the estimated intake of 5-methyl-2-thiophene-carboxyaldehyde when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

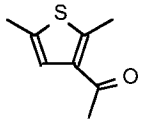
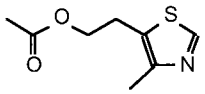
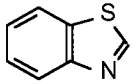
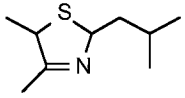
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
3-Acetyl-2,5-dimethylthiophene	1051	2530-10-1 	No B side	No Europe: 22 USA: 0.2	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 700 000 times the estimated intake of 3-acetyl-2,5-dimethylthiophene when used as a flavouring agent.	No safety concern
4-Methyl-5-thiazoleethanol acetate	1054	656-53-1 	Yes[e] A side	No Europe: 10 USA: 3	N/R	N/R	No safety concern
Structural class III							
Benzothiazole	1040	95-16-9 	No B side	No Europe: 1 USA: 0.2	N/R	B5 Yes. The NOEL of 5.1 mg/kg bw per day is > 20 000 times the estimated intake of benzothiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl-2-isobutyl-3-thiazoline	1045	65894-83-9 	No B side	No Europe: 0.01 USA: 4	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day for the related flavouring agent No. 1059 is > 10 000 times the estimated intake of 4,5-dimethyl-2-isobutyl-3-thiazoline when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

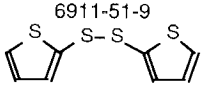
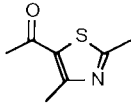
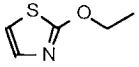
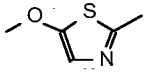
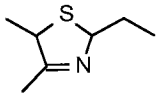
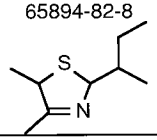
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Thienyl mercaptan	1052	7774-74-5 	No B side	No Europe: 0.01 USA: 0.03	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day for the related flavouring agent No. 1053 is > 700 million times the estimated intake of 2-thienyl mercaptan when used as a flavouring agent.	No safety concern
2-Thienyl disulfide	1053	6911-51-9 	No B side	No Europe: ND USA: 0.07	N/R	B5 Yes. The NOEL of 290 mg/kg bw per day is > 200 million times the estimated intake of 2-thienyl disulfide when used as a flavouring agent.	No safety concern
2,4-Dimethyl-5-acetylthiazole	1055	38205-60-6 	No B side	No Europe: 0.01 USA: 2	N/R	B5 Yes. The NOEL of 24 mg/kg bw per day is > 10 million times the estimated intake of 2,4-dimethyl-5-acetylthiazole when used as a flavouring agent.	No safety concern
2-Ethoxythiazole	1056	15679-19-3 	No B side	No Europe: 0.01 USA: 0.12	N/R	B5 Yes. The NOEL of 50 mg/kg bw per day for the related flavouring agent No. 1041 is > 20 million times the estimated intake of 2-ethoxythiazole when used as a flavouring agent.	No safety concern

Table 5 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3 /B3*[a,b] Does intake exceed threshold for structural class?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5 / B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Methyl-5-methoxythiazole	1057	38205-64-0 	No B side	No Europe: ND USA: 0.01	N/R	B5 Yes. The NOEL of 8.6 mg/kg bw per day is > 40 million times the estimated intake of 2-methyl-5-methoxythiazole when used as a flavouring agent.	No safety concern
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	76788-46-0 	No B side	No Europe: ND USA: 0.01	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day for the related flavouring agent No. 1059 is > 6 million times the estimated intake of 4,5-dimethyl-2-ethyl-3-thiazoline when used as a flavouring agent.	No safety concern
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	65894-82-8 	No B side	No Europe: ND USA: 5	N/R	B5 Yes. The NOEL of 1.2 mg/kg bw per day is > 10 000 times the estimated intake of 2-(2-butyl)-4,5-dimethyl-3-thiazoline when used as a flavouring agent.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation

[a] Step 1. Twenty-one flavouring agents are in structural class II and nine flavouring agents are in structural class III.

[b] The thresholds for human intake for structural classes II and III are 540 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intake of flavouring agents in structural class II is 3100 µg/day in Europe and 1600 µg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 1 µg/day in Europe and 11 µg/day in the USA.

[c] Enzymatically cleaved to yield 4-methyl-5-thiazoleethanol (No. 1031) and 2-methyl-4-amino-5-hydroxymethylpyrimidine. The thiazole and pyrimidine fragments are further oxidized to yield 4-methylthiazole-4-acetic acid and the 5-pyrimidine carboxylic acid derivative, respectively, which, together with thiamine, are excreted in the urine. Can also be converted to 2-methyl-4-amino-5-formylaminopyrimidine and thiamine acetic acid.

[d] Oxidized to yield 4-methylthiazole-4-acetic acid and excreted in the urine (See note c)

[e] Hydrolysed to yield 4-methylthiazole-4-acetic acid and excreted in urine (See note c)

Dithiazine is a six-membered non-aromatic heterocyclic compound containing two sulfur atoms and one nitrogen atom in the 1-, 3- and 5-positions, respectively (Nos 1046–1049). Thiazoline (partially reduced thiazole) is a five-membered non-aromatic heterocyclic compound containing sulfur and nitrogen atoms in the 1- and 3-positions, respectively (Nos 1045, 1058 and 1059). Thiophene is a five-membered aromatic heterocyclic compound containing a sulfur atom in the 1-position (Nos 1050–1053). Except for thiazole (No. 1032), all the flavouring agents in this group are ring-substituted with one or more of the following functional groups: alkyl, alkenyl, aryl, alcohol, keto, thiol and disulfide.

The Committee has not previously evaluated any of the members of this group.

Eighteen flavouring agents in the group have been detected as natural components of food, and quantitative data have been reported on the natural occurrence of eight agents (Nos 1032, 1034, 1036, 1040, 1041, 1043 and 1050). The foods in which one or more of these flavouring agents can be found include lean meat, beans, nuts, whole-grain cereals, fish, coffee, milk, beer, peanuts, popcorn, pork liver, shrimp, tomato, potato, grapes and apples.

Estimated daily per capita *intake*

The total annual volume of production of the 30 sulfur-containing heterocyclic compounds in this group was reported to be 22 000 kg in Europe and 12 000 kg in the USA. These values are equivalent to total daily *per capita* intakes of 3100 μg in Europe and 1600 μg in the USA.

In both Europe and the USA, thiamine hydrochloride (vitamin B1; No. 1030) and its principal metabolite, 4-methyl-5-thiazoleethanol (No. 1031), accounted for approximately 98% of the total *per capita* dietary intake of the flavouring agents in this group. In Europe, thiamine hydrochloride and 4-methyl-5-thiazoleethanol accounted for approximately 92% (2900 μg/day) and 6% (170 μg/day), respectively, of the total *per capita* intake. In the USA, thiamine hydrochloride and 4-methyl-5-thiazoleethanol accounted for approximately 75% (1200 μg/day) and 23% (380 μg/day), respectively, of the total *per capita* intake of the flavouring agents in this group.

The estimated intakes of the remaining flavouring agents are between 0.01 and 22 μg/day in both Europe and the USA, with most intakes below 4 μg/day.

Absorption, distribution, metabolism and elimination

Thiazole and its derivatives are metabolized primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms; however, other routes of metabolism, involving ring cleavage, are possible. Derivatives of dithiazine, which are cyclic sulfides, are expected to be metabolized primarily by

S-oxidation to yield the corresponding sulfoxides and sulfones. Thiazoline is predicted to be similarly metabolized. Thiamine hydrochloride (No. 1030) at an intake of less than 5 mg/day is readily absorbed in the small intestine by an active transport system, followed by conversion to the coenzyme, thiamine pyrophosphate. Thiamine is metabolized primarily to a pyrimidine and a thiazole (4-methyl-5-thiazoleethanol, No. 1031) fragment. These fragments are further oxidized and excreted in the urine.

Thiophene derivatives are metabolized primarily by S-oxidation, followed by conjugation with glutathione; however, other routes of metabolism, involving ring cleavage, are also possible. The resulting mercapturic acid derivative is eliminated in the urine. The aromatic-fused thiazole, 2-acetylthiazole (benzothiazole; No. 1041), which lacks ring substituents, is metabolized by thiazole ring cleavage, yielding a series of free and conjugated *ortho*-aminophenyl sulfone and sulfoxide metabolites.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

The stepwise evaluation of the 30 sulfur-containing heterocyclic compounds is described in detail below and summarized in Table 5.

Step 1. All the flavouring agents in this group are heterocyclic compounds, and five of these (Nos 1040, 1050, 1051, 1052 and 1053) are also aromatic[1]. One of the aromatic heterocyclic flavouring agents, benzothiazole (No. 1040) is unsubstituted and was therefore placed in structural class III. Of the other four substituted compounds, two (Nos 1050 and 1051) are common constituents of food and were therefore classified in structural class II, while the other two (Nos 1052 and 1053) are not common constituents of food and were therefore placed in structural class III.

Of the 25 non-aromatic heterocyclic flavouring agents, 19 (Nos 1030–1039, 1041–1044, 1046–1049 and 1054) are or are structurally closely related to common constituents of food and were therefore placed in structural class II. The remaining six flavouring agents (Nos 1045 and 1055–1059) were classified in structural class III.

Step 2. The data on most individual members of the group were, in most cases, insufficient to allow conclusions about their probable metabolic fate. The metabolism of thiamine hydrochloride (No. 1030) and its metabolite, 4-methyl-5-thiazoleethanol (No. 1031), is well characterized, and both are metabolized to innocuous products. 4-Methyl-5-thiazoleethanol acetate (No. 1054) is expected to be readily hydrolysed to 4-methyl-5-thiazole-

[1] According to the decision-tree process, an 'aromatic' compound has at least one benzene, furan, thiophene, pyridine or pyrole ring, however substituted and regardless of whether it is fused to another ring.

ethanol. The evaluation of all three flavouring agents therefore proceeded via the A (left-hand) side of the scheme. For all other flavouring agents in the group, the evaluation proceeded via the B (right-hand) side of the scheme.

Step A3. With respect to those flavouring agents evaluated via the A side of the scheme, the estimated daily *per capita* intakes of 4-methyl-5-thiazoleethanol (No. 1031) and 4-methyl-5-thiazoleethanol acetate (No. 1054) are below the threshold for human intake for structural class II (540 μg), and therefore these flavouring agents are not expected to raise any safety concern. The daily *per capita* intake of thiamine hydrochloride (No. 1030) is 2900 μg in Europe and 1200 μg in the USA. The intake of this flavouring agent therefore exceeds the threshold for human intake of compounds in structural class II, and its evaluation proceeded to step A4.

Step A4. Thiamine hydrochloride (No. 1030) is not endogenous; therefore, its evaluation proceeded to step A5.

Step A5. The NOEL of 36 mg/kg bw per day for thiamine hydrochloride (No. 1030) in a 90-day dietary study in rats is more than 500 times the estimated intake of this substance from its use as a flavouring agent in Europe (48 μg/kg bw per day) and more than 1000 times the estimated intake in the USA (22 μg/kg bw per day). Thiamine hydrochloride would therefore not be expected to be a safety concern.

Step B3 With regard to those flavouring agents evaluated via the B side of the scheme, the estimated daily *per capita* intakes of all 18 flavouring agents in structural class II (Nos 1032–1039, 1041–1044 and 1046–1051) are below the threshold for human intake for structural class II (540 μg per person per day). Similarly, the estimated daily *per capita* intakes of all nine flavouring agents in structural class III (Nos 1040, 1045, 1052, 1053 and 1055–1059) are below the threshold for human intake for compounds in this class (90 μg per person per day). The evaluation of all of these flavouring agents therefore proceeded to step B4.

Step B4 The NOEL for 2,4-dimethyl-5-vinylthiazole (No. 1039) in a 90-day dietary study in rats was 0.92 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agents, thiazole (No. 1032), 2-(1-methylpropyl)thiazole (No. 1033), 2-isobutylthiazole (No. 1034), 4,5-dimethylthiazole (No. 1035), 2,4,5-trimethylthiazole (No. 1036), 2-isopropyl-4-methylthiazole (No. 1037), 4-methyl-5-vinylthiazole (No. 1038), 4-methylthiazole (No. 1043) and 2-ethyl-4-methylthiazole (No. 1044).

The NOEL for 2-acetylthiazole (No. 1041) in a 90-day dietary study in rats was 50 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agent, 2-propionylthiazole (No. 1042).

The NOEL for 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) in a 90-day dietary study in rats was 1.2 mg/kg bw per day, and this NOEL is appropriate for the structurally related flavouring agents, 4,5-dimethyl-2-isobutyl-3-thiazoline (No. 1045) and 4,5-dimethyl-2-ethyl-3-thiazoline (No. 1058).

The NOEL for a mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1046) and of a mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1047) in a 14-day dietary study in rats was 11 mg/kg bw per day, and this NOEL is appropriate for the structurally related agents, 2,4,6-triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine (No. 1048) and 2,4,6-trimethyldihydro-4*H*-1,3,5-dithiazine (No. 1049).

The NOEL for 2-thienyl disulfide (No. 1053) in a 90-day dietary study in rats was 290 mg/kg bw per day, and this NOEL is appropriate for the structurally related agents 5-methyl-2-thiophene carboxyaldehyde (No. 1050), 3-acetyl-2,5-dimethylthiophene (No. 1051) and 2-thienyl mercaptan (No. 1052).

The NOEL for benzothiazole (No. 1040) in a 90-day dietary study in rats was 5.1 mg/kg bw per day.

The NOEL for 2,4-dimethyl-5-acetylthiazole (No. 1055) in a dietary study in rats was 24 mg/kg bw per day.

The NOEL for 2-methyl-5-methoxythiazole (No. 1057) in a 90-day dietary study in rats was 8.6 mg/kg bw per day.

For all the above-mentioned flavouring agents (Nos 1032–1053, 1055–1059), therefore, a NOEL exists either for the substance itself, which provides an adequate margin of safety under the intended conditions of use, or for a structurally related substance, which is high enough to accommodate any perceived difference in toxicity between the substance itself and the related substance.

The NOEL used to evaluate each flavouring agent and the margins of safety they provide on the basis of current levels of intake are summarized in Table 6. On the basis of these data, these flavouring agents would not be expected to be a safety concern.

Consideration of combined intakes from use as flavouring agents

Although the 30 flavouring agents in this group are unlikely to produce common metabolites, consideration of their combined intake is appropriate since all are likely to be conjugated with glutathione before excretion.

Table 6

Margins of safety for thesulfur-containing heterocyclic compounds used as flavouring agents proceeding via the B side of the Procedure

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
Structural class II						
Thiazole	1032	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.001	> 900 000
2-(1-Methylpropyl)thiazole	1033	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.0005	> 1 million
2-Isobutylthiazole	1034	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.05	> 10 000
4,5-Dimethylthiazole	1035	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.004	> 200 000
2,4,5-Trimethylthiazole	1036	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.01	> 90 000
2-Isopropyl-4-methylthiazole	1037	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.4	> 2000
4-Methyl-5-vinylthiazole	1038	2,4-dimethyl-5-vinylthiazole	1039	0.92[a]	0.04	> 20 000
2,4-Dimethyl-5-vinylthiazole	1039	NA	NA	0.92[a]	0.0001	> 9 million
2-Acetylthiazole	1041	NA	NA	50[a]	0.2	> 200 000
2-Propionylthiazole	1042	2-Acetylthiazole	1041	50[a]	0.003	> 10 million
4-Methylthiazole	1043	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.002	> 400 000
2-Ethyl-4-methylthiazole	1044	2,4-Dimethyl-5-vinylthiazole	1039	0.92[a]	0.06	> 10 000
2-Isobutyl-4,6-dimethyldihdro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1046	NA	NA	11[b]	0.002	> 5 million
2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1047	NA	NA	11[b]	0.001	> 10 million
2,4,6-Triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine	1048	A mixture of 2-Isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	1046	11[b]	0.04	> 200 000
2,4,6-Trimethyldihydro-4*H*-1,3,5-dithiazine	1049	A mixture of 2-Isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	1046	11[b]	0.002	> 5 million
5-Methyl-2-thiophene-carboxyaldehyde	1050	2-Thienyl disulfide	1053	290[a]	0.01	> 20 million
3-Acetyl-2,5-dimethylthiophene	1051	2-Thienyl disulfide	1053	290[a]	0.4	> 700 000

Table 6 *(continued)*

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (µg/kg bw per day)	Margin of safety
5-Methyl-2-thiophene-carboxyaldehyde	1050	2-Thienyl disulfide	1053	290[a]	0.01	> 20 million
3-Acetyl-2,5-dimethylthiophene	1051	2-Thienyl disulfide	1053	290[a]	0.4	> 700 000
Structural class III						
Benzothiazole	1040	NA	NA	5.1[a]	0.02	> 20 000
4,5-Dimethyl-2-isobutyl-3-thiazoline	1045	2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	1.2[a]	0.07	> 10 000
2-Thienyl mercaptan	1052	2-Thienyl disulfide	1053	290[a]	0.0004	> 700 million
2-Thienyl disulfide	1053	NA	NA	290[a]	0.001	> 200 million
2,4-Dimethyl-5-acetylthiazole	1055	NA	NA	24[a]	0.03	> 10 million
2-Ethoxythiazole	1056	2-Acetylthiazole	1041	50[a]	0.002	> 20 million
2-Methyl-5-methoxythiazole	1057	NA	NA	8.6[a]	0.0002	> 40 million
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	1.2[a]	0.0002	> 6 million
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	NA	NA	1.2[a]	0.09	> 10 000

NA, not applicable
[a]90-day study in rats
[b]14-day study in rats

In the unlikely event that all 21 flavouring agents in structural class II were to be consumed simultaneously on a daily basis, the estimated combined *per capita* intakes (3100 μg/day in Europe and 1600 μg/day in the USA) would exceed the threshold of human intake for their structural class (540 μg per person). Since, at these levels of intake, the conjugation capacity of the glutathione pool is unlikely to be depleted, this level of intake is not anticipated to be a safety concern.

In the unlikely event that the nine flavouring agents in structural class III were to be consumed simultaneously on a daily basis, the estimated combined per capita intakes (1 μg/day in Europe and 11 μg/day in the USA) would not exceed the threshold for human intake for their structural class (90 μg per person).

Conclusions

On the basis of the predicted metabolism and the NOELs for some members of the group, the Committee concluded that the 30 sulfur-containing heterocyclic compounds in this group would not raise safety concerns at current levels of intake.

The Committee noted that, for the three flavouring agents that were predicted to be metabolized to innocuous products, the available data on toxicity were consistent with the results of the safety evaluation.

A toxicological monograph was prepared.

4.1.4 *Sulfur-substituted furan derivatives*

The Committee evaluated a group of 33 flavouring agents that includes thiofuran and thiofurfuryl derivatives (see Table 7), using the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). The Committee has not previously evaluated any member of the group.

Eighteen of the 33 flavouring agents in this group have been reported to occur naturally in foods. Thiofurfuryl and thiofuran derivatives have been detected mainly in coffee and cooked meats.

Estimated daily per capita *intake*

The total annual volume of production of the 33 sulfur-substituted furan derivatives is approximately 370 kg in Europe and 210 kg in the USA. Approximately 65% of the total annual volume in Europe and 40% of that in the USA arises from use of one substance, furfuryl mercaptan (No. 1072), with annual volumes of 240 kg in Europe and 82 kg in the USA. The estimated *per capita* intake of furfuryl mercaptan is 40 μg/day in Europe and 11 μg/day in the USA. The estimated daily intakes of each of the other 32 flavouring agents in this group are less than 5 μg per day in Europe and in the USA.

Table 7

Summary of results of safety evaluations of sulfur-substituted furan derivatives used as flavouring agents[a]

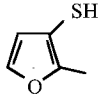
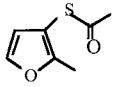
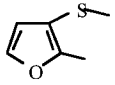
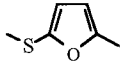
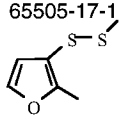
Flavouring agent	No.	CAS No. and structure	*Step B3*[b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Structural class II					
2-Methyl-3-furanthiol	1060	28588-74-1 	No Europe: 0.6 USA: 0.9	Yes. The NOEL of 5 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-furanthiol when used as a flavouring agent.	No safety concern
Ethanoic acid, S-(2-methyl-3-furanyl) ester	1069	55764-25-5 	No Europe: 0.01 USA: 0.07	Yes. The NOEL of 5 mg/kg bw per day for the related substance No. 1060 is > 1 million times the estimated intake of ethanoic acid, *S*-(2-methyl-3-furanyl) ester when used as a flavouring agent.	No safety concern
2-Methyl-3-(methylthio)furan	1061	63012-97-5 	No Europe: 1 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of 2-methyl-3-(methylthio)furan when used as a flavouring agent.	No safety concern
2-Methyl-5-(methylthio)furan	1062	13678-59-6 	No Europe: 1 USA: 0.02	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of 2-methyl-5-(methylthio)furan when used as a flavouring agent.	No safety concern
Methyl 2-methyl-3-furyl disulfide	1064	65505-17-1 	No Europe: 0.9 USA: 0.05	Yes. The NOEL of 1.2 mg/kg bw per day is > 10 000 times the estimated intake of methyl 2-methyl-3-furyl disulfide when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

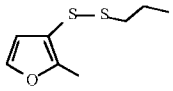
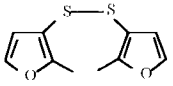
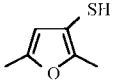
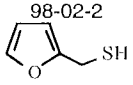
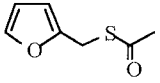
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Propyl 2-methyl-3-furyl disulfide	1065	61197-09-9 	No Europe: ND USA: 0.7	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 100 000 times the estimated intake of propyl 2-methyl-3-furyl disulfide when used as a flavouring agent.	No safety concern
Bis(2-methyl-3-furyl) disulfide	1066	28588-75-2 	No Europe: 0.3 USA: 0.7	Yes. The NOEL of 0.45 mg/kg bw per day is > 10 000 times the estimated intake of bis(2-methyl-3-furyl) disulfide when used as a flavouring agent.	No safety concern
2,5-Dimethyl-3-furanthiol	1063	55764-23-3 	No Europe: 0.03 USA: 0.7	Yes. The NOEL of 5 mg/kg bw per day for the related substance No. 1060 is > 100 000 times the estimated intake of 2,5-dimethyl-3-furanthiol when used as a flavouring agent.	No safety concern
Furfuryl mercaptan	1072	98-02-2 	No Europe: 40 USA:11	Yes. The NOEL of 3 mg/kg bw per day is > 1000 times the estimated intake of furfuryl mercaptan when used as a flavouring agent.	No safety concern
Furfuryl thioacetate	1074	13678-68-7 	No Europe: 0.5 USA: 0.05	Yes. The NOEL of 0.83 mg/kg bw per day is > 100 000 times the estimated intake of furfuryl thioacetate when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

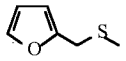
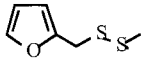
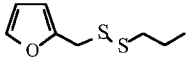
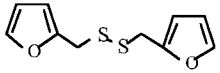
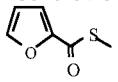
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
Furfuryl methyl sulfide	1076	1438-91-1 	No Europe: 1 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day for the related substance No. 1077 is > 10 000 times the estimated intake of furfuryl methyl sulfide when used as a flavouring agent.	No safety concern
Methyl furfuryl disulfide	1078	57500-00-2 	No Europe: 1 USA: 0.04	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 10 000 times the estimated intake of methyl furfuryl disulfide when used as a flavouring agent.	No safety concern
Furfuryl propyl disulfide	1079	2527366-36-0 	No Europe: ND USA: 3	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance No. 1064 is > 10 000 times the estimated intake of furfuryl propyl disulfide when used as a flavouring agent.	No safety concern
2,2′-(Dithiodimethylene) difuran	1081	4437-20-1 	No Europe: 4 USA: 0.7	Yes. The NOEL of 3 mg/kg bw per day for the related substance No. 1072 is > 10 000 times the estimated intake of 2,2′-(dithiodimethylene) difuran when used as a flavouring agent.	No safety concern
Methyl thiofuroate	1083	13679-61-3 	No Europe: 0.4 USA: 0.1	Yes. The flavouring agent is expected to hydrolyse to methanethiol and furoic acid. The NOEL of 0.74 mg/kg bw per day for the related substance No. 1071, which also hydrolyses to furoic acid, is > 100 000 times the estimated intake of methyl thiofuroate when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

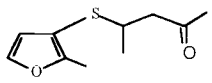
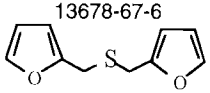
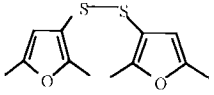
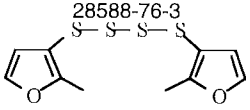
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084	180031-78-1 	No Europe: ND USA: 0.6	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 is > 100 000 times the estimated intake of 4-[(2-methyl-3-furyl)thio]-2-pentanone when used as a flavouring agent.	No safety concern
2-Methyl-3-tetrahydrofuranthiol	1090	57124-87-5 	No Europe: 4 USA: 0.7	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of 2-methyl-3-tetrahydrofuranthiol when used as a flavouring agent.	No safety concern
2,2´-(Thiodimethylene) difuran	1080	13678-67-6 	No Europe: 0.9 USA: 0.005	Yes. The NOEL of 10 mg/kg bw per day is > 100 000 times the estimated intake of 2,2´-(thiodimethylene) when used as a flavouring agent.	No safety concern
Structural class III					
Bis(2,5-dimethyl-3-furyl)disulfide	1067	28588-73-0 	No Europe: 0.01 USA: 0.7	Yes. The NOEL of 0.45 mg/kg bw per day for the related substance No. 1066 is > 10 000 times the estimated intake of bis(2,5-dimethyl-3-furyl)disulfide when used as a flavouring agent.	No safety concern
Bis(2-methyl-3-furyl) tetrasulfide	1068	28588-76-3 	No Europe: ND USA: 0.7	Yes. The NOEL of 0.56 mg/kg bw per day is > 10 000 times the estimated intake of bis(2-methyl-3-furyl) tetrasulfide when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

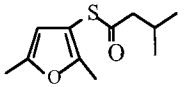
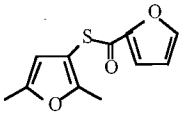
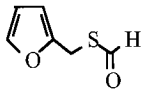
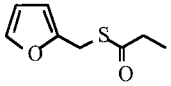
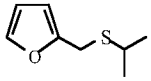
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2,5-Dimethyl-3-furan thioisovalerate	1070	55764-28-8 	No Europe: ND USA: 0.7	Yes. The NOEL of 0.73 mg/kg bw per day is > 10 000 times the estimated intake of 2,5-dimethyl-3-furan thioisovalerate when used as a flavouring agent.	No safety concern
S-2,5-Dimethyl-3-thiofuroyl furan	1071	65505-16-0 	No Europe: ND USA: 0.01	Yes. The NOEL of 0.74 mg/kg bw per day is > 1 million times the estimated intake of *S*-2,5-dimethyl-3-thiofuroyl furan when used as a flavouring agent.	No safety concern
Furfuryl thioformate	1073	59020-90-5 	No Europe: 2 USA: 0.02	Yes. The NOELs of 3 and 0.83 mg/kg bw per day for the related substances Nos 1072 and 1074 are > 1 million and > 10 000 times, respectively, the estimated intake of furfuryl thioformate when used as a flavouring agent.	No safety concern
Furfuryl thiopropionate	1075	59020-85-8 	No Europe: 0.01 USA: 0.005	Yes. The NOELs of 3 and 0.83 mg/kg bw per day for the related substances Nos 1072 and 1074 are > 1 million times the estimated intake of furfuryl thiopropionate when used as a flavouring agent.	No safety concern
Furfuryl isopropyl sulfide	1077	1883-78-9 	No Europe: 0.001 USA: 0.1	Yes. The NOEL of 1.3 mg/kg bw per day is > 100 000 times the estimated intake of furfuryl isopropyl sulfide when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

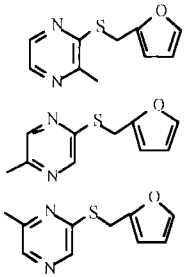
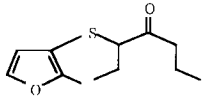
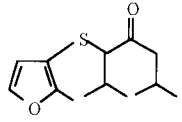
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082	65530-53-2 	No Europe: 0.4 USA: 0.7	Yes. The NOEL of 1.7 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-, 5- or 6-(furfurylthio)pyrazine when used as a flavouring agent.	No safety concern
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085	61295-41-8 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day is > 100 000 times the estimated intake of 3-[(2-methyl-3-furyl)thio]-4-heptanone when used as a flavouring agent.	No safety concern
2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086	61295-51-0 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 is > 100 000 times the estimated intake of 2,6-dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

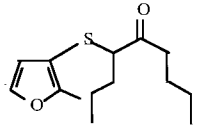
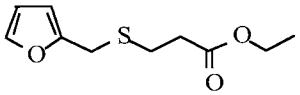
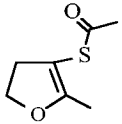
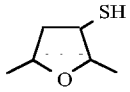
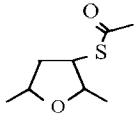
Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087	61295-50-9 	No Europe: ND USA: 0.7	Yes. The NOEL of 3.8 mg/kg bw per day for the related substance No. 1085 was > 100 000 times the estimqted intake of 4-[(2-methyl-3-furyl)thio]-5-nonanone when used as a flavouring agent.	No safety concern
Ethyl 3-(furfuryl-thio)propionate	1088	94278-27-0 	No Europe: 0.01 USA: 0.2	Yes. The NOEL of 17 mg/kg bw per day is > 1 million times the estimated intake of ethyl 3-(furfurylthio)propionate when used as a flavouring agent.	No safety concern
2-Methyl-3-thioacetoxy-4,5-dihydrofuran	1089	26486-14-6 	No Europe: ND USA: 0.7	Yes. The NOEL of 8.3 mg/kg bw per day is > 100 000 times the estimated intake of 2-methyl-3-thioacetoxy-4,5-dihydrofuran at when used as a flavouring agent.	No safety concern
cis- and *trans*-2,5-Dimethyl-3-tetrahydro-furanthiol	1091	26486-21-5 	No Europe: ND USA: 0.9	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of *cis*- and *trans*-2,5-dimethyl-3-tetrahydrofuranthiol when used as a flavouring agent.	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step B3*[a,b] Does intake exceed threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related chemical?	Conclusion based on current intake
cis- and *trans*-2,5-Dimethyltetrahydro-3-furyl thioacetate	1092	252736-39-3 	No Europe: ND USA: 2	Yes. The NOEL of 8.3 mg/kg bw per day for the related substance No. 1089 is > 100 000 times the estimated intake of *cis*- and *trans*-2,5-dimethyl-tetrahydro-3-furyl thioacetate when used as a flavouring agent.	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported

[a] Step 2: None of the flavouring agents in this group was predicted to be metabolized to innocuous products.

[b] The human intake threshold is 540 μg/person per day for class II and 90 μg/person per day for class III. All intake levels are expressed in μg/person per day. The combined intake of flavouring agents in class II is 56 and 20 μg/person per day in Europe and the USA, respectively. The combined intake of flavouring age in class III is 2.4 and 8.8 μg/person per day in Europe and the USA, respectively.

Absorption, distribution, metabolism and elimination

No data on the metabolism of these flavouring agents were available; however, all the members of the group contain a reactive divalent sulfur atom attached to the heteroaromatic or heterocyclic ring. Therefore, these flavouring agents are probably metabolized via reactions of the divalent sulfur, like other heteroaromatic ring systems containing sulfur as a side-chain substituent. However, in the absence of data on the disposition of these substances, the metabolic fate of this group of flavouring agents is unknown.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Twenty-nine of the 33 flavouring agents in this group are aromatic heterocyclic compounds bearing ring substituents. Twenty-two of the 29 heteroaromatic *S*-substituted furan derivatives containing only one aromatic ring were assigned to structural class II if they were naturally occurring or to structural class III if they did not occur naturally in foods. The seven remaining heteroaromatic substances contain two aromatic rings. Of these, five agents (Nos 1066, 1067, 1068, 1071 and 1081) would be hydrolysed (thioester) or readily reduced (di- or tetra-sulfides) to mononuclear residues. They were assigned to structural class II or III depending on their natural occurrence. The two remaining substances (Nos 1080 and 1082), which are not readily converted to mononuclear residues, were placed in structural class III.

The remaining four flavouring agents are heterocyclic but not heteroaromatic (Nos 1089–1092). One agent, 2-methyl-3-tetrahydrofuranthiol (No. 1090), occurs naturally in foods and was placed in structural class II. The other three agents were placed in structural class III.

In summary, 18 of the 33 flavouring agents were assigned to structural class II because they are common components of food, while 15 that are not common components of food were assigned to structural class III.

Step 2. None of the flavouring agents in this group was predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded down the B (right-hand) side of the decision tree.

Step B3. The current estimated daily *per capita* intakes of each of the 33 flavouring agents in this group are below the threshold for human intake for the respective structural classes (540 mg/day for structural class II and 90 mg/day for structural class III). Accordingly, the evaluation of all 33 substances in the group proceeded to step B4.

Step B4. The NOEL for 2-methyl-3-furanthiol (No. 1060) in a 90-day dietary study in rats was 5 mg/kg bw per day. This NOEL is appropriate for ethanoic acid, *S*-(2-methyl-3-furanyl) ester (No. 1069) because the acetate ester would

be hydrolysed to 2-methyl-3-furanthiol (No. 1060). This NOEL is also appropriate for the closely structurally related agent, 2,5-dimethyl-3-furanthiol (No. 1063).

The NOEL for furfuryl isopropyl sulfide (No. 1077) in a 90-day dietary study in rats was 1.3 mg/kg bw per day. This NOEL is also appropriate for three structurally related sulfides, 2-methyl-3-(methylthio)furan (No. 1061), 2-methyl-5-(methylthio)furan (No. 1062) and furfuryl methyl sulfide (No. 1076), which would be expected to participate in the same metabolic pathways as furfuryl isopropyl sulfide.

The NOEL for methyl 2-methyl-3-furyl disulfide (No. 1064) in a 90-day dietary study in rats was 1.2 mg/kg bw per day. This NOEL is also appropriate for three structurally related disulfides, propyl 2-methyl-3-furyl disulfide (No. 1065), methyl furfuryl disulfide (No. 1078) and furfuryl propyl disulfide (No. 1079).

The NOEL for bis(2-methyl-3-furyl) disulfide (No. 1066) in a 90-day dietary study in rats was 0.45 mg/kg bw per day. This NOEL is also appropriate for a structurally related bis-disulfide, bis(2,5-dimethyl-3-furyl) disulfide (No. 1067).

The NOEL for furfuryl mercaptan (No. 1072) in a multiple-dose, 13-week study in rats treated by gavage was 3 mg/kg bw per day; and the NOEL for furfuryl thioacetate (No. 1074) in a 90-day dietary study in rats was 0.83 mg/kg bw per day. These NOELs are also appropriate for the esters furfuryl thioformate (No. 1073) and furfuryl thiopropionate (No. 1075), because they are either close structural relatives of furfuryl thioacetate (No. 1074) or are expected to be hydrolysed to furfuryl mercaptan (No. 1072). The NOEL of 3 mg/kg bw per day for furfuryl mercaptan (No. 1072) is also appropriate for 2,2´-(dithiodimethylene) difuran (No. 1081), because this chemical is anticipated to be readily reduced to furfuryl mercaptan.

The NOEL for 2,5-dimethyl-3-thiofuroyl furan (No. 1071) in a 90-day dietary study in rats was 0.74 mg/kg bw per day. This NOEL is appropriate for methyl thiofuroate (No. 1083), because both 2,5-dimethyl-3-thiofuroyl furan and methyl thiofuroate would be hydrolysed to furoic acid.

The NOEL for 3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1085) in a 90-day dietary study in rats was 3.8 mg/kg bw per day. This NOEL is also appropriate for three structurally related thioketones, 4-[(2-methyl-3-furyl)thio]-2-pentanone (No. 1084), 2,6-dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1086) and 4-[(2-methyl-3-furyl)thio]-5-nonanone (No. 1087).

The NOELs for 2,2´-(thiodimethylene) difuran (No. 1080), bis(2-methyl-3-furyl) tetrasulfide (No. 1068), 2,5-dimethyl-3-furan thioisovalerate (No.

1070), 2-methyl-3-, 5- or 6-(furfurylthio)pyrazine (No. 1082) and ethyl 3-(furfurylthio)propionate (No. 1088) in 14-day or 90-day dietary studies in rats were 10, 0.56, 0.73, 1.7 and 17 mg/kg bw per day, respectively.

The NOEL for 2-methylthioacetoxy-4,5-dihydrofuran (No. 1089) in a 1-year dietary study in rats was 8.3 mg/kg bw per day. This NOEL is also appropriate for three structurally related analogues, 2-methyl-3-tetrahydrofuranthiol (No. 1090), *cis*- and *trans*-2,5-dimethyl-3-tetrahydrofuranthiol (No. 1091) and *cis*- and *trans*-2,5-dimethyltetrahydro-3-furyl thioacetate (No. 1092), because these chemicals are expected to be hydrolysed to the corresponding dihydro- or tetrahydrofuranthiol.

The NOEL(s) used to evaluate each flavouring agent and the margins of safety they provide, on the basis of current intake levels, are summarized in Table 8.

Consideration of combined intakes from use as flavouring agents

The hydrolysis of furfuryl thioesters (Nos 1073, 1074 and 1075) or reduction of the disulfides (Nos 1078, 1079 and 1081) in this group would yield the common metabolite furfuryl mercaptan (No. 1072). In the unlikely event that these seven flavouring agents were to be consumed simultaneously on a daily basis, the estimated combined intakes (48 and 15 μg per person per day in Europe and the USA, respectively) would not exceed the threshold for daily human intake of compounds in structural class II (540 μg per person) or III (90 μg per person).

Likewise, hydrolysis of 2-methyl-3-furan thioester (No. 1069) or reduction of the corresponding disulfides (Nos 1064, 1065 and 1066) or tetrasulfide (No. 1068) would yield the common metabolite 2-methyl-3-furanthiol (No. 1060). In the unlikely event that these six flavouring agents were to be consumed simultaneously on a daily basis, the estimated combined daily intake would be less than 5 μg per person in Europe and the USA, which would not exceed the threshold for human daily intake of compounds in structural class II (540 μg per person) or III (90 μg per person).

On the basis of the evaluation of the collective data, the Committee identified no safety concern from combined intake.

Conclusions

The Committee concluded that use of flavouring agents in this group of 33 thiofurfuryl and thiofuran derivatives at current levels of intake would not present a safety concern. In the Procedure, data on toxicity were required for all the evaluations. The Committee noted the absence of data on the metabolic fate of these substances but considered that their potential

Table 8

NOELs and margins of safety for sulfur-substituted furan derivatives used as flavouring agents

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (μg/kg bw per day)	Margin of safety
Structural class II						
2-Methyl-3-furanthiol	1060			5[a]	0.015	> 100 000
		Ethanoic acid, *S*-(2-methyl-3-furanyl) ester	1069		0.001	> 1 000 000
		2,5-Dimethyl-3-furanthiol	1063		0.01	> 100 000
Methyl 2-methyl-3-furyl disulfide	1064			1.2[a]	0.014	> 10 000
		Propyl 2-methyl-3-furyl disulfide	1065		0.01	> 100 000
		Methyl furfuryl disulfide	1078		0.017	> 10 000
		Furfuryl propyl disulfide	1079		0.04	> 10 000
Bis(2-methyl-3-furyl) disulfide	1066			0.45[a]	0.01	> 10 000
		Bis(2,5-dimethyl-3-furyl) disulfide	1067		0.01	> 10 000
Furfuryl mercaptan	1072			3[a]	0.58	> 1 000
		Furfuryl thioformate	1073		0.026	> 100 000
		Furfuryl thiopropionate	1075		0.0002	> 1 000 000
		2,2′-(Dithiodimethylene) difuran	1081		0.064	> 10 000
Furfuryl thioacetate	1074			0.83[a]	0.008	> 100 000
		Furfuryl thioformate	1073		0.026	> 10 000
		Furfuryl thiopropionate	1075		0.0002	> 1 000 000
4-[(2-Methyl-3-furyl)thio]-2-pentanone	1084			54[b,c]	0.01	> 1 000 000
		2-Methyl-3-tetrahydrofuranthiol	1090	12[b,c]	0.07	> 100 000
2,2′-(Thiodimethylene) difuran	1080			10[b]	0.014	> 100 000

Table 8 *(continued)*

Flavouring agent	No.	Related flavouring agent(s)	No.	NOEL (mg/kg bw per day)	Highest estimated intake (μg/kg bw per day)	Margin of safety
Structural class III						
Bis(2-methyl-3-furyl) tetrasulfide	1068			0.56[a]	0.01	> 10 000
2,5-Dimethyl-3-furan thioisovalerate	1070			0.73[a]	0.01	> 10 000
S-2,5-Dimethyl-3-thiofuroyl furan	1071			0.74[a]	0.0002	> 1 000 000
		Methyl thiofuroate	1083		0.002	> 100 000
Furfuryl isopropyl sulfide	1077			1.3[a]	0.002	> 100 000
		2-Methyl-3-(methylthio)furan	1061		0.024	> 10 000
		2-Methyl-5-(methylthio)furan	1062		0.021	> 10 000
		Furfuryl methyl sulfide	1076		0.02	> 10 000
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082			1.7[a]	0.01	> 100 000
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085			3.8[a]	0.01	> 100 000
		4-[(2-Methyl-3-furyl)thiol]-2-pentanone	1084		0.01	> 100 000
		2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086		0.01	> 100 000
		4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087		0.01	> 100 000
Ethyl 3-(furfurylthio)propionate	1088			17[a]	0.003	> 1 000 000
2-Methyl-3-thioacetoxy-4,5-dihydrofuran	1089			8.3[d]	0.01	> 100 000
		2-Methyl-3-tetrahydrofuranthiol	1090		0.07	> 100 000
		cis- and *trans*-2,5-Dimethyl-3-tetrahydrofuranthiol	1091		0.02	> 100 000
		cis- and *trans*-2,5-Dimethyltetrahydro-3-furyl thioacetate	1092		0.03	> 100 000

[a]90-day study
[b]14-day study
[c]This study was not used in the evaluation, as a 90-day study on a structurally related flavouring agent was available.
[d]1-year study

metabolism via the reactive divalent sulfur atom and their very low levels of use as flavouring agents were consistent with the outcome of the evaluations.

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

4.1.5 *Alicyclic ketones, secondary alcohols and related esters*

The Committee evaluated a group of 25 flavouring agents consisting of alicyclic ketones, secondary alcohols and related esters (see Table 9). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). None of these agents has been evaluated previously by the Committee.

Twelve of the 25 substances (Nos 1093, 1098, 1100–1104, 1107, 1108, 1110, 1112 and 1114) have been reported to occur naturally in foods. They have been detected in fruits, vegetables, cheese, meats, seafood, grains, alcoholic beverages, coffee and tea.

Estimated daily per capita *intake*

The total annual volume of production of the 25 alicyclic ketones, secondary alcohols and related esters is approximately 520 kg in Europe and 310 kg in the USA. The reported annual production of only one agent, 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (No. 1114), is greater than 100 kg (110 kg in Europe). The daily *per capita* intake of each of the agents in this group is $\leq$ 15 μg in Europe and in the USA.

Absorption, distribution, metabolism and elimination

Esters (Nos 1093–1098) in this group are hydrolysed to their corresponding alcohols and carboxylic acids by carboxylesterases, which are found abundantly in hepatocytes. The resulting alicyclic secondary alcohols are conjugated with glucuronic acid and excreted mainly in the urine. Side-chain oxidation of methyl groups may also occur.

Alicyclic ketones (Nos 1100–1117) are reduced to the corresponding secondary alcohol and excreted primarily as glucuronic acid conjugates. If a double bond is present, the chemical may be reduced to the corresponding dihydro derivative. Reduction of the double bond in metabolites excreted in the bile is probably associated with the action of gut microflora. Endocyclic double bonds (Nos 1105, 1107 and 1110–1115) are more prone to reduction than exocyclic double bonds (Nos 1098 and 1106). Alicyclic ketones containing an alkyl side-chain (Nos 1098, 1099 and 1102–1117) can not only follow reductive pathways but can undergo oxidation of the side-chain to form poly-oxygenated metabolites, which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

Table 9
Summary of results of the safety evaluation of alicyclic ketones, secondary alcohols and related esters used as flavouring agents[a]

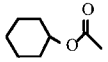
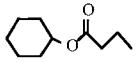
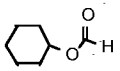
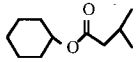
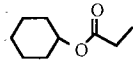
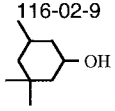
Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Cyclohexyl acetate	1093	622-45-7 	No Europe: 14 USA: 10	See note 1.	No safety concern
Cyclohexyl butyrate	1094	1551-44-6 	No Europe: ND USA: 0.1	See note 1.	No safety concern
Cyclohexyl formate	1095	4351-54-6 	No Europe: 0.01 USA: 0.2	See note 1.	No safety concern
Cyclohexyl isovalerate	1096	7774-44-9 	No Europe: 0.3 USA: 0.05	See note 1.	No safety concern
Cyclohexyl propionate	1097	6222-35-1 	No Europe: 0.01 USA: 0.05	See note 1.	No safety concern
3,3,5-Trimethyl cyclohexanol	1099	116-02-9 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern

Table 9 *(continued)*

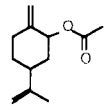
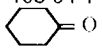
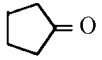
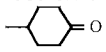
Flavouring agent	No.	CAS No. and structure	*Step A3*[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class II					
cis- and *trans-para*-1(7),8-Menthadien-2-yl acetate	1098	71660-03-2 	No Europe: ND USA: 0.6	See note 1.	No safety concern
Cyclohexanone	1100	108-94-1 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
Cyclopentanone	1101	120-92-3 	No Europe: 0.02 USA: 0.02	See note 2.	No safety concern
2-Methylcyclohexanone	1102	583-60-8 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
3-Methylcyclohexanone	1103	591-24-2 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern
4-Methyl cyclohexanone	1104	589-92-4 	No Europe: 0.1 USA: 0.1	See note 2.	No safety concern

Table 9 *(continued)*

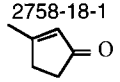
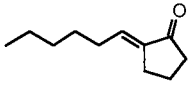
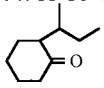
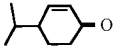
Flavouring agent	No.	CAS No. and structure	*Step A3*[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
1-Methyl-1-cyclopenten-3-one	1105	2758-18-1 	No Europe: 0.07 USA: ND	See note 3.	No safety concern
Structural class II					
2-Hexylidene cyclopentanone	1106	17373-89-6 	No Europe: 0.3 USA: 0.01	See notes 3 and 4.	No safety concern
3-Methyl-2-cyclohexen-1-one	1107	1193-18-6 	No Europe: 0.01 USA: 0.1	See note 3.	No safety concern
2,2,6-Trimethylcyclo-hexanone	1108	2408-37-9 	No Europe: 2.4 USA: 0.04	See note 2.	No safety concern
2-*sec*-Butylcyclohexanone	1109	14765-30-1 	No Europe: 6 USA: ND	See note 4.	No safety concern
4-Isopropyl-2-cyclo-hexenone	1110	500-02-7 	No Europe: 0.001 USA: 0.001	See note 3.	No safety concern

Table 9 *(continued)*

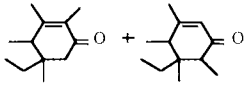
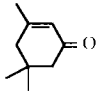
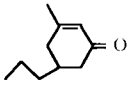
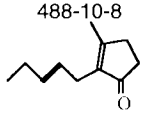
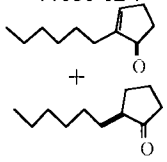
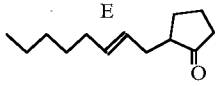
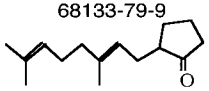
Flavouring agent	No.	CAS No. and structure	*Step A3*[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Tetramethyl ethyl cyclohexenone (mixture of isomers)	1111	17369-60-7 	No Europe: 9 USA: 0.2	See note 3.	No safety concern
Isophorone	1112	78-59-1 	No Europe: 5.4 USA: 0.1	See note 3.	No safety concern
3-Methyl-5-propyl-2-cyclohexen-1-one	1113	3720-16-9 	No Europe: 0.1 USA: 4.1	See note 3.	No safety concern
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114	488-10-8 	No Europe: 16 USA: 7.2	See notes 3 and 4.	No safety concern
Isojasmone	1115	11050-62-7 	No Europe: 0.4 USA: 0.01	See notes 3 and 4.	No safety concern

Table 9 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
(E)-2-(2-Octenyl) cyclopentanone	1116	65737-52-2 	No Europe: 7 USA: 6.6	See note 4.	No safety concern
2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone	1117	68133-79-9 	No Europe: 7.1 USA: 6.6	See note 4.	No safety concern

CAS: Chemical Abstracts Service; ND: No intake data reported

[a] Step 2: All of the flavouring agents in this group were predicted to be metabolized to innocuous products.

[b] The threshold for human intake is 1800 μg/person per day for class I and 540 μg/person per day for class II. All intake levels are expressed in μg/person per day. The combined intake of flavouring agents in class I is 14 and 10 μg per person per day in Europe and the USA, respectively. The combined intake of flavouring agents in class II is 54 and 26 μg per person per day in Europe and the USA, respectively.

Notes:

1. Detoxicated by hydrolysis of ester and glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid
2. Detoxicated by reduction of the ketone followed by glucuronic acid conjugation of the corresponding alcohol
3. Detoxicated by reduction of the ketone functional group followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone
4. Detoxicated by reduction of the ketone and alkyl side-chain oxidation and excretion

The nine chemicals that are α,β-unsaturated ketones (Nos 1105–1107 and 1110–1116) are subject to glutathione conjugation, with subsequent elimination in the urine as mercapturic acids.

As the α,β-unsaturated carbonyl group is a structural alert for toxicity, the Committee, at previous meetings, has devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference *154*) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with use of such compounds as flavouring agents. With respect to α,β-unsaturated ketones, these protective processes include reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid) and conjugation with glutathione. These processes operate for the aliphatic ketones used as flavouring agents.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned six (Nos 1093–1097 and 1099) of the 25 substances to structural class I. These agents are either alicyclic secondary alcohols or are readily hydrolysed to secondary alcohols and simple short-chain carboxylic acids. They are simple aliphatic substances containing secondary hydroxyl groups, which have low toxicity. The remaining 19 compounds, which are monocyclic alkanones (Nos 1100–1117) or have a secondary alcohol attached to a vinyl group (No. 1098), were assigned to structural class II.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded *via* the A (left-hand) side of the decision tree.

Step A3. The estimated daily *per capita* intake of each of the six flavouring agents in structural class I and each of the 19 agents in structural class II is below the threshold for daily human intake of compounds in the respective structural class (i.e., 1800 μg per person for class I and 540 μg per person for class II). According to the Procedure, the safety of these 25 flavouring agents raises no concern when consumed at currently estimated levels.

Table 9 summarizes the evaluations of acyclic ketones, secondary alcohols and related esters used as flavouring agents.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the flavouring agents in structural class I or II were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake of either class.

Conclusions

The Committee concluded that none of the flavouring agents in this group of alicyclic ketones, secondary alcohols and related esters would raise a safety concern at current estimated levels of intake. Other data on the toxicity of alicyclic ketones, secondary alcohols and related esters were consistent with the results of the safety evaluation.

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

4.1.6 *Aliphatic secondary alcohols, ketones and related esters*

The Committee evaluated a group of 39 flavouring agents that includes aliphatic acyclic secondary alcohols and ketones and esters derived from aliphatic secondary alcohols (see Table 10), using the Procedure for the Safety Evaluation of Flavouring Agents (See Figure 1). The Committee had not previously evaluated any of these agents.

Twenty-six of the 39 substances in this group of flavouring agents (Nos 1118, 1120, 1122–1132, 1134–1136, 1139, 1142, 1144, 1147, 1148, 1150–1152, 1154 and 1156) have been reported to occur naturally in foods. They have been found in fruits, juices, spices, vegetables, cocoa, coffee and tea.

Estimated daily per capita *intake*

The total annual volume of production of the 39 aliphatic secondary alcohols and ketones in this group is approximately 3900 kg in Europe and 1100 kg in the USA. Approximately 73% and 47% of the total annual production volume in Europe and the USA, respectively, is accounted for by 6-methyl-5-hepten-2-one (No. 1120) and 1-octen-3-ol (No. 1152). The daily *per capita* intake of 6-methyl-5-hepten-2-one (No. 1120) and 1-octen-3-ol (No. 1152) was 120 μg and 290 μg in Europe and 44 μg and 23 μg, respectively, in the USA, respectively.

Absorption, distribution, metabolism and elimination

In general, the aliphatic esters in this group hydrolyse to the corresponding secondary alcohols. The secondary alcohols and their corresponding ketones are interconvertible under physiological conditions. In the principal excretion pathway, the ketones are reduced to the corresponding secondary alcohols, which are subsequently conjugated with glucuronic acid and excreted.

When the ketone carbonyl function is located at the 2-position (i.e. a methyl ketone), the methyl group may undergo α-hydroxylation and subsequent oxidation, to yield a corresponding ketocarboxylic acid. The ketoacids are intermediary metabolites (e.g. α-ketoacids), which can undergo oxidative decarboxylation to yield carbon dioxide and simple aliphatic carboxylic acids.

Table 10

Summary of the results of safety evaluations of aliphatic secondary alcohols, ketones and related esters used as flavouring agents[a]

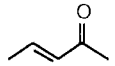
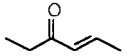
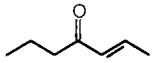
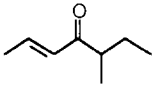
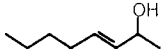
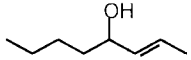
Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
3-Penten-2-one	1124	625-33-2 	No Europe: 0.3 USA: ND	See notes 1, 3 and 4.	No safety concern
4-Hexen-3-one	1125	2497-21-4 	No Europe: 15 USA: 1	See notes 1 and 3.	No safety concern
2-Hepten-4-one	1126	4643-25-8 	No Europe: 0.01 USA: ND	See notes 1 and 3.	No safety concern
5-Methyl-2-hepten-4-one	1133	81925-81-7 	No Europe: 7 USA: 1	See notes 1 and 3.	No safety concern
3-Octen-2-ol	1140	76649-14-4 	No Europe: 1 USA: ND	See note 1.	No safety concern
(E)-2-Octen-4-ol	1141	20125-81-9 	No Europe: ND USA: 1	See note 1.	No safety concern

Table 10 *(continued)*

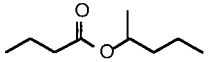
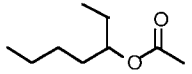
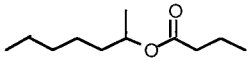
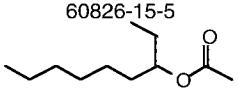
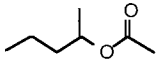
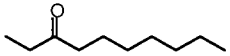
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Pentyl butyrate	1142	60415-61-4 	No Europe: ND USA: 1	See note 2.	No safety concern
(±) Heptan-3-yl acetate	1143	5921-83-5 	No Europe: 4 USA: 3	See note 2.	No safety concern
(±) Heptan-2-yl butyrate	1144	39026-94-3 	No Europe: 4 USA: 3	See note 2.	No safety concern
(±) Nonan-3-yl acetate	1145	60826-15-5 	No Europe: 4 USA: 3	See note 2.	No safety concern
2-Pentyl acetate	1146	626-38-0 	No Europe: 3 USA: 3	See note 2.	No safety concern
Structural class II					
3-Decanone	1118	928-80-3 	No Europe: 4 USA: 3	See notes 1 and 3.	No safety concern

Table 10 *(continued)*

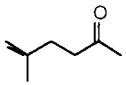
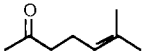
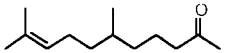
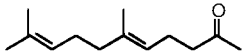
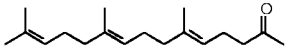
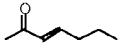
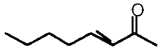
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
5-Methyl-5-hexen-2-one	1119	3240-09-3 	No Europe: ND USA: 0.3	See notes 1, 3 and 4.	No safety concern
6-Methyl-5-hepten-2-one	1120	110-93-0 	No Europe: 120 USA: 44	See notes 1 and 4.	No safety concern
3,4,5,6-Tetrahydro-pseudoionone	1121	4433-36-7 	No Europe: ND USA: 0.01	See notes 1 and 4.	No safety concern
6,10-Dimethyl-5,9-undecadien-2-one	1122	3796-70-1 	No Europe: 49 USA: 2	See notes 1 and 4.	No safety concern
2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one	1123	762-29-8 	No Europe: 0.1 USA: ND	See notes 1 and 4.	No safety concern
3-Hepten-2-one	1127	1119-44-4 	No Europe: 0.2 USA: 0.07	See notes 1, 3 and 4.	No safety concern
3-Octen-2-one	1128	1669-44-9 	No Europe: 1 USA: 1	See notes 1, 3 and 4.	No safety concern

Table 10 *(continued)*

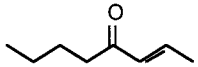
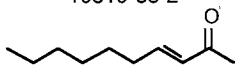
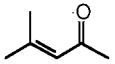
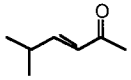
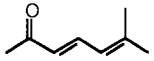
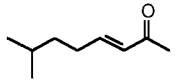
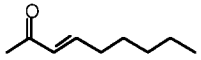
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Octen-4-one	1129	4643-27-0 	No Europe: 1 USA: 3	See notes 1 and 3.	No safety concern
3-Decen-2-one	1130	10519-33-2 	No Europe: 0.01 USA: ND	See notes 1, 3 and 4.	No safety concern
4-Methyl-3-penten-2-one	1131	141-79-7 	No Europe: 0.4 USA: ND	See notes 1 and 4.	No safety concern
5-Methyl-3-hexen-2-one	1132	5166-53-0 	No Europe: ND USA: 0.1	See notes 1, 3 and 4.	No safety concern
6-Methyl-3,5-heptadien-2-one	1134	1604-28-0 	No Europe: 15 USA: 5	See notes 1, 3 and 4.	No safety concern
(E)-7-Methyl-3-octen-2-one	1135	33046-81-0 	No Europe: ND USA: 2	See notes 1, 3 and 4.	No safety concern
3-Nonen-2-one	1136	14309-57-0 	No Europe: 14 USA: 13	See notes 1, 3 and 4.	No safety concern

Table 10 *(continued)*

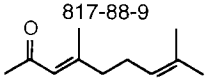
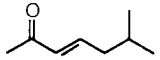
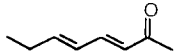
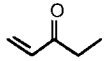
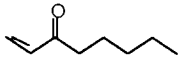
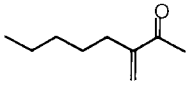
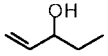
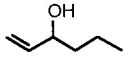
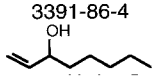
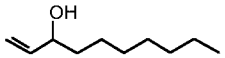
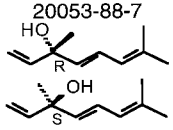
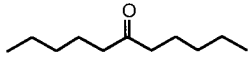
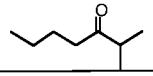
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
(E) & (Z)-4,8-Dimethyl-3,7-nonadien-2-one	1137	817-88-9 	No Europe: 7 USA: 7	See notes 1, 3 and 4.	No safety concern
(E)-6-Methyl-3-hepten-2-one	1138	20859-10-3 	No Europe: 4 USA: 3	See notes 1, 3 and 4.	No safety concern
(E,E)-3,5-Octadien-2-one	1139	30086-02-3 	No Europe: 4 USA: 3	See notes 1, 3 and 4.	No safety concern
1-Penten-3-one	1147	1629-58-9 	No Europe: 0.3 USA: 0.1	See notes 1 and 3.	No safety concern
1-Octen-3-one	1148	4312-99-6 	No Europe: 1 USA: 0.1	See notes 1 and 3.	No safety concern
2-Pentyl-1-buten-3-one	1149	63759-55-7 	No Europe: 0.2 USA: ND	See notes 1, 3 and 4.	No safety concern
1-Penten-3-ol	1150	616-25-1 	No Europe: 2 USA: 1	See note 1.	No safety concern

Table 10 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
1-Hexen-3-ol	1151	4798-44-1 	No Europe: 1 USA: 2	See note 1.	No safety concern
1-Octen-3-ol	1152	3391-86-4 	No Europe: 290 USA: 23	See note 1.	No safety concern
1-Decen-3-ol	1153	51100-54-0 	No Europe: ND USA: 0.1	See note 1.	No safety concern
(E,R)-3,7-Dimethyl-1,5,7-octatrien-3-ol	1154	20053-88-7 	No Europe: ND USA: 6	See note 1.	No safety concern
6-Undecanone	1155	927-49-1 	No Europe: 4 USA: 3	See note 1.	No safety concern
2-Methylheptan-3-one	1156	13019-20-0 	No Europe: 4 USA: 3	See note 1.	No safety concern

CAS: Chemical Abstracts Service; ND: No intake data reported

[a] Step 2: All the flavouring agents in this group were predicted to be metabolized to innocuous products.

[b] The human intake threshold is 1800 μg per person per day for class I and 540 μg per person per day for class II. All intake levels are expressed in μg per person per day. The combined intake of flavouring agents in class I is 58 and 16 μg per person per day in Europe and the USA, respectively. The combined intake of flavouring agents in class II is 520 and 120 μg per person per day in Europe and the USA, respectively.

Notes:

1. Detoxicated by reduction of the ketone followed by glucuronic acid conjugation of the corresponding alcohol or direct glucuronic acid conjugation of the secondary alcohol
2. Detoxicated by hydrolysis of the ester and glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid
3. Detoxicated by reduction of the ketone functional group followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone
4. Detoxicated by reduction of the ketone and alkyl side-chain oxidation and excretion

The acid may be completely metabolized in the fatty acid pathway and citric acid cycle. When the substance is an α,β-unsaturated ketone (Nos 1124–1139 and 1147–1149) or secondary alcohol (Nos 1140–1141 and 1150–1154) which is oxidized to an α,β-unsaturated ketone, it can conjugate with glutathione. The glutathione conjugates are converted to the corresponding mercapturic acid derivatives (*N*-acetylcysteine derivative) and excreted.

As the α,β-unsaturated carbonyl group is a structural alert for toxicity, the Committee, at previous meetings, has devoted considerable attention to the safety of flavouring agents containing this reactive moiety. The Committee concluded at its fifty-seventh meeting (Annex 1, reference *154*) that the presence of protective processes in cells provides adequate detoxication capacity at the low doses associated with use of such compounds as flavouring agents. With respect to α,β-unsaturated ketones, these protective processes include reduction of the ketone to the corresponding alcohol (followed by conjugation of the alcohol with glucuronic acid) and conjugation with glutathione. These processes operate for the aliphatic ketones used as flavouring agents.

Application of Procedure for the Safety Evaluation of Flavouring Substances

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these chemicals, the Committee assigned 11 of the 39 agents to structural class I on the basis that they are acyclic saturated and unsaturated aliphatic ketones with no other functional groups and three or fewer carbons on either side of the keto group (Nos 1124–1126 and 1133) and secondary alcohols (Nos 1140–1141) or esters of secondary alcohols (Nos 1142–1146). The remaining 28 flavouring agents, which are acyclic aliphatic ketones with four or more carbons on either side of the keto group (Nos 1118–1123, 1127–1132, 1134–1139, 1148, 1155 and 1156) and either a ketone (Nos 1147 and 1149) or a secondary alcohol (Nos 1150–1154) attached to a terminal vinyl group, were assigned to structural class II.

Step 2. All the flavouring agents in this group were predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded *via* the A (left-hand) side of the decision tree.

Step A3. The estimated daily *per capita* intakes of the 11 flavouring agents in structural class I and the 28 flavouring agents in structural class II are below the threshold for human intake for each class (i.e., 1800 μg per person for structural class I and 540 μg per person for structural class II). According to the Procedure, the safety of these 39 flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 10 summarizes the evaluation of aliphatic secondary alcohols, ketones and related esters used as flavouring agents.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the flavouring agents in this group were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for structural class I or II.

Conclusions

The Committee concluded that none of the flavouring agents in this group of 39 aliphatic secondary alcohols, ketones and related esters would raise a safety concern at the current estimated levels of intake. Other data on the toxicity of aliphatic secondary alcohols, ketones and related esters are consistent with the results of the safety evaluation.

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

4.2 Evaluation of secondary components of flavouring agents

The Committee had previously considered the procedure for evaluating flavouring agents for which the minimum assay figures were below 95%. It has followed the general principle that specifications should be designated as tentative if less than 95% of the material in the commercial product consists of the named compound, and known secondary components should be taken into account in the safety evaluation.

At its present meeting, the Committee considered information on the presence of known secondary components in all 51 flavouring agents evaluated at its forty-sixth through fifty-fifth meetings, for which the minimum assay was less than 95%, in order to assess whether these were covered by the existing safety evaluations of the named compounds.

In most cases, the secondary components of known chemical identity were found to be normal metabolites of the named compound, or to have chemical structures that are sufficiently similar that they fit within the same group evaluations. In two cases, the secondary components were not closely related to the named compound: the safety of 6-hydroxy-3,7-dimethyloctanoic acid (No. 237) was evaluated by reference to the NOEL for a similar compound, while that of the secondary component in 2,3-pentadione (No. 410) was assessed by reference to its likely metabolism .

In the light of all the available data on secondary components present in materials of commerce containing less than 95% of the named compound, the Committee concluded that the secondary components do not raise safety concerns and confirmed its conclusion that the flavouring agents listed in Annex 4 do not present any safety concern at the current estimated levels of intake.

The specifications for the 51 flavouring agents listed in Annex 4 that were originally evaluated at the forty-sixth to the fifty-fifth meetings will be reviewed at a future meeting.

4.3 Chemicals requiring confirmation of flavour use

The Committee received information from the flavour industry (*4*) concerning 18 flavouring agents for which the evaluations could not be finalized at the fifty-seventh meeting (Annex 1, reference *154*), pending information on whether they were in current use as flavouring agents.

Glycerol (No. 909) and *propylene glycol (No. 925)* have a long history of use as solvents and humectants (inter alia). With respect to their use as flavouring agents, the Committee was informed only that these substances provide 'a sweet taste', although propylene glycol was also reported to produce 'a warm burning sensation in the mouth' in certain aqueous applications. With the exception of the category 'meats', the reported flavouring uses of these compounds require high levels of addition, in many cases greater than 10 000 mg/kg (1%), and any 'flavouring' effect might either be a corollary of a more significant technical effect as a carrier or humectant or be related to the acknowledged sweetening properties. On the basis of the information provided, the Committee was not convinced that the reported uses of glycerol and propylene glycol are those of a flavouring agent. The Committee did not finalize the evaluations pending development of a definition of 'flavouring agent'.

Benzoic acid (No 860) and benzoate salts have known uses as preservatives, particularly in certain beverages. The flavouring properties of benzoic acid appear to be well established, and the Committee accepted that this chemical can be added to some foods primarily for its flavouring effect. The Committee noted that the levels used as a flavouring agent are lower than those generally associated with the preservative uses of benzoates, although the possibility of a secondary preservative effect in some flavouring applications cannot be excluded. The group ADI of 0–5 mg/kg bw for benzoic acid and related compounds was maintained at the forty-sixth meeting (Annex 1, reference *122*). The contribution of the intake of benzoic acid arising from its use as a flavouring agent to the total intake of benzoic acid and benzoates does not represent a safety concern.

The remaining 15 chemicals were reported to be added to foods as flavouring agents with the expectation that they will be hydrolysed to release flavour molecules, either under storage or during processing of the food. In some cases, hydrolysis is followed by further chemical changes (decarboxylation or oxidation) to generate the ultimate flavouring species. Because the end-products which are ingested by the consumer have been defined (see section

2.2.1), the Committee concluded that it is appropriate to evaluate these 15 chemicals by the Procedure for the Safety Evaluation of Flavouring Agents.

At its fifty-seventh meeting (Annex 1, reference *154*), the Committee predicted that all 15 chemicals would be metabolized to innocuous products, and they were therefore evaluated via the left-hand side of the decison tree.

Glyceryl tribenzoate (No. 861) and *propylene glycol dibenzoate (No. 862)* were considered by the Committee at its fifty-seventh meeting in the group of benzyl derivatives (Annex 1, reference *154*). The estimated intakes were below the threshold for human intake of their structural class (Step A3). They are partially hydrolysed in food under acidic conditions, giving rise to benzoic acid and glycerol and benzoic acid and propylene glycol. The group ADI of 0–5 mg/kg bw for benzoic acid and related compounds was maintained at the forty-sixth meeting (Annex 1, reference *122*). An ADI 'not specified'[1] was established for glycerol by the Committee at its twentieth meeting (Annex 1, reference *41*), and an ADI of 0–25 mg/kg bw was established for propylene glycol at the seventeenth meeting (Annex 1, reference *32*). The intakes of the hydrolysis products resulting from the flavouring uses of these agents are included in the relevant ADI. There are no safety concerns in relation to use of these chemicals as flavouring agents.

Butyl para-*hydroxybenzoate (No. 870)* was considered at the fifty-seventh meeting in the group of hydroxy- and alkoxy-substituted benzyl derivatives (Annex 1, reference *154*). The estimated intake was below the threshold for human intake of the structural class (step A3). When baked goods containing this chemical are heated, it is partially hydrolysed to butyl alcohol and *para*-hydroxybenzoic acid. Butyl alcohol (No. 85) was evaluated as a flavouring agent at the forty-ninth meeting and was considered not to be a safety concern (Annex 1, reference *131*). The intake of butyl alcohol from the flavouring use of butyl *para*-hydroxybenzoate would not significantly increase the total intake of this alcohol. *para*-Hydroxybenzoic acid was evaluated as a flavouring agent at the fifty-seventh meeting and was considered not to be a safety concern. The formation of *para*-hydroxybenzoic acid from the hydrolysis of butyl *para*-hydroxybenzoate would not significantly increase the total intake of this acid. There is no safety concern in relation to use of this chemical as a flavouring agent.

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

3-Oxodecanoic acid glyceride (No. 914), 3-oxododecanoic acid glyceride (No. 915), 3-oxotetradecanoic acid glyceride (No. 916) and *3-oxohexadecanoic acid glyceride (No. 917)* were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference *154*). The estimated intake of No. 914 was above the threshold for human intake of its structural class, class III, while the intakes of the others were below the thresholds for their corresponding structural classes (I and III) (step A3). After absorption, the glycerides would be hydrolysed to glycerol and the corresponding β-keto acid, which are endogenous. All these agents are hydrolysed in food, and flavour arises from the corresponding keto acid and glycerol. The keto acids are unstable and would undergo decarboxylation to the corresponding ketones (2-nonanone, 2-undecanone, 2-tridecanone and 2-pentadecanone, respectively). After absorption, the ketones would be metabolized by reduction to the corresponding aliphatic secondary alcohol, followed by conjugation with glucuronic acid. The ketones (Nos 292, 296, 298 and 299) were assigned to structural class II when they were evaluated at the fifty-first meeting (Annex 1, reference *137*) and considered not to be of safety concern at their estimated intakes as flavouring agents. The additional intake arising from intake of agents Nos 914–917 would not result in intakes of the ketones above the threshold for structural class II. There are no safety concerns in relation to use of these chemicals as flavouring agents.

Glyceryl monostearate (No. 918), glyceryl monooleate (No. 919) and *propylene glycol monostearate (No. 926)* were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference *154*). The estimated intake of propylene glycol monostearate (No. 926) was above the threshold for human intake of structural class I, while the intakes of the others were below the intake threshold (Step A3). After absorption, the glyceryl esters would be hydrolysed to glycerol and the corresponding saturated or unsaturated fatty acid, which are endogenous. Mono- and diglycerides (including glycerol monostearate and glyceryl monooleate) were allocated an ADI 'not limited' at the seventeenth meeting of the Committee (Annex 1, reference *32*). Propylene glycol monostearate (No. 926) would be hydrolysed to stearic acid and propylene glycol, for which an ADI of 0–25 mg/kg bw was established at the seventeenth meeting. All these agents are hydrolysed in food to the corresponding fatty acid and glycerol and, under certain ageing conditions, may be partially oxidized, imparting a rancid note to certain food flavours. The intake of the hydrolysis products resulting from the flavouring uses of these agents are included in the relevant ADI. There is no safety concern in relation to use of these chemicals as flavouring agents.

Triacetin (No. 920) was considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference *154*). The estimated intake exceeded the threshold for human intake of its structural class (Step A3), and triacetin was evaluated under Step A4. When used in foods that are heated or otherwise processed, triacetin would be hydrolysed to glycerol and acetic acid, both of which are endogenous. An ADI 'not specified' was established for glyceryl acetates by the Committee at its twentieth meeting (Annex 1, reference *41*), and the flavouring use of triacetin is included in this ADI. There is no safety concern in relation to the intake of triacetin or its hydrolysis products arising from its use as a flavouring agent.

Glyceryl tripropionate (No. 921) and *tributyrin (No. 922)* were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference *154*). The estimated intakes were below the threshold for human intake of their structural class (Step A3). With heating, the substances are partially or completely hydrolysed to glycerol and the corresponding carboxylic acid. Propionic and butyric acids were evaluated at the forty-ninth meeting (Annex 1, reference *131*), and their use as flavouring agents (Nos 84 and 87, respectively) was considered not to represent a safety concern. Glycerol and both acids are endogenous, and the intakes arising from flavouring use of substances Nos 921 and 922 would not significantly alter the total intake of either acid. There is no safety concern in relation to use of these chemicals as flavouring agents.

Glycerol 5-hydroxydecanoate (No. 923) and *glycerol 5-hydroxydodecanoate (No. 924)* were considered at the fifty-seventh meeting in the group of aliphatic acyclic diols, triols and related substances (Annex 1, reference *154*). The estimated intakes were below the threshold for human intake of their structural class (step A3). With heating, the substances are partially or completely hydrolysed to glycerol and the corresponding carboxylic acid. The acids are unstable and would rapidly cyclize to δ-decalactone and δ-dodecalactone, respectively. These lactones were evaluated at the forty-ninth meeting (Annex 1, reference *131*), and their uses as flavouring agents (Nos 232 and 236, respectively) were considered not to represent a safety concern, on the basis of the availability of a NOEL for a structural analogue. The intakes of δ-decalactone and δ-dodecalactone arising from flavouring use of Nos 923 and 924 would not significantly alter the total intakes of either lactone. There is no safety concern in relation to use of these chemicals as flavouring agents.

The conclusions of the present meeting relating to the flavouring properties of these chemicals are summarized in Table 11.

Table 11
Information on flavouring properties of chemicals evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

No.	Chemical	Level of use as a flavouring agent (mg/kg)	Reported flavouring properties	Evaluation based on current intake as a flavouring agent
850	Benzoic acid[a]	≤ 100	Aromatic acid with a sweet–sour, acrid note or a balsamic note at concentrations up to 100 mg/kg	No safety concern[b]
861	Glyceryl tribenzoate	≤ 100 (beverages only)	Flavour depends on hydrolysis to *benzoic acid* and *glycerol/propylene glycol*	No safety concern[b]
862	Propylene glycol dibenzoate	≤ 100 (beverages only)	Flavour depends on hydrolysis to *benzoic acid* and *glycerol/propylene glycol*	No safety concern[b]
870	Butyl-*para*-hydroxybenzoate	20 (baked goods) 1000 (beverages)	Flavour depends on hydrolysis to *butyl alcohol* and *para-hydroxybenzoic acid*	No safety concern[b]
909	Glycerol[c]	40 (meats) 500–23 000 (other foods)	Provides a sweet taste (0.6 times as sweet as cane sugar)	Evaluation not finalized, pending definition of 'flavouring agent'
914	3-Oxodecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form *2-nonanone* (up to 50 mg/kg).	No safety concern[b]
915	3-Oxododecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form *2-undecanone* (up to 50 mg/kg).	No safety concern[b]
916	3-Oxotetradecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form *2-tridecanone* (up to 50 mg/kg).	No safety concern[b]
917	3-Oxohexadecanoic acid glyceride	75 (baked goods and imitation dairy products)	Flavour depends on hydrolysis on heating or during storage. The resulting 3-oxo acid is unstable and decarboxylates to form *2-pentadecanone* (up to 50 mg/kg).	No safety concern[b]
918	Glyceryl monostearate[d]	500–3900	Flavour depends on hydrolysis on heating or during storage. The resulting *stearic acid* has flavouring properties. It may be further oxidized to produce 'rancid' flavour notes.	No safety concern[b]

Table 11 *(continued)*

No.	Chemical	Level of use as a flavouring agent (mg/kg)	Reported flavouring properties	Evaluation based on current intake as a flavouring agent
919	Glyceryl monooleate[d]	≤ 100	Flavour depends on hydrolysis on heating or during storage. The resulting *oleic acid* has flavouring properties. It may be further oxidized to produce 'rancid' flavour notes.	No safety concern[b]
920	Triacetin[e]	350 (jams and jellies) ≤ 100 (other foods)	Flavour depends on hydrolysis in heated or otherwise processed foods to *glycerol* and *acetic acid*	No safety concern[b]
921	Glyceryl tripropionate (Tripropionin)	≤ 50	Flavour depends on hydrolysis in heated or otherwise processed foods to *glycerol* and *propionic acid*	No safety concern[b]
922	Tributyrin	≤ 100	Flavour depends on hydrolysis in heated or otherwise processed foods to *glycerol* and *butyric acid*	No safety concern[b]
923	Glycerol 5-hydroxy-decanoate	25	Flavour depends on hydrolysis in heated foods and spontaneous cyclization of the 5-hydroxy acid to δ-*decalactone* (up to 20 mg/kg)	No safety concern[b]
924	Glycerol 5-hydroxy-dodecanoate	25	Flavour depends on hydrolysis in heated foods and spontaneous cyclization of the 5-hydroxy acid to δ-*dodecalactone* (up to 20 mg/kg)	No safety concern[b]
925	Propylene glycol[f]	10 (meats) 690–100 000 (other foods)	Provides a mildly sweet taste. In an aqueous environment, may leave a warm burning sensation in the mouth	Evaluation not finalized, pending definition of 'flavouring agent'
926	Propylene glycol stearate[g]	3000–7000	Flavour depends on hydrolysis on heating or during maturation. The resulting *stearic acid* has flavouring properties. It may be further oxidized to produce a 'rancid' flavour note.	No safety concern[b]

[a] Previously evaluated for use as a preservative
[b] The flavouring uses of this agent are included in the relevant ADI of the agent and/or its hydrolysis products.
[c] Previously evaluated for use as a carrier and humectant and for other uses
[d] Previously evaluated (as mono- and di-glycerides) for use as an emulsifier
[e] Previously evaluated for use as a carrier
[f] Previously evaluated for use as a carrier and humectant and for other uses
[g] Previously evaluated for use as an emulsifier

At its fifty-seventh meeting, the Committee mistakenly included *isoamyl pyruvate (No. 939)* in its evaluation of the aliphatic acyclic diols, triols and related substances, even though there was no information on its current use as a flavouring agent (Annex 1, reference *154*). Since the fifty-seventh meeting, the flavour industry has provided information that the reported annual volume is approximately 1 kg per year in the USA (*5*). On the basis of the equation in section 4.1, this corresponds to a daily *per capita* intake of 0.1 μg, which is below the threshold of concern for its structural class (class I, 1800 μg). Therefore, the present Committee confirmed that there is no safety concern in relation to the use of isoamyl pyruvate as a flavouring agent.

4.4 Specifications of purity for flavouring agents

4.4.1 *Specifications for flavouring agents evaluated for the first time*

Specifications were prepared for 196 flavouring agents being evaluated for the first time and for six flavouring agents that were evaluated for safety at the fifty-seventh meeting but for which specifications were not prepared (Annex 1, reference *154*). At the present meeting, none of the 202 specifications was designated 'tentative'. This is the first time since the review of flavouring agents began that no tentative designations were made for new submissions for specifications for flavouring agents and reflects the high quality of the information received.

4.4.2 *Reconsideration of existing specifications for flavouring agents*

Since 1996, a number of specifications for flavouring agents have been classified as 'tentative' either because of missing physical data or of uncertainty about their use as flavouring agents. In addition the Codex Committee on Food Additives and Contaminants referred 29 specifications of flavouring agents evaluated at the fifty-fifth meeting (Annex 1, reference *151*) to the Committee for reconsideration of certain parameters.

The call for data for the present meeting did not include all these substances, but they had been included in previous calls for data and in the reports of previous meetings. As data had been received before the present meeting, the Committee added them to the agenda.

Chemicals used as flavouring agents

At its fifty-seventh meeting, the Committee questioned whether a number of chemicals were used as flavouring agents (Annex 1, reference *154*). At its present meeting, it agreed that some of these substances have a flavouring function (see section 4.3), and therefore the tentative designation was deleted. Additionally, data missing on some of the substances had subsequently been supplied. Nevertheless, the specifications for glycerol monostearate (No.

918) and glycerol monooleate (No. 919) were maintained as tentative because of missing or inconsistent data. The specifications for glycerol-5-hydroxydecanoate (No. 923) and glycerol 5-hydroxydodecanoate (No. 924) were also maintained as tentative because the assay method was called into question.

The Committee did not finalize its evaluations of glycerol (No. 909) and propylene glycol (No. 925), pending development of a definition of 'flavouring agent'. The Committee agreed that benzoic acid (No. 850) was used as a flavouring agent but that in this use it should comply with the specifications established for benzoic acid used as a preservative. The Committee recommended that the existing specifications for glycerol, propylene glycol, triacetin (No. 920) and benzoic acid be reviewed at a future meeting, especially in relation to the functional uses of these food additives.

The status of the specifications of these chemicals is listed in Table 12.

Missing physical data

In the request for data for the present meeting, 12 flavouring agents with 'tentative' specifications were listed, with a request for the missing data. These data were provided, and the specifications of these flavouring agents were revised and the tentative status deleted. The Committee also considered the specifications of 10 flavouring agents that were designated 'tentative' at the fifty-seventh meeting and for which data were supplied. The specifications were revised and the tentative designation removed, except in the case of

Table 12
Specifications of chemicals for which functional use as flavouring agents was considered at the present meeting

No.	Chemical	Specifications[a]
861	Glyceryl tribenzoate	S
862	Propylene glycol dibenzoate	S
870	Butyl *para*-hydroxybenzoate	S
914	3-Oxodecanoic acid glyceride	R
915	3-Oxododecanoic acid glyceride	R
916	3-Oxotetradecanoic acid glyceride	R
917	3-Oxohexadecanoic acid glyceride	R
918	Glyceryl monostearate	R,T
919	Glyceryl monooleate	R,T
921	Glyceryl tripropanoate	S
922	Tributyrin	S
923	Glycerol 5-hydroxydecanoate	S,T
924	Glycerol 5-hydroxydodecanoate	S,T
926	Propylene glycol stearate	R

[a] R, existing specifications revised: S, specifications exist, revision not considered or required; T, the existing or revised specifications are tentative and comments are invited.

pyruvaldehyde (No. 937), which was maintained as tentative. The status of the specifications of these flavouring agents is provided in Table 13.

Specifications referred to the Committee

Table 14 summarizes the specifications of flavouring agents that were referred to the Committee by the Codex Committee on Food Additives and Contaminants for reconsideration.

5. Future work

1. The Committee concluded that it is not desirable to develop dual specifications, as has been done, for example, for chemicals that were evaluated both by the Procedure for the Safety Evaluation of Flavouring Agents and also as food additives. The Committee recommended that the existing specifications for such chemicals be reviewed at a future meeting, with a view to consolidating and revising them to incorporate all functional uses and new information.

Table 13
Specifications of flavouring agents previously designated as 'tentative'

No.	Chemical	Specifications[a]
111	Lauric acid	R
113	Myristic acid	R
115	Palmitic acid	R
116	Stearic acid	R
390	γ-Ionone	R
490	Allyl thiopropionate	R
814	α-Methylphenethylbutyrate	R
817	4-(*para*-Tolyl)-2-butanone	R
847	Benzyl 2,3-dimethylcrotonate	R
866	Tolualdehydes (mixed *ortho*, *meta* and *para*)	R
872	Anisyl formate	R
609	1,4-Nonanediol diacetate	R
627	Aconitic acid	R
642	3-Phenylpropyl hexanoate	R
678	α-Amylcinnamyl isovalerate	R
729	Dihydroxyacetophenone	R
752	2-Phenyl3-carboethoxyfuran	R
910	3-Oxohexanoic acid glyceride	R
911	3-Oxooctanoic acid glyceride	R
937	Pyruvaldehyde	S,T
943	Acetaldehyde ethyl *cis*-3-hexenyl acetal	R
954	Ethyl vanillin propylene glycol acetal	R

[a]R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing specifications are tentative and comments are invited.

Table 14
Specifications of flavouring agents referred to the Committee

No.	Chemical	Specifications[a]
182	Isoamyl laurate	R
230	Hydroxynonanoic acid δ-lactone	S
238	δ-Tetradecalactone	S
250	γ-Methyldecalactone	S
310	Isopropyl isovalerate	R
455	Butyl sulfide	R
476	Ethyl 3-methylthiopropionate	R
483	Ethyl thioacetate	R
492	Methylthio 2-(acetyloxy)propionate	R
493	Methylthio 2-(propionyloxy)propionate	R
540	1,6-Hexanedithiol	R
542	1,9-Nonanedithiol	R
551	2-Mercaptopropionic acid	R
553	Ethyl 3-mercaptopropionate	R
566	Propyl disulfide	R
601	Ethyl 3-hydroxyhexanoate	R
602	Ethyl 3-oxohexanoate	R
606	Laevulinic acid	R
608	Butyl laevulinate	R
614	Diethyl malonate	R
616	Dimethyl succinate	R
617	Diethyl succinate	R
622	Diethyl tartrate	R
624	Diethyl sebacate	R
625	Dibutyl sebacate	R
626	Ethylene brassylate	R
743	Furfuryl 3-methylbutanoate	S
745	5-Methylfurfural	R

[a]R, existing specifications revised; S, specifications exist, revision not considered or required.

2. The Committee recommended that the acute toxicity of nitrite be evaluated at a future meeting.

3. The Committee agreed to continue its activity to update the heavy metals specifications in food additives and concluded that specifications for antioxidants and other functional uses should be reviewed at the next meeting on food additives.

4. The Committee recognized the need for a working definition of the term 'flavouring agent' and recommended that such a definition be agreed at a future meeting of the Committee.

5. As noted in section 2.7, the present Committee considered several guidelines that had been prepared by the Joint Secretariat and made comments and suggestions for improvement, including the following:

   — The Technical Data Sheets prepared by Committee experts, which summarize the available technological information, should

provide authors of working papers on intake with information on the levels of use of the additive in current practice.

— Data for intake assessments of food additives should preferably be submitted in a standard format. The Joint Secretariat should publish a *Format for submission of intake data* as a separate document and encourage its use when calling for data.

— The Secretariat should publish amended versions of guidelines for working papers on both the FAO and the WHO Internet sites for further comments and suggestions.

— The Committee recommended that the *Guidelines for the preparation of working papers (monographs) on flavouring agents* be updated to include consideration of the evaluation of flavouring agents that are members of groups that have been evaluated previously.

6. Recommendations

In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role of the recommendations of the Committee in the development of international food standards and of regulations in many countries and the need to maintain consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.

Acknowledgements

This report of the Committee is dedicated to Dr John L. Herrman, who joined WHO as Joint Secretary of the Joint FAO/WHO Joint Committee on Food Additives in 1988. From his first meeting, the thirty-third, he demonstrated the unique management skills required of his position. He has served as Joint Secretary at 27 meetings, culminating in the present one, his last before retirement.

Over the course of his career, he has steadfastly supported the work of the Committee and is largely responsible for the emergence of JECFA as the leading international body on food safety.

As WHO Joint Secretary he has displayed immense dedication and leadership, resulting in his international recognition as a leading scientist in food safety. His timely introduction to the Committee of new concepts in safety evaluation has kept the Committee at the forefront of science, has contributed to its continued success and ensures that its advice is based on the best possible science.

The Committee wishes John Herrman a happy and successful retirement.

The Committee wished to thank E. Heseltine, St Léon-sur-Vézère, France, for her assistance in the preparation of the report.

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4. Adams TB. *The flavouring properties of glycerol, propylene glycol, benzoic acid and 15 related glyceryl and benzoate esters.* Report from the Flavour and Extract Manufacturers Association of America, 2001.

5. Private communication dated 11 June 2002 from T.B. Adams, Flavour and Extract Manufacturers Association of America, to J. Herrman, WHO.

Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
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3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
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10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
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12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
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19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
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57. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 16, 1981.
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63. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 28, 1983.
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Annex 2

Acceptable daily intakes, other recommendations and information on specifications

Food additives evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Alitame	R	0–1 mg/kg bw[b]
Cross-linked sodium carboxymethyl cellulose	N	Included in the group ADI 'not specified'[c] with other modified celluloses
Mineral oils (low- and medium-viscosity)		
Class I[d]	R	0–10 mg/kg bw
Class II[e] and Class III[f]	R	0–0.01 mg/kg bw (group ADI) (temporary)[g]
Nitrite	S	0–0.07 mg/kg bw (expressed as nitrite ion)
Nitrate	S	0–3.7 mg/kg bw (expressed as nitrate ion)
Salatrim (short- and long-chain acyltriglyceride molecules)	R	Adequate information was not available to evaluate safety and nutritional effects.

[a] N, new specifications prepared; R, existing specifications revised; S, existing specifications were not considered.

[b] ADI was established at the forty-sixth meeting (WHO Technical Report Series No, 868, 1997). Evaluation of a 90-day study of teolerance in patients with diabetes was postponed.

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

[d] Including P70(H) oil.

[e] Including N70(H) and N70(A) oils.

[f] Including P15(H), N15(H), and N10(A) oils

[g] See Annex3.

Food additive considered for specifications only

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

[a] R, existing specifications revised

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Cyclohexanecarboxylic acid	961	N	No safety concern
Methyl cyclohexanecarboxylate	962	N	No safety concern
Ethyl cyclohexanecarboxylate	963	N	No safety concern
Cyclohexaneethyl acetate	964	N	No safety concern
Cyclohexaneacetic acid	965	N	No safety concern
Ethyl cyclohexanepropionate	966	N	No safety concern
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	N	No safety concern
cis-5-Isopropenyl-*cis*-2-methylcyclopentan-1-carboxaldehyde	968	N	No safety concern
Campholene acetate	969	N	No safety concern
α-Campholenic alcohol	970	N	No safety concern
para-Menth-1-en-9-al	971	N	No safety concern
1-*para*-Menthen-9-yl acetate	972	N	No safety concern
para-Mentha-1,8-dien-7-al	973	N	No safety concern
para-Mentha-1,8-dien-7-ol	974	N	No safety concern
para-Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
1,2,5,6-Tetrahydrocuminic acid	976	N	No safety concern
2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	977	N	No safety concern
2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	978	N	No safety concern
2,6,6-Trimethyl-1&2-cyclohexen-1-carboxaldehyde	979	N	No safety concern
2-Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (Myrtenal)	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	N	No safety concern
6,6-Myrtenyl formate	983	N	No safety concern
Santalol (α and β)	984	N	No safety concern
Santalyl acetate (α and β)	985	N	No safety concern
10-Hydroxymethylene-2-pinene	986	N	No safety concern

[a]N, new specifications prepared

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

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Phenethyl alcohol	987	N	No safety concern
Phenethyl formate	988	N	No safety concern
Phenethyl acetate	989	N	No safety concern
Phenethyl propionate	990	N	No safety concern
Phenethyl butyrate	991	N	No safety concern
Phenethyl isobutyrate	992	N	No safety concern
Phenethyl 2-methylbutyrate	993	N	No safety concern
Phenethyl isovalerate	994	N	No safety concern
Phenethyl hexanoate	995	N	No safety concern
Phenethyl octanoate	996	N	No safety concern
Phenethyl tiglate	997	N	No safety concern
Phenethyl senecioate	998	N	No safety concern
Phenethyl phenylacetate	999	N	No safety concern
Acetaldehyde phenethyl propyl acetal	1000	N	No safety concern
Acetaldehyde butyl phenethyl acetal	1001	N	No safety concern
Phenylacetaldehyde	1002	N	No safety concern
Phenylacetaldehyde dimethyl acetal	1003	N	No safety concern
Phenylacetaldehyde glyceryl acetal	1004	N	No safety concern
Phenylacetaldehyde 2,3-butylene glycol acetal	1005	N	No safety concern
Phenylacetaldehyde diisobutyl acetal	1006	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Phenylacetic acid	1007	N	No safety concern
Methyl phenylacetate	1008	N	No safety concern
Ethyl phenylacetate	1009	N	No safety concern
Propyl phenylacetate	1010	N	No safety concern
Isopropyl phenylacetate	1011	N	No safety concern
Butyl phenylacetate	1012	N	No safety concern
Isobutyl phenylacetate	1013	N	No safety concern
Isoamyl phenylacetate	1014	N	No safety concern
Hexyl phenylacetate	1015	N	No safety concern
3-Hexenyl phenylacetate	1016	N	No safety concern
Octyl phenylacetate	1017	N	No safety concern
Rhodinyl phenylacetate	1018	N	No safety concern
Linalyl phenylacetate	1019	N	No safety concern
Geranyl phenylacetate	1020	N	No safety concern
Citronellyl phenylacetate	1021	N	No safety concern
Santalyl phenylacetate (α and β)	1022	N	No safety concern
para-Tolylacetaldehyde	1023	N	No safety concern
para-Isopropylphenylacetaldehyde	1024	N	No safety concern
Methyl *para-tert*-butylphenylacetate	1025	N	No safety concern
Phenoxyacetic acid	1026	N	No safety concern
Ethyl (*para*-tolyloxy)acetate	1027	N	No safety concern
2-Phenoxyethyl isobutyrate	1028	N	No safety concern
Sodium 2-(4-methoxyphenoxy)propanoate	1029	N	No safety concern

[a]N, new specifications prepared

C. Sulfur-containing heterocyclic compounds

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Thiamine hydrochloride	1030	N	No safety concern
4-Methyl-5-thiazoleethanol	1031	N	No safety concern
Thiazole	1032	N	No safety concern
2-(1-Methylpropyl)thiazole	1033	N	No safety concern
2-Isobutylthiazole	1034	N	No safety concern
4,5-Dimethylthiazole	1035	N	No safety concern
2,4,5-Trimethylthiazole	1036	N	No safety concern
2-Isopropyl-4-methylthiazole	1037	N	No safety concern
4-Methyl-5-vinylthiazole	1038	N	No safety concern
2,4-Dimethyl-5-vinylthiazole	1039	N	No safety concern
Benzothiazole	1040	N	No safety concern
2-Acetylthiazole	1041	N	No safety concern
2-Propionylthiazole	1042	N	No safety concern
4-Methylthiazole	1043	N	No safety concern
2-Ethyl-4-methylthiazole	1044	N	No safety concern
4,5-Dimethyl-2-isobutyl-3-thiazoline	1045	N	No safety concern
2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1046	N	No safety concern
2-Isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	1047	N	No safety concern
2,4,6-Triisobutyl-5,6-dihydro-4*H*-1,3,5-dithiazine	1048	N	No safety concern
2,4,6-Trimethyldihydro-4*H*-1,3,5-dithiazine	1049	N	No safety concern
5-Methyl-2-thiophenecarboxaldehyde	1050	N	No safety concern
3-Acetyl-2,5-dimethylthiophene	1051	N	No safety concern
2-Thienylmercaptan	1052	N	No safety concern
2-Thienyl disulfide	1053	N	No safety concern
4-Methyl-5-thiazoleethanol acetate	1054	N	No safety concern
2,4-Dimethyl-5-acetylthiazole	1055	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
2-Ethoxythiazole	1056	N	No safety concern
2-Methyl-5-methoxythiazole	1057	N	No safety concern
4,5-Dimethyl-2-ethyl-3-thiazoline	1058	N	No safety concern
2-(2-Butyl)-4,5-dimethyl-3-thiazoline	1059	N	No safety concern

[a]N, new specifications prepared

D. Sulfur-substituted furan derivatives

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
2-Methyl-3-furanthiol	1060	N	No safety concern
2-Methyl-3-(methylthio)furan	1061	N	No safety concern
2-Methyl-5-(methylthio)furan	1062	N	No safety concern
2,5-Dimethyl-3-furanthiol	1063	N	No safety concern
Methyl 2-methyl-3-furyl disulfide	1064	N	No safety concern
Propyl 2-methyl-3-furyl disulfide	1065	N	No safety concern
Bis(2-methyl-3-furyl) disulfide	1066	N	No safety concern
Bis(2,5-dimethyl-3-furyl) disulfide	1067	N	No safety concern
Bis(2-methyl-3-furyl) tetrasulfide	1068	N	No safety concern
2-Ethanethoic acid, *S*-(2-methyl-3-furanyl) ester	1069	N	No safety concern
2,5-Dimethyl-3-furan thioisovalerate	1070	N	No safety concern
2,5-Dimethyl-3-thiofuroylfuran	1071	N	No safety concern
Furfuryl mercaptan	1072	N	No safety concern
S-Furfuryl thioformate	1073	N	No safety concern
S-Furfuryl thioacetate	1074	N	No safety concern
S-Furfuryl thiopropionate	1075	N	No safety concern
Furfuryl methyl sulfide	1076	N	No safety concern
Furfuryl isopropyl sulfide	1077	N	No safety concern
Methyl furfuryl disulfide	1078	N	No safety concern
Propyl furfuryl disulfide	1079	N	No safety concern
2,2'-(Thiodimethylene)difuran	1080	N	No safety concern
2,2'-(Dithiodimethylene)difuran	1081	N	No safety concern
2-Methyl-3-, 5- or 6-(furfurylthio)pyrazine	1082	N	No safety concern
S-Methyl thiofuroate	1083	N	No safety concern
4-[(2-Furanmethyl)thio]-2-pentanone	1084	N	No safety concern
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085	N	No safety concern
2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	1086	N	No safety concern
4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087	N	No safety concern
Ethyl 3-(furfurylthio)propionate	1088	N	No safety concern
2-Methyl-3-thioacetoxy-4,5-dihydrofuran	1089	N	No safety concern
2-Methyl-3-tetrahydrofuranthiol	1090	N	No safety concern
2,5-Dimethyl-3-tetrahydrofuranthiol, *cis* and *trans* isomers	1091	N	No safety concern
2,5-Dimethyl-3-thioacetoxy-tetrahydrofuran, *cis* and *trans* isomers	1092	N	No safety concern

[a]N, new specifications prepared

E. Alicyclic ketones, secondary alcohols and related esters

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

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
3,3,5-Trimethyl cyclohexanol	1099	N	No safety concern
Cyclohexanone	1100	N	No safety concern
Cyclopentanone	1101	N	No safety concern
2-Methylcyclohexanone	1102	N	No safety concern
3-Methylcyclohexanone	1103	N	No safety concern
4-Methylcyclohexanone	1104	N	No safety concern
1-Methyl-1-cyclopenten-3-one	1105	N	No safety concern
2-Hexylidene cyclopentanone	1106	N	No safety concern
3-Methyl-2-cyclohexen-1-one	1107	N	No safety concern
2,2,6-Trimethylcyclohexanone	1108	N	No safety concern
2-*sec*-Butylcyclohexanone	1109	N	No safety concern
4-Isopropyl-2-cyclohexenone	1110	N	No safety concern
Tetramethylethylcyclohexenone (mixture of isomers)	1111	N	No safety concern
Isophorone	1112	N	No safety concern
3-Methyl-5-propyl-2-cyclohexen-1-one	1113	N	No safety concern
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114	N	No safety concern
Isojasmone	1115	N	No safety concern
(*E*)-2-(2-Octenyl)cyclopentanone	1116	N	No safety concern
2-(3,7-Dimethyl-2,6-octadienyl)cyclopentanone	1117	N	No safety concern

[a]N, new specifications prepared

F. Aliphatic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
3-Decanone	1118	N	No safety concern
5-Methyl-5-hexen-2-one	1119	N	No safety concern
6-Methyl-5-hepten-2-one	1120	N	No safety concern
3,4,5,6-Tetrahydropseudoionone	1121	N	No safety concern
6,10-Dimethyl-5,9-undecadien-2-one	1122	N	No safety concern
2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one	1123	N	No safety concern
3-Penten-2-one	1124	N	No safety concern
4-Hexen-3-one	1125	N	No safety concern
2-Hepten-4-one	1126	N	No safety concern
3-Hepten-2-one	1127	N	No safety concern
3-Octen-2-one	1128	N	No safety concern
2-Octen-4-one	1129	N	No safety concern
3-Decen-2-one	1130	N	No safety concern
4-Methyl-3-penten-2-one	1131	N	No safety concern
5-Methyl-3-hexen-2-one	1132	N	No safety concern
5-Methyl-2-hepten-4-one	1133	N	No safety concern
6-Methyl-3,5-heptadien-2-one	1134	N	No safety concern
(*E*)-7-Methyl-3-octen-2-one	1135	N	No safety concern
3-Nonen-2-one	1136	N	No safety concern
(*E*) and (Z)-4,8-Dimethyl-3,7-nonadien-2-one	1137	N	No safety concern
(*E*)-6-Methyl-3-hepten-2-one	1138	N	No safety concern
(*E*,*E*)-3,5-Octadien-2-one	1139	N	No safety concern
3-Octen-2-ol	1140	N	No safety concern
(*E*)-2-Octen-4-ol	1141	N	No safety concern
2-Pentyl butyrate	1142	N	No safety concern
(+/–)Heptan-3-yl acetate	1143	N	No safety concern
(+/–)Heptan-2-yl butyrate	1144	N	No safety concern
(+/–)Nonan-3-yl acetate	1145	N	No safety concern
2-Pentyl acetate	1146	N	No safety concern
1-Penten-3-one	1147	N	No safety concern
1-Octen-3-one	1148	N	No safety concern
2-Pentyl-1-buten-3-one	1149	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
1-Penten-3-ol	1150	N	No safety concern
1-Hexen-3-ol	1151	N	No safety concern
1-Octen-3-ol	1152	N	No safety concern
1-Decen-3-ol	1153	N	No safety concern
(*E*,R)-3,7-Dimethyl-1,5,7-octatrien-3-ol	1154	N	No safety concern
6-Undecanone	1155	N	No safety concern
2-Methylheptan-3-one	1156	N	No safety concern

[a]N, new specifications prepared

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
111	Lauric acid	R
113	Myristic acid	R
115	Palmitic acid	R
116	Stearic acid	R
182	Isoamyl laurate	R
310	Isopropyl isovalerate	R
390	γ-Ionone	R
455	Butyl sulfide	R
476	Ethyl 3-methylthiopropionate	R
483	Ethyl thioacetate	R
490	Allyl thiopropionate	R
492	Methylthio 2-(acetyloxy)propionate	R
493	Methylthio 2-(propionyloxy) propionate	R
540	1,6-Hexanedithiol	R
542	1,9-Nonanedithiol	R
551	2-Mercaptopropionic acid	R
553	Ethyl 3-mercaptopropionate	R
564	Dimethyl disulfide	R
566	Propyl disulfide	R
601	Ethyl 3-hydroxyhexanoate	R
602	Ethyl 3-oxohexanoate	R
606	Laevulinic acid	R
608	Butyl levulinate	R
609	1 4-Nonanediol diacetate	R
614	Diethyl malonate	R
616	Dimethyl succinate	R
617	Diethyl succinate	R
622	Diethyl tartrate	R
624	Diethyl sebacate	R
625	Dibutyl sebacate	R
626	Ethylene brassylate	R
627	Aconitic acid	R
642	3-Phenylpropyl hexanoate	R
678	α-Amylcinnamyl isovalerate	R
729	Dihydroxyacetophenone	R
745	5-Methylfurfural	R
752	2-Phenyl-3-carbethoxyfuran	R
814	α-Methylphenethyl butyrate	R
817	4-(*para*-Tolyl)-2-butanone	R
847	Benzyl 2 3-dimethylcrotonate	R
861	Glyceryl tribenzoate	R
862	Propylene glycol dibenzoate	S
866	Tolualdehydes (mixed *ortho meta* and *para*)	R
870	Butyl *para*-hydroxybenzoate	S
872	Anisyl formate	R

No.	Flavouring agent	Specifications[a]
910	3-Oxohexanoic acid diglyceride	R
911	3-Oxooctanoic acid glyceride	R
914	3-Oxodecanoic acid glyceride	R
915	3-Oxododecanoic acid glyceride	R
916	3-Oxotetradecanoic acid glyceride	R
917	3-Oxohexadecanoic acid glyceride	R
918	Glyceryl monostearate	R,T
919	Glyceryl monooleate	R,T
921	Glyceryl tripropanoate	S
922	Tributyrin	S
923	Glycerol 5-hydroxydecanoate	S,T
924	Glycerol 5-hydroxydodecanoate	S T
926	Propylene glycol stearate	R
937	Pyruvaldehyde	S,T
943	Acetaldehyde ethyl cis-3-hexenyl acetal	R
954	Ethyl vanillin propylene glycol acetal	R
955	4-Hydroxybenzyl alcohol	N
956	4-Hydroxybenzaldehyde	N
957	4-Hydroxybenzoic acid	N
958	2-Hydroxybenzoic acid	N
959	4-Hydroxy-3-methoxy benzoic acid	N
960	Vanillin erythro- and threo-butan-2 3-diol acetal	N

[a]N, new specifications prepared; R, existing specifications revised; S, existing specifications were maintained; T, the existing, new, or revised specifications are tentative and new information is required.

Flavouring agents for which confirmation of flavour use was required

The Committee could not complete its evaluation of 18 substances at its fifty-seventh meeting, pending confirmation that they were in current use as flavouring agents. The Committee received information from the flavour industry on these substances, and concluded that flavour uses have been established for 16 of them. The conclusions of the Committee are summarized in the following table.

No.	Flavouring agent	Evaluation based on current intake as a flavouring agent
850	Benzoic acid[a]	No safety concern
861	Glyceryl tribenzoate	No safety concern
862	Propylene glycol dibenzoate	No safety concern
870	Butyl-*para*-hydroxybenzoate	No safety concern
909	Glycerol[b]	Evaluation not finalized, pending definition of 'flavouring agent'
914	3-Oxodecanoic acid glyceride	No safety concern
915	3-Oxododecanoic acid glyceride	No safety concern
916	3-Oxotetradecanoic acid glyceride	No safety concern
917	3-Oxohexadecanoic acid glyceride	No safety concern
918	Glyceryl monostearate[c]	No safety concern
919	Glyceryl monooleate[c]	No safety concern
920	Triacetin[d]	No safety concern
921	Glyceryl tripropionate (Tripropionin)	No safety concern
922	Tributyrin	No safety concern
923	Glycerol 5-hydroxydecanoate	No safety concern
924	Glycerol 5-hydroxydodecanoate	No safety concern
925	Propylene glycol[e]	Evaluation not finalized, pending definition of 'flavouring agent'
926	Propylene glycol stearate[f]	No safety concern

Annex 3
Further information required or desired

Mineral oils (low- and medium-viscosity), class II and class III

Information on the relevance to humans of the response of Fischer 344 and Sprague-Dawley rats to these materials is required for evaluation in 2006. In order that the data be applicable to as wide a range of mineral oils as possible, the Committee suggested that commercial mineral oils of the lowest viscosity be used in such studies. Additional studies might be required, depending on the outcome of these studies.

Aluminium powder, iron oxides and titanium dioxide

The high levels of heavy metals set in the present specifications for these inorganic colours should be reconsidered. The Committee maintained the existing limits but decided to call for data on the raw materials and manufacturing methods and analytical data on impurities for review at a future meeting.

Annex 4

Summary of conclusions on flavouring agents with minimum assay values of 95% or less

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
42	Isoamyl formate	92	4–8% isoamyl alcohol	Isoamyl alcohol (No. 52) has been evaluated by JECFA.	Secondary components do not raise safety concern.
53	Citronellyl formate	86	5–10% citronellol	NOEL for citronellol is > 50 mg/kg bw per day in rats (Trubek Laboratories, Inc., 1958); oral LD_{50} in rats: 3400 mg/kg bw (Moreno, 1973)	Secondary components do not raise safety concern.
54	Geranyl formate	85 (total esters)	8–10% geraniol; 2–4% nerol	Data for geraniol in rats: 28-week NOEL: 50 mg/kg bw per day (Hagan et al., 1967) 13-week NOEL: 500 mg/kg bw per day (Hagan et al., 1967) 16-week NOEL: 50 mg/kg bw per day (Food and Drug Administration, 1954) Oral LD_{50}: 3600 mg/kg bw (Jenner et al., 1964) Geranyl and citronellyl acetate had no effect at 2000 mg/kg bw per day for 2 years in F344 rats or $B6C3F_1$ mice (National Toxicology Program, 1987). Nerol is metabolized by oxidation of the primary alcohol and ω-oxidation to yield polar, excretable metabolites. Oral LD_{50} in rats: 4500 mg/kg bw (Moreno, 1972)	Secondary components do not raise safety concern.
55	Neryl formate	85	4–6% geraniol; 1–3% nerol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
56	Rhodinyl formate	85 (total esters)	10–13% rhodinol	Rhodinol is metabolized by oxidation of the primary alcohol and ω-oxidation to yield polar, excretable metabolites. Acute toxicity in rats treated orally: 3/10 deaths at 5000 mg/kg bw (Moreno, 1973)	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
57	Citronellyl acetate	92 (total esters)	4–6% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
60	Rhodinyl acetate	87 (total esters)	9–12% rhodinol	For data on rhodinol, see No. 56 above.	Secondary components do not raise safety concern.
61	Citronellyl propionate	90 (total esters)	5–8% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
62	Geranyl propionate	92 (total esters)	3–4% geraniol; 1–2% nerol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
65	Citronellyl butyrate	90 (total esters)	6–8% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
66	Geranyl butyrate	92 (total esters)	3–5% geraniol; 1% nerol	For data on geraniol, and nerol, see No. 54 above.	Secondary components do not raise safety concern.
68	Rhodinyl butyrate	90	3–5% rhodinol	For data on rhodinol, see No. 56 above.	Secondary components do not raise safety concern.
71	Citronellyl isobutyrate	92 (total esters)	3–5% citronellol	For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
73	Neryl isobutyrate	92	2–5% nerol; 1–2% geraniol	For data on geraniol and nerol, see No. 54 above.	Secondary components do not raise safety concern.
95	Heptanal	92	4–7% 2-methylhexanal	2-Methylhexanal is oxidized to 2-methylhexanoic acid, which is oxidized in the fatty acid pathway similarly to 2-methyldecanoic acid (see No. 107)	Secondary components do not raise safety concern.
98	Octanal	92	4–7% 2-methylheptanal	2-Methylheptanal is oxidized to 2-methylheptanoic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
101	Nonanal	92	4–8% 2-methyloctanal	2-Methyloctanal (No. 270) has been evaluated by JECFA.	Secondary components do not raise safety concern.
104	Decanal	92	4–7% 2-methylnonanal	2-Methylnonanal is oxidized to 2-methylnonanoic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
107	Undecanal	92	4–8% 2-methyldecanal	2-Methyldecanal is oxidized to the corresponding acid, which undergoes β-oxidation to yield a propionic acid fragment and octanoyl coenzyme A, which is metabolized to carbon dioxide and water. Acute toxicity Rats: 0/10 deaths at 5000 mg/kg bw (Moreno, 1975) Rabbits: 1/10 deaths at 5000 mg/kg bw (Moreno, 1975)	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
110	Lauric aldehyde	90	3–6% tetradecanal; 2–5% decanal; 1–2% hexadecanal	Tetradecanal (No. 112) has been evaluated by JECFA. Decanal (No. 104) has been evaluated by JECFA. Hexadecanal is oxidized to palmitic acid and enters the fatty acid pathway, where it is completely metabolized.	Secondary components do not raise safety concern.
112	Myristaldehyde	85	10–12% dodecanal, hexadecanal and octadecanal	Dodecanal (No. 110) has been evaluated by JECFA. Hexadecanal and octadecanal are oxidized to palmitic and stearic acid, respectively, and metabolized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
117	Propyl formate	94	4–6% propyl alcohol	Propyl alcohol (No. 82) has been evaluated by JECFA.	Secondary components do not raise safety concern.
119	n-Amyl formate	92	4–8% *n*-amyl alcohol	Amyl alcohol (No. 88) has been evaluated by JECFA.	Secondary components do not raise safety concern.
124	Isobutyl formate	94	4–6% isobutyl alcohol	Isobutyl alcohol (No. 251) has been evaluated by JECFA.	Secondary components do not raise safety concern.
170	n-Amyl heptanoate	93	4–7% *n*-amyl 2-methylhexanoate	*n*-Amyl-2-methylhexanoate is hydrolysed to amyl alcohol (No. 88) and 2-methylhexanoic acid (No. 265), which have been evaluated by JECFA.	Secondary components do not raise safety concern.
180	Methyl laurate	90	3–6% methyl tetradecanoate; 2–5% methyl decanoate; 1–2% methyl hexadecanoate	Methyl tetradecanoate (No. 183) has been evaluated by JECFA. A 90-day study of a mixture of methyl tetradecanoate, methyl decanoate and methyl hexadecanoate in rats showed no effects with any of the materials at 1% of the diet, equivalent to 500 mg/kg bw per day (Alfin-Slater et al., 1965).	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
205	Methyl 2-methylbutyrate	92	5–7% methyl isovalerate	Methyl isovalerate (No. 195) has been evaluated by JECFA.	Secondary components do not raise safety concern.
212	2-Methylbutyl 2-methylbutyrate	90	5–7% 2-methylbutyl 3-methylbutyrate	2-Methylbutyl 3-methylbutyrate (No. 204) has been evaluated by JECFA.	Secondary components do not raise safety concern.
237	6-Hydroxy-3,7-dimethyloctanoic acid lactone	90	5–6% 6-hydroxy-3,7-dimethyl-2-octenoic acid lactone	The secondary component differs from the primary component only in the presence of a double bond at the 2-position of the secondary component. The NOEL for a similar 2-alkenoic acid lactone (5-hydroxy-2,4-decadienoic acid-$^{\delta}$-lactone) in a 90-day study in rats was 12 mg/kg bw per day (Cox et al., 1974a).	Secondary components do not raise safety concern.
272	3,7-Dimethyl-1-octanol	90	5–7% geraniol and citronellol	For data on geraniol, see No. 54 above; for data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
302	2,6-Dimethyl-4-heptanone	80	15–17% 4,6-dimethyl-2-heptanone	4,6-Dimethyl-2-heptanone is reduced in vivo, and the resulting alcohol is conjugated with glucuronic acid and excreted in the urine. Oral LD_{50} in rats: 5800 mg/kg bw (Bar & Griepentrog, 1967)	Secondary components do not raise safety concern.
303	2,6-Dimethyl-4-heptanol	90	8–9% 2-heptanol	2-Heptanol (No. 284) has been evaluated by JECFA.	Secondary components do not raise safety concern.
322	*cis*-5-Octen-1-ol	90	7–9% *trans*-5-octen-1-ol	*trans*-5-Octen-1-ol is oxidized to *trans*-5-octenoic acid, which is oxidized in the fatty acid pathway and the citric acid cycle.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
323	*cis*-5-Octenal	85	10–15% *trans*-5-octenal	*trans*-5-Octenal is oxidized to the corresponding carboxylic acid, which is oxidized in the fatty acid pathway and citric acid cycle.	Secondary components do not raise safety concern.
325	*cis*-6-Nonenal	90	6–9% *trans*-6-nonenal	*trans*-6-Nonenal is oxidized to 6-nonenoic acid, which is completely metabolized in the fatty acid pathway.	Secondary components do not raise safety concern.
332	Linoleic acid	62	44–46% linolenic acid; 18–20% linoleic acid; 22–25% stearic and oleic acid; 7–8% palmitic acid	Stearic acid (No. 116), oleic acid (No. 333) and palmitic acid (No. 115) have all been evaluated by JECFA.	Secondary components do not raise safety concern.
346	Methyl linoleate and methyl linolenate	60	44–46% methyl linolenate; 18–20% methyl linoleate; 22–25% methyl stearate and methyl oleate; 7–8% methyl palmitate	The NOELs for methyl stearate, methyl oleate and methyl palmitate fed to rats for 90 days at 1% of the diet was 500 mg/kg bw per day (Alfin-Slater et al., 1965).	Secondary components do not raise safety concern.
348	2,6-Dimethyl-6-hepten-1-ol	90	5–10% 6-methyl-5-hepten-2-one	6-Methyl-5-hepten-2-one (No. 1120) was evaluated at the present meeting.	Secondary components do not raise safety concern.
349	2,6-Dimethyl-5-heptenal	85	9–10% 6-methyl-5-hepten-2-one; 1–2% 2,6-dimethyl-6-heptenal	For data on 6-methyl-5-hepten-2-one, see No. 348 above. 2,6-Dimethyl-6-heptenal is oxidized to the corresponding acid, which undergoes β-oxidation.	Secondary components do not raise safety concern.
358	Linalyl formate	90	6–8% linalool	Linalool (No. 356) has been evaluated by JECFA.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
360	Linalyl propionate	92	2–5% linalool	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.
384	β-Damascone	90 (sum of *cis* and *trans* isomers)	5–8% α- and δ-damascone	α-Damascone (No. 385) and δ-damascone (No. 386) have been evaluated by JECFA.	Secondary components do not raise safety concern.
385	α-Damascone	98 (sum of *cis* and *trans* isomers)	92–96% *trans*, 4–8% *cis* isomers	The NOEL in a 90-day study with α-damascone containing a *trans*- and *cis*-isomer mixture listed in specifications was 2 mg/kg bw per day (Posternak & Vodoz, 1975). The position of the double bond and the presence of *cis* and *trans* isomers is not expected to alter metabolic detoxication pathways. α-Damascone is metabolized by reduction of the side-chain ketone group to yield the corresponding alcohol, which is excreted as the glucuronic acid conjugate.	Secondary components do not raise safety concern.
399	Methyl-β-ionone	88 (sum of *cis* and *trans* isomers of α-, β- and γ-methylionone)	7–10% α- and β-isomethylionone	α- and β-Isomethylionone and α- and β-methylionone are prepared by methylation of ionone. Metabolic detoxication proceeds by reduction of the ketone followed by conjugation of the resulting alcohol with glucuronic acid; the presence of a methyl group is not anticipated to alter the metabolism significantly.	Secondary components do not raise safety concern.
410	2,3-Pentadione	93	2–3% 2,5-diethylcyclohexadien-1,4-dione	2,3-Pentadione dimerizes to 2,5-diethylcyclohexadien-1,4-dione, which is expected to be reduced to the corresponding alcohol and excreted or conjugated with glutathione.	Secondary components do not raise safety concern.
419	Ethyl cyclopentenolone	90	5–10% 3-ethylcyclopentan-1,2-dione (enolic form)	The secondary component is the enol form of the principal substance, ethyl cyclopentenolone (keto form), which is a diketone in equilibrium with the corresponding enol.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
435	*para*-Menth-1-en-3-one	94	2–3% menthol and menthone	Menthol (No. 427) and menthone (No. 429) have been evaluated by JECFA.	Secondary components do not raise safety concern.
592	Citronellyloxy-acetaldehyde	75	20–21% geranyloxyacetaldehyde; 1–2% citronellol	The structures of geranyloxyacetaldehyde and citronellyloxyaldehyde differ only in the presence of a second double-bond at the 2-position of geranyloxyaldehyde. The safety evaluation of the named flavouring agent also applies to the secondary component. Acute toxicity of oral citronellyloxyaldehyde in rats: no deaths at 5000 mg/kg bw (Moreno, 1976). For data on citronellol, see No. 53 above.	Secondary components do not raise safety concern.
604	3-(Hydroxymethyl)-2-heptanone	90	5–8% 1-hydroxy-3-ocatanone	The secondary component, 1-hydroxy-3-octanone, is oxidized to 3-oxooctanoic acid, which is predicted to be metabolized in the fatty acid pathway.	Secondary components do not raise safety concern.
625	Dibutyl sebacate	93	2–4% butyl esters of C14, C16 and C18 fatty acids	As the acid is prepared by oxidative cleavage of unsaturated fatty acids, small amounts of fatty acids are secondary components of sebacate esters. The butyl esters of C14, C16 and C18 fatty acids are hydrolysed and metabolized as endogenous fatty acids.	Secondary components do not raise safety concern.
668	Linalyl cinnamate	94	3–5% linalool	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
978	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	92	2.6% β-cyclocitral; 0.5% β-ionone; 3% methyl β-homocyclogeranate; 0.6% ethyl β-homocyclogeranate	β-Cyclocitral is a mixture of predominantly 2,6,6-trimethyl-1-cyclohexene-1-aldehyde (No. 979) and 2,6,6-trimethyl-2-cyclohexene-1-aldehyde. The NOEL for the structurally related 2,2,3-trimethylcyclopent-3-ene-1-aldehyde (No. 967) in a 90-day study in rats was 12 mg/kg bw per day (BIBRA, 1976). β-Ionone (No. 392) has been evaluated by JECFA. Methyl β-homocyclogeranate and ethyl β-homocyclogeranate are esters of (2,6,6-trimethyl-2-cyclohexen-1-yl)acetic acid (CAS No. 24739-72-8), a simple cyclohexylacetic acid derivative. The esters are expected to be hydroysed to the corresponding acid in vivo. The acid may be excreted directly as a glucuronic acid conjugate or undergo β-oxidation of the side-chain.	Secondary components do not raise safety concern.
1005	Phenylacetaldehyde 2,3-butylene glycol acetal	93	2–3% butane-2,3-diol	Butanediol is the diol formed by hydrolysis of phenylacetaldehyde 2,3-butanediol acetal. The diol is excreted as the glucuronide conjugate.	Secondary components do not raise safety concern.
1046	A mixture of 2-isobutyl-4,6-dimethyl and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	64% 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine; 18% 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine	12% 2,4,6-trimethyldihydro-1,3,5-dithiazine; 4% 2,4-diisobutyl-6-methyl-1,3,5-dithiazine; 2% 2,6-dimethyl-4-butyldihydro-1,3,5-dithiazine; < 1% substituted 1,3,5-thiadiazine	The cyclic compound 2-methyl-1,3-dithiolane has a similar structure to these heterocyclic compounds, and the NOEL in a 90-day study in rats was 7 mg/kg bw per day (Griffiths et al., 1979). The NOELs in dietary 14-day studies on the structural analogues 2-isobutyl-4,6-dimethyldihydrothiazine (Rush, 1989a) and 2-isopropy-4,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989b) were 11 mg/kg bw per day and 10 mg/kg bw per day, respectively. The secondary components are all structural isomers or homologues of the principal components and were present in the 14-day studies on the flavouring agent. Metabolic pathways for these substances include *S*-oxidation to polar sulfoxides and sulfones.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
1047	Mixture of 2-isopropyl-4,6-dimethyl and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine	44% 2-isopropyl-4,6-dimethyldi-hydro-1,3,5-dithiazine; 27% 4-isopropyl-2,6-dimethyldi-hydro-1,3,5-dithiazine	13% 2,4,6-trimethyldihydro-1,3,5-dithiazine; 8% 6-methyl-2,4-diisopropyl-1,3,5-dithiazine; 4% 4-methyl-2,6-diisopropyl-1,3,5-dithiazine; 2% 2,4,6-triisopropyl-dihydro-1,3,5-dithiazine	See No. 1046 above.	Secondary components do not raise safety concern.
1069	2-Ethanoic acid, *S*-(2-methyl-3-furanyl) ester	92	< 7% *cis*- and *trans*-2-methyl-3-tetrahydrofuranthiol acetate	This secondary component is an ester that is hydrolysed to 2-methyl-3-tetrahydrofuranthiol (No. 1090), which has been evaluated by JECFA.	Secondary components do not raise safety concern.
1086	2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	94	5.5% 2-[(2-methyl-3-furyl)thio]-4-heptanone isomer	The NOEL for the structural analogue 3-[(2-methyl-3-furyl)thio]-4-heptanone in a 90-day study in rats was 3.8 mg/kg bw per day (Gallo et al., 1976).	Secondary components do not raise safety concern.
1092	2,5-Dimethyl-3-thioacetoxy-tetrahydrofuran, *cis* and *trans* isomers	90% as mixture of 4 stereoisomers (2 pairs of diastereomers)	< 4% 2,5-dimethyltetrahydrofuran-3-thiol; < 2% dimethyltetrahydro-3-furyl dithioacetate	The structural analogue 2-methyl-3-tetrahydrofuranthiol (No. 1090) has been evaluated by JECFA.	Secondary components do not raise safety concern.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components	Evaluation
1109	2-*sec*-Butylcyclohexanone	94	2–2.5% 2-isobutylcyclohexanone	In a 90-day study in rats, the gas chromatography data for the *2-sec*-butylcyclohexanone tested showed the presence of 2-isobutylcyclohexanone as a secondary component. As the secondary component was present in the test material, this would not be a safety concern (Hummler, 1969). The NOEL in a 90-day study on a structurally related 2-alkyl-substituted cyclopentanone, 2-hexylidenecyclopentanone, was 2.9 mg/kg bw per day (Posternak et al., 1969).	Secondary components do not raise safety concern.
1128	3-Octen-2-one	94	4–6% 4-octen-2-one	The NOEL for 2-octen-4-one (No. 1129) and 1-octene-3-one in a 90-day oral study in rats was 7.5 mg/kg bw per day (Cox, 1974a,b).	Secondary components do not raise safety concern.
1135	(*E*)-7-Methyl-3-octen-2-one	94	2–4% 7-methyl-4-octen-2-one; 5,6-dimethyl-3-hepten-2-one; 3-nonen-2-one	The secondary components are all aliphatic α,β-unsaturated ketones and are metabolized by reaction with glutathione to a mercapturic acid derivative and reduction to the corresponding alcohol. The NOELs in 90-day studies in rats with three structurally related α,β-unsaturated ketones were 6 mg/kg bw per day with 4-hexene-3-one (Shellenberger, 1970), 7.5 mg/kg bw per day with 2-octen-4-one (Cox et al., 1974a) and 7.5 mg/kg bw per day with 1-octen-3-one (Cox et al., 1974b). 3-Nonen-2-one is a straight-chain analogue of these unsaturated ketones.	Secondary components do not raise safety concern.
1137	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadien-2-one	94	3–4% 4,8-dimethyl-3,7-nonadien-2-ol	As the secondary component is an alcohol which is interconvertible in vivo with the ketone, the safety evaluation appplies to both.	Secondary components do not raise safety concern.
1154	(*E,R*)-3,7-Dimethyl-1,5,7-octatrien-3-ol	93	3–5% linalool and lesser quantities of linalool oxide and nerol oxide	For data on linalool, see No. 358 above.	Secondary components do not raise safety concern.

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SAFETY DATA SHEET

Version 6.3
Revision Date 01/14/2020
Print Date 02/08/2020

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Phenylacetic acid

Product Number : P16621
Brand : Aldrich
CAS-No. : 103-82-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 Spruce Street
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Eye irritation (Category 2A), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word Warning

Hazard statement(s)
H319 Causes serious eye irritation.

Precautionary statement(s)
P264 Wash skin thoroughly after handling.
P280 Wear eye protection/ face protection.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes.

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	Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical advice/ attention.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS

Stench.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Benzeneacetic acid α-Tolylic acid PAA
Formula	:	$C_8H_8O_2$
Molecular weight	:	136.15 g/mol
CAS-No.	:	103-82-2
EC-No.	:	203-148-6

Component	Classification	Concentration
Phenylacetic acid		
	Eye Irrit. 2A; H319	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

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SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.
Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

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SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Do not let product enter drains.

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SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: crystalline Colour: beige
b)	Odour	Stench.
c)	Odour Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	Melting point/range: 76 - 78 °C (169 - 172 °F)
f)	Initial boiling point and boiling range	265 °C 509 °F - lit.
g)	Flash point	132 °C (270 °F)
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	1 hPa at 97 °C (207 °F)
l)	Vapour density	No data available
m)	Relative density	1.081 g/cm3 at 25 °C (77 °F)
n)	Water solubility	ca.15 g/l
o)	Partition coefficient: n-octanol/water	log Pow: 1.37 - 1.43
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information

Solubility in other solvents	Ethanol - soluble

SECTION 10: Stability and reactivity

10.1 Reactivity

No data available

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10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents, Strong bases, Strong reducing agents

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - No data available
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - 2,250 mg/kg
Inhalation: No data available
LD50 Dermal - Rabbit - > 5,000 mg/kg
LD50 Intraperitoneal - Rat - 1,600 mg/kg
LD50 Intraperitoneal - Mouse - 2,270 mg/kg
LD50 Subcutaneous - Mouse - 1,500 mg/kg

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Moderate eye irritation - 24 h
(Draize Test)
Eyes - Rabbit
Result: Moderate eye irritation

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
No data available

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Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: AJ2430000

Nausea, Dizziness, Headache, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	LC50 - other fish - 1,273 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	Remarks: No data available(Phenylacetic acid)
Toxicity to algae	Remarks: No data available(Phenylacetic acid)
Toxicity to bacteria	Remarks: No data available(Phenylacetic acid)

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

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SECTION 14: Transport information

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
UN number: 3335 Class: 9 Packing group: III
Proper shipping name: Aviation regulated solid, n.o.s. (Phenylacetic acid)

SECTION 15: Regulatory information

SARA 302 Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Phenylacetic acid	103-82-2	

SECTION 16: Other information

Further information
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European Food Safety Authority

The EFSA Journal (2009) 1024, 1-42

SCIENTIFIC OPINION

Flavouring Group Evaluation 53, Revision 1 (FGE.53Rev1):

Consideration of phenethyl alcohol, aldehyde, acid and related acetals and esters evaluated by JECFA (59th meeting) and structurally related to phenethyl alcohol, aldehyde, esters and related phenylacetic acid esters evaluated by EFSA in FGE.14Rev1 (2009) and one phenoxyethyl ester evaluated in FGE.23Rev1 (2008)

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2009-00482)

ADOPTED ON 26 MARCH 2009

PANEL MEMBERS

Arturo Anadon, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle.

SUMMARY

The Scientific Panel on Food Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to 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 consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217 EC and its consecutive amendments.

The JECFA has evaluated a group of 43 flavouring substances consisting of phenethyl alcohol, aldehyde, acid and related acetals and esters. Two of the JECFA evaluated substances [FL-no:

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09.704 and 09.712] may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these two substances have been given special considerations.

The remaining 41 flavouring substances have originally been considered by the European Food Safety Authority (EFSA) in the Flavouring Group Evaluation (FGE) 53 (EFSA, 2008z).

The genotoxicity of one of the alpha,beta-unsaturated substances, geranyl phenylacetate [FL-no: 09.704] has been considered in FGE.202 (EFSA, 2009ac). The structural alert for genotoxicity is present in the metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl phenylacetate can be evaluated through the Procedure in this FGE.

For the second substance, santalyl phenylacetate [FL-no: 09.712], considered in subgroup 2.1 of FGE.19 (EFSA, 2008b), concern with respect to genotoxicity could not be ruled out and additional data were requested. Accordingly, this substance will not be considered in the present FGE.

The present FGE.53Rev1 therefore only deals with 42 flavouring substances.

The Panel concluded that all the 42 substances in the JECFA flavouring group of phenethyl alcohol, aldehyde, acid and related acetals and esters are structurally related to the group of 13 phenethyl alcohol, aldehyde, esters and related phenylacetic acid esters evaluated by EFSA in the Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1) and one phenoxyethyl ester evaluated in the Flavouring Group Evaluation 23, Revision1 (FGE.23Rev1).

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for the 42 phenylethyl derivatives. However, for four substances [FL-no: 06.027, 09.702, 09.783 and 16.041] the JECFA evaluation is only based on Maximised Survey-derived Daily Intake (MSDI) values derived from production figures from the USA. Accordingly, the safety in use in Europa could not be assessed using the Procedure, so EU production figures are needed in order to finalise the evaluation of these four substances.

For all 42 substances use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 42 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for 39 of the 42 materials of commerce. For two substances [FL-no: 06.007 and 06.027] information on the stereoisomeric composition is lacking and for three substances [FL-no: 06.007, 06.027 and 09.805] further information on the composition of mixture is requested. Thus, for six substances [FL-no: 06.007, 06.027, 09.702, 09.783, 09.805 and 16.041] the Panel has reservations (no European production volumes available, preventing them to be evaluated using the Procedure, and/or stereoisomerism/composition of mixture).

For the remaining 36 substances [FL-no: 02.019, 05.030, 05.042, 05.044, 06.006, 06.016, 06.024, 06.036, 08.038, 08.049, 09.031, 09.083, 09.137, 09.168, 09.261, 09.262, 09.407, 09.427, 09.466, 09.487, 09.496, 09.538, 09.703, 09.704, 09.707, 09.758, 09.772, 09.784, 09.785, 09.786, 09.787, 09.788, 09.789, 09.791, 09.797 and 09.804] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEYWORDS

Phenethyl, phenoxyethyl, JECFA 59th meeting, FGE.14Rev1, FGE.23Rev1.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 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, 1999).

Commision Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

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ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of

structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by the JECFA or if it has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be

amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

At its 59th meeting the JECFA evaluated a group of 43 flavouring substances consisting of phenethyl alcohol, aldehyde, acid and related acetals and esters. Two of the substances evaluated by the JECFA [FL-no: 09.704 and 09.712] may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), they have been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The remaining 41 flavouring substances have originally been considered by EFSA in the FGE.53 (EFSA, 2008z).

FGE.19 contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship ((Q)SAR) prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b)and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220).

History of FGE.53:

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.53	April 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178710471471.htm	41
FGE.53Rev1	March 2009		42

The present Revision of FGE.53, FGE.53Rev1, includes the assessment of one additional substance [FL-no: 09.704] originally considered in FGE.202 (subgroup 1.1.3 in FGE.19) and for which the Panel concluded that the genotoxicity data available do not preclude its evaluation through the Procedure.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 43 flavouring substances consisting of phenethyl alcohol, aldehyde, acid and related acetals and esters.

1.1.2. EFSA Considerations

Fourty-one of 43 flavouring substances have originally been considered by EFSA in the FGE.53 (EFSA, 2008z).The remaining two of the JECFA evaluated substances, geranyl phenylacetate [FL-no: 09.704] and santalyl phenylacetate [and 09.712] may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these two substances have been given special considerations.

The genotoxicity of one of the alpha,beta-unsaturated substances, geranyl phenylacetate [FL-no: 09.704], has been considered in FGE.202 (EFSA, 2009ac). The structural alert for genotoxicity, relevant to this substance is present in the metabolite citral and the Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl phenylacetate [FL-no: 09.704] can be evaluated through the Procedure.

For the second substance, santalyl phenylacetate [FL-no: 09.712], considered with respect to genotoxicity in FGE.202 (EFSA, 2009ac), subgroup 2.1 of FGE.19 (EFSA, 2008b), a final conclusion as to its genotoxic properties could not be reached and additional data were requested. Accordingly, this substance will not be considered in this FGE.

The present FGE.53Rev1 therefore only deals with 42 flavouring substances (see Table 1).

The Panel concluded that the 42 substances in the JECFA flavouring group of phenethyl alcohol, aldehyde, acid and related acetals and esters are structurally related to the group of ten phenethyl alcohol, aldehyde, esters and related phenylacetic acid esters evaluated by EFSA in the Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1) and one phenoxyethyl ester evaluated in the Flavouring Group Evaluation 23, Revision1 (FGE.23Rev1).

1.2. Isomers

1.2.1. JECFA Status

Twelve Register substances in the group of the JECFA evaluated phenethyl alcohol, aldehyde, acid, and related acetals and esters have possibility for stereoisomerism [FL-no: 06.007, 06.016, 06.027, 06.036, 09.496, 09.538, 09.704, 09.772, 09.785, 09.791, 09.805 and 16.041].

1.2.2. EFSA Considerations

Information is lacking about the stereoisomerism for two substance [FL-no: 06.007 and 06.027].

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 42 substances (JECFA, 2001c). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for 39 substances. Information on stereoisomerism is lacking for two [FL-no: 06.007 and 06.027], see Section 1.2, and further compositional information for three substances [FL-no: 06.007, 06.027 and 09.805] is requested.

2. Intake Estimations

2.1. JECFA Status

For 38 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the remaining four substances production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for four substances, MSDI values for the EU cannot be calculated for these [FL-no: 06.027, 09.702, 09.783 and 16.041].

3. Genotoxicity Data

3.1. Genotoxicity Studies - Text Taken[1] from the JECFA (JECFA, 2003a)

Tests for genotoxicity have been performed on seven representative phenethyl alcohol derivatives and three phenoxyethyl alcohol derivatives.

In vitro

Phenethyl alcohol [FL-no: 02.019], phenylacetaldehyde [FL-no: 05.030], phenylacetic acid [FL-no: 08.038], ethyl phenylacetate [FL-no: 09.784], isobutyl phenylacetate [FL-no: 09.788], isoamyl phenylacetate [FL-no: 09.789] and *para*-tolylacetaldehyde [FL-no: 05.042] have been tested for their ability to induce reverse mutation in various strains of *Salmonella typhimurium* (e.g., TA98, TA100, TA1535, TA1537 and TA1538) in the presence or absence of an exogenous metabolic activation system. None of the compounds was mutagenic when tested at concentrations up to 5000 µg/ml or 50 mg/plate (Oda et al., 1979; Florin et al., 1980; Rapson et al., 1980; Ishidate et al., 1984; Heck et al., 1989; Kato et al., 1989; Fujita et al., 1994). No reverse mutation was seen when various strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) were incubated with

[1] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed*.

ethyl (*para*-tolyloxy)acetate [FL-no: 09.797] at up to 3600 µg per plate (Wild et al., 1983) 2-phenoxyethyl isobutyrate [FL-no: 09.487] at 3600 µg per plate (Wild et al., 1983) or sodium 2-(4-methoxyphenoxy)propanoate [FL-no: 16.041] at up to 5000 µg per plate (Varley, 1985), with or without metabolic activation.

Tests of the ability of ethyl phenylacetate [FL-no: 09.784] and isoamyl phenylacetate [FL-no: 09.789] to induce mutation in *Bacillus subtilis* H17 and M45 were inconclusive. In a study in which ethyl phenylacetate was incubated with *B. subtilis* H17 and M45 at 21 µg per disc, the difference in the zone of inhibition (0.8 mm) between the two strains indicated that it was not active (Oda et al., 1979). In a study with a lower concentration, ethyl phenylacetate was incubated at a concentration of 20 µl per disc with *B. subtilis* H17 and M45 in the same assay. The difference in the zone of inhibition (> 8 mm) between the two strains was considered to provide evidence of mutagenicity (Yoo, 1986). Contradictory data have also been reported with isoamyl phenylacetate [FL-no: 09.789]. When 20 µg per disc were incubated with *B. subtilis* H17 and M45, a weak (2–5 mm difference) positive response was reported by Oda et al. (Oda et al., 1979), while Yoo (Yoo, 1986) reported a negative response with 20 µl per disc.

Phenylacetaldehyde [FL-no: 05.030] was tested in *E. coli* strain WP2uvrA/pKM101 with preincubation (Kato et al., 1989), and *para*-tolylacetaldehyde [FL-no: 05.042] was studied in *E. coli* strain PQ37 (Ohshima et al., 1989), both at unspecified concentrations. There was no evidence of mutagenicity in either assay. In another assay, 200–1600 µg per plate of ethyl phenylacetate showed no evidence of mutagenicity when incubated with *E. coli* WP2uvrA (Yoo, 1986). The contradictory results reported by Yoo (Yoo, 1986) and Oda et al. (Oda et al., 1979), the negative result in the WP2 uvrA strain and the fact that phenethyl alcohol is bactericidal in *E. coli* (Treick & Konetzka, 1964; Brunner & Treick, 1982) support the conclusion that the results with *B. subtilis* H17 and M45 should not be used in the overall assessment of the genotoxic potential of these substances.

No increase in sister chromatid exchange frequency was observed when human whole blood lymphocyte cultures were exposed to 2-phenethyl alcohol [FL-no: 02.019] for 72 h (Norppa & Vainio, 1983). Also, no increase in unscheduled DNA synthesis was noted when rat hepatocytes were incubated with phenylacetic acid (Heck et al., 1989). Incubation of ethyl phenylacetate at 1000 µg/ml with Chinese hamster fibroblasts for 48 h caused chromosomal aberrations in 3% of cells. On the basis of a threshold of positivity of > 10%, ethyl phenylacetate gave negative results in this assay (Ishidate et al., 1984).

In vivo

The results of tests for genotoxicity *in vivo* with phenylacetate ester and 2-phenoxyethyl isobutyrate [FL-no: 09.487] and sodium 2-(4-methoxyphenoxy)propanoate [FL-no: 16.041] were negative. No significant increase in the number of micronucleated polychromatic erythrocytes was seen in mice given intraperitoneal injections of 2-phenoxyethyl isobutyrate at 620–1900 mg/kg bw (Wild et al., 1983). In another test, sodium 2-(4-methoxyphenoxy) propanoate was given to mice by gavage at doses of 500–2000 mg/kg bw. An increased frequency of micronucleated polychromatic erythrocytes was found in males at 500 mg/kg bw at 24 h (Asquith & Pickering, 1985).

A phenyl acetate ester that was not included in this group of substances, isoeugenol phenylacetate, was also tested for its ability to induce micronucleus formation. Groups of male and female NMRI

mice were given the compound at doses of 1100–2800 mg/kg bw by intraperitoneal injection. After 30 h, they were killed, and the mean number of micronucleated polychromatic erythrocytes per 1000 normochromatic erythrocytes was calculated. No effect was seen at any dose. Furthermore, the frequency of sex-linked lethal mutations was not increased when *Drosophila melanogaster* were fed a solution of isoeugenol phenylacetate at 25 mmol/l for 3 days (Wild et al., 1983).

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA, see Table 2.1.

3.2. Genotoxicity Studies - Text Taken from EFSA FGE.14Rev1 (EFSA, 2009f)

In vitro / in vivo

Valid *in vitro* mutagenicity and/or genotoxicity data are available for the EFSA evaluated substance [FL-no: 02.166] and for two JECFA evaluated substances [FL-no: 02.019 and 09.784]. There are neither *in vivo* mutagenicity/genotoxicity data available for the EFSA evaluated substances of the present flavouring group evaluation nor for the substances previously evaluated by the JECFA.

Valid *in vitro* and limited *in vivo* mutagenicity data are available for isoeugenyl phenylacetate, a phenyl acetate ester structurally related to the EFSA evaluated substances in this evaluation (Wild et al., 1983).

For the EFSA evaluated substance 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166] there are data available from a Comet assay in oxidative stress sensitive PC human prostate cancer cells (PC3) in which the substance at any of the concentrations tested did not increase the value of oxidative DNA damage (DNA strand breaks) as compared to control cells. On the contrary, at relatively high concentrations the substance was found to decrease DNA damage induced by hydrogen peroxide. However, results indicated that the substance induced lipid peroxidation and decreased the antioxidant capacity of the cells. These effects on enzymes may be attributed to a pro-oxidant activity of 2-(4-hydroxyphenyl)ethan-1-ol (Quiles et al., 2002).

Data on phenethyl alcohol[2] (syn. 2-phenylethan-1-ol) [FL-no: 02.019] and ethyl phenylacetate [FL-no: 09.784][3] are considered representative for some of the EFSA evaluated substances (see footnotes). They have been tested for their ability to induce reverse mutations in various strains of *Salmonella typhimurium* (e.g. TA92, TA94, TA97, TA98, TA100, TA1535, TA1537 and TA1538) in the presence or absence of an exogenous metabolic activation system. None of the compounds was mutagenic in any of the tester strains when tested at concentrations up to 5000 microgram/plate.

There are some positive findings with two of the potential hydrolysis products of the two EFSA evaluated acetals [FL-no: 06.078 and 06.080] *in vitro* and *in vivo*, ethanol and acetaldehyde. The genotoxicity of these two compounds is well known. However, they both do occur naturally in many foods in mg amount (apart from alcoholic beverages) (TNO, 2000) and, based on the MSDI approach, the estimated intakes of EFSA evaluated flavouring substances which might be expected to be hydrolysed to the corresponding alcohols and aldehydes are much lower. Further, ethanol and acetaldehyde are endogenous. So, the daily *in vivo* formation of ethanol has been estimated to be 40-80 mg/kg body weight/day (JECFA, 1997a).

[2] *EFSA evaluated (in FGE.14) 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166].*

[3] *EFSA evaluated (in FGE.14) pentyl phenylacetate [FL-no: 09.761], menthyl phenylacetate [FL-no: 09.620].*

For the JECFA evaluated substances, there are *in vitro* genotoxicity studies available from test systems other than bacterial, which were reported to be negative: no increase in sister chromatid exchange frequency was reported in human whole blood lymphocyte cultures exposed to phenethyl alcohol [FL-no: 02.019] for 72 hours; and ethyl phenylacetate [FL-no: 09.784] did not cause chromosomal aberrations in Chinese hamster fibroblasts when incubated for 48 hours.

From the available *in vitro* and *in vivo* mutagenicity data on the additional structurally related substance isoeugenyl phenylacetate there is no indication of a mutagenic activity: a negative result was reported in an Ames Test in various strains of *Salmonella typhimurium* (e.g. TA98, TA100, TA1535, TA1537 and TA1538) with and without metabolic activation and the substance was reported not to induce sex-linked recessive (lethal) mutations in *Drosophila melanogaster in vivo* (Wild et al., 1983).

There are no genotoxicity studies available on 2-phenethyl acetals, neither from the group of EFSA evaluated nor of JECFA-evaluated substances.

Conclusion on Genotoxicity

Overall the genotoxicity data available are not sufficient to evaluate the genotoxicity adequately. However, the data available on candidate and supporting substances do not give rise to concern with respect to genotoxicity of the candidate substances in FGE.14Rev1.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Table 2.2 and Table 2.3.

3.3. Genotoxicity and Conclusion on Genotoxicity and Carcinogenicity – Text from FGE.202 (EFSA, 2009ac)

"There are ten *in vitro* studies and three *in vivo* study available on citral [FL-no: 05.020] and on 3-methylcrotonaldehyde (3-methyl-2-butenal) [FL-no: 05.124].

3-Methylcrotonaldehyde was found to be mutagenic in a valid modified Ames test, i.e. the liquid suspension assay, both in the absence and, to a lower extent, in the presence of metabolic activation (S9-mix), in TA100 *S. typhimurium* strain (BASF, 1991b). Of doubtful relevance was a slight increase (factor 2.1) in the number of revertants observed with TA98 strain, only in the absence of S9 at the highest concentration (2500 microgram/plate). It was found negative in a valid bone marrow micronucleus assay in mice, treated orally at 175, 350 and 750 mg/kg body weight, with signs of toxicity at the highest dose, as shown by the ratio of polychromatic to normochromatic erythrocytes (BASF, 1992c). Moreover, it was found negative in a valid *in vivo* unscheduled DNA synthesis (UDS) assay, carried out on hepatocytes from rats treated orally at dose levels of 350 and 700 mg/kg body weight (BASF, 2001). In conclusion, based on the negative results in two valid *in vivo* assays (rat liver UDS and mouse bone marrow micronucleus), the positive result observed in the modified Ames test is considered of limited relevance for the overall evaluation. Therefore, for this substance, the Panel considers that genotoxicity is of no concern.

Citral was not mutagenic in several valid Ames tests (Ishidate et al., 1984; Zeiger et al., 1987; Gomes-Carneiro et al., 1998; NTP, 2003e), and it did not induce chromosome aberrations in a valid *in vitro* study with chinese hamster ovary (CHO) cells (NTP, 2003e). Moreover, it was negative in a valid *in vivo* mouse bone marrow micronucleus assay (NTP, 2003e). The positive results in an *in vitro* test for sister chromatid exchanges (SCE) (NTP, 2003e) and in inappropriate test systems like the *Rec* assay in *B. subtilis* (Yoo, 1986) and the induction of the tumour suppressor protein p53

(Duerksen-Hughes et al., 1999) are considered of limited relevance for the overall evaluation. The Panel concluded that for citral genotoxicity is not of concern.

Overall, the Panel concluded that the genotoxicity data available do not give rise to concern for the 37 substances in FGE.202 using the Procedure.

Study validation and results are presented in Table 2.4 and 2.5.

Conclusion on Genotoxicity and Carcinogenicity

Based on the available data, the Panel concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity for the 37 alpha,beta-unsaturated substances presented in this FGE."

For a summary of genotoxicity data see Table 2.4: Genotoxicity data (*in vitro*) EFSA / FGE.202 and Table 2.5 Genotoxicity data (*in vivo*) EFSA / FGE.202.

3.4. EFSA Considerations

In the FGE.53 there were valid *in vitro* genotoxicity data available for one [FL-no: 02.166] of the ten substances evaluated in FGE.14 and valid *in vitro* and a limited number of *in vivo* mutagenicity data available for two of the substances evaluated by the JECFA and on a further structurally related substance (isoeugenyl phenylacetate).

For 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166] evaluated by the EFSA in FGE.14Rev1, the only available study gave no indication of a genotoxic potential *in vitro*.

From the various studies carried out with the substances evaluated by the JECFA, there is no indication of a genotoxic activity of the phenethyl alcohols, phenylacetic acids and related esters in bacterial mutation assays.

There are no genotoxicity studies available for the phenoxyethyl ester [FL-no: 09.687] evaluated in FGE.23Rev1.

Overall the Panel concluded that the genotoxicity data available are not sufficient to evaluate the genotoxicity adequately. However, the data available do not preclude evaluation of the 41 JECFA evaluated phenetyl alcohol, aldehyde, acid and related acetals and esters through the Procedure.

Based on the available data on citral evaluated in FGE.202 the Panel concluded that there would be no safety concern with respect to genotoxicity and carcinogenicity of geranyl phenylacetate [FL-no: 09.704] and therefore the 42 flavouring substances consisting of phenethyl alcohol, aldehyde, acid, and related acetals and esters could be evaluated through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 42 Phenylethyl Alcohol and Related Substances Evaluated by the JECFA (JECFA, 2003a):

According to the JECFA 38 of the substances belong to structural class I and four to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 41 of the substances at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural classes I and III (step A3).

One substance was concluded at step A5 – i.e. the intake is above the threshold for the structural class, the substance is not endogenous, but a NOAEL is available that can provide an adequate margin of safety to the estimated intake of the substance [FL-no: 09.487].

In conclusion, the JECFA evaluated all 42 substances as to be of no safety concern at the estimated levels of intakes as flavouring substances based on the MSDI approach.

The evaluations of the 42 phenetyl alcohol, aldehyde, acid and related acetals and esters are summarised in Table 3.1: Summary of Safety Evaluation of 42 Phenylethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters (JECFA, 2003a).

4.2. Application of the Procedure to Ten Phenethyl Derivatives by EFSA (EFSA, 2009f):

Thirteen candidate substances were evaluated in FGE.14Rev1. Twelve substances are classified into structural class I and one substance into structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The 13 substances were all concluded at step A3 – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class (step A3).

In conclusion, the Panel evaluated all 13 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The substance in FGE.23Rev1 (EFSA, 2008bg) was concluded at step A3 – i.e. the substance is expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class III (step A3).

The stepwise evaluations of the 13 substances evaluated in FGE.14Rev1 and the phenoxythyl ester evaluated in FGE.23Rev1 are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.14Rev1 and One Phenoxyethyl Ester Evaluated in FGE.23Rev1).

4.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all the 42 substances in the group of phenylethyl alcohol, aldehyde, acid and related acetals and esters and related substances.

However, for four substances [FL-no: 06.027, 09.702, 09.783 and 16.041] no European production figures were available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for these four substances.

5. Conclusion

The JECFA has evaluated a group of 43 flavouring substances consisting of phenethyl alcohol, aldehyde, acid and related acetals and esters. Two of the JECFA evaluated substances [FL-no: 09.704 and 09.712] may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these two substances have been given special considerations.

The remaining 41 flavouring substances have originally been considered by EFSA in the FGE.53 (EFSA, 2008z).

The genotoxicity of one of the alpha,beta-unsaturated substances, geranyl phenylacetate [FL-no: 09.704] has been considered in FGE.202. The structural alert for genotoxicity is present in the metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl phenylacetate can be evaluated through the Procedure.

For the second substance, santalyl phenylacetate [FL-no: 09.712], considered in subgroup 2.1 of FGE 19 (EFSA, 2008b), concern with respect to genotoxicity could not be ruled out and additional data were requested. Accordingly, this substance will not be considered in the present FGE.

The present FGE.53Rev1 therefore only deals with 42 flavouring substances.

The Panel concluded that all the 42 substances in the JECFA flavouring group of phenethyl alcohol, aldehyde, acid and related acetals and esters are structurally related to the group of 13 phenethyl alcohol, aldehyde, esters and related phenylacetic acid esters evaluated by EFSA in FGE.14Rev1 and one phenoxyethyl ester evaluated in FGE.23Rev1.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for the 42 phenylethyl derivatives. However, for four substances [FL-no: 06.027, 09.702, 09.783 and 16.041] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. Accordingly, the safety in use in Europa could not be assessed using the Procedure, so EU production figures are needed in order to finalise the evaluation of these four substances.

For all 42 substances use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 42 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for 39 of the 42 materials of commerce. For two substances [FL-no: 06.007 and 06.027] information on the stereoisomeric composition is lacking and for three substances [FL-no: 06.007, 06.027 and 09.805] further information on the composition of mixture is requested. Thus, for six substances [FL-no: 06.007, 06.027, 09.702, 09.783, 09.805 and 16.041] the Panel has reservations (no European production volumes available, preventing them to be evaluated using the Procedure, and/or stereoisomerism/composition of mixture).

For the remaining 36 substances [FL-no: 02.019, 05.030, 05.042, 05.044, 06.006, 06.016, 06.024, 06.036, 08.038, 08.049, 09.031, 09.083, 09.137, 09.168, 09.261, 09.262, 09.407, 09.427, 09.466, 09.487, 09.496, 09.538, 09.703, 09.704, 09.707, 09.758, 09.772, 09.784, 09.785, 09.786, 09.787, 09.788, 09.789, 09.791, 09.797 and 09.804] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP (JECFA, 2002D)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

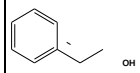
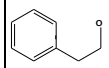
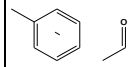
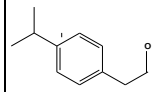
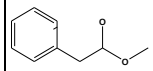
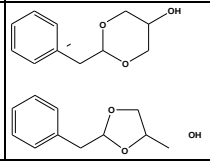
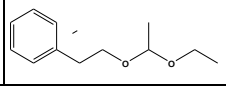
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.019 987	2-Phenylethan-1-ol		2858 68 60-12-8	Liquid $C_8H_{10}O$ 122.17	Slightly soluble 1 mL in 2 mL 50% ethanol	219-221 IR 98 %	1.529-1.535 1.017-1.020	
05.030 1002	Phenylacetaldehyde		2874 116 122-78-1	Liquid C_8H_8O 120.15	Slightly soluble 1 mL in 2 mL 80% ethanol	195 NMR 95 %	1.524-1.545 1.023-1.045	
05.042 1023	p-Tolylacetaldehyde		3071 130 104-09-6	Liquid $C_9H_{10}O$ 134.18	Insoluble Miscible	210 NMR 95 %	1.530-1.549 1.010-1.016	
05.044 1024	p-Isopropyl phenylacetaldehyde		2954 132 4395-92-0	Liquid $C_{11}H_{14}O$ 162.23	Insoluble Miscible	230-243 NMR 97 %	1.515-1.525 0.965-0.975	
06.006 1003	1,1-Dimethoxy-2-phenylethane		2876 40 101-48-4	Liquid $C_{10}H_{14}O_2$ 166.22	Insoluble 1 mL in 2 mL 70% ethanol	219 IR 95 %	1.492-1.498 1.000-1.006	
06.007 1004	Phenylacetaldehyde glyceryl acetal 6)		2877 41 29895-73-6	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	358 NMR 95 %	1.524-1.536 1.158-1.168	CASrn in Register refers to named substance; "Incompletely Defined Substance".
06.016 1000	1-Phenylethoxy-1-propoxy ethane		2004 511 7493-57-4	Liquid $C_{13}H_{20}O_2$ 208.30	Miscible	272 NMR 96 %	1.475-1.483 0.944-0.950	Racemate.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

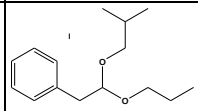
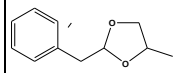
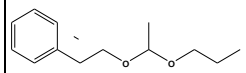
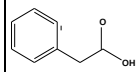
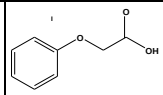
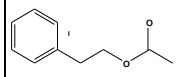
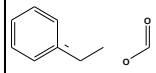
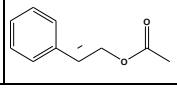
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
06.024 1006	1,1-Di-isobutoxy-2-phenylethane		3384 595 68345-22-2	Liquid $C_{16}H_{26}O_2$ 250.38	Insoluble Miscible	240 IR 97 %	1.468-1.476 0.928-0.936	
06.027 1005	4,5-Dimethyl-2-benzyl-1,3-dioxolan 6)		2875 669 5468-06-4	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble Miscible	118 (13 hPa) NMR 93 %	1.496-1.512 1.030-1.040	According to the JECFA: Min. assay value is "93 %" and secondary components "butane-2,3-diol".
06.036 1001	1-Butoxy-1-(2-phenylethoxy)ethane		3125 10007 64577-91-9	Liquid $C_{14}H_{22}O_2$ 222.33	Miscible	280-282 NMR 97 %	1.467-1.481 0.923-0.935	Racemate.
08.038 1007	Phenylacetic acid		2878 672 103-82-2	Solid $C_8H_8O_2$ 136.15	Slightly soluble Soluble	265 76-78 IR 99 %	n.a. n.a.	
08.049 1026	Phenoxyacetic acid		2872 2005 122-59-8	Solid $C_8H_8O_3$ 152.15	Slightly soluble Soluble	285 98-103 NMR 98 %	n.a. n.a.	
09.031 989	Phenethyl acetate		2857 221 103-45-7	Liquid $C_{10}H_{12}O_2$ 164.20	Insoluble 1 mL in 2 mL 70% ethanol	232 IR 98 %	1.496-1.502 1.030-1.034	
09.083 988	Phenethyl formate		2864 350 104-62-1	Liquid $C_9H_{10}O_2$ 150.18	Slightly soluble Miscible	226 NMR 96 %	1.503-1.513 1.056-1.065	
09.137 990	Phenethyl propionate		2867 418 122-70-3	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	244-245 NMR 97 %	1.489-1.499 1.010-1.021	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

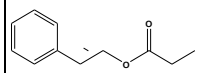
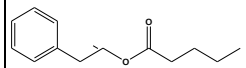
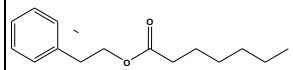
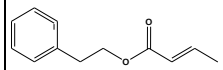
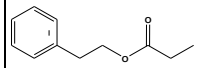
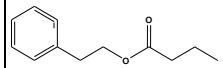
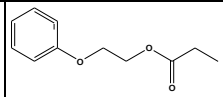
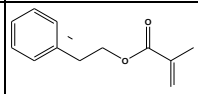
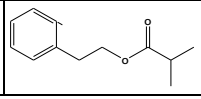
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.168 991	Phenethyl butyrate		2861 506 103-52-6	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble Miscible	238 NMR 97 %	1.487-1.493 0.991-0.994	
09.261 995	2-Phenethyl hexanoate		3221 10882 6290-37-5	Liquid $C_{14}H_{20}O_2$ 220.31	Insoluble Miscible	263 NMR 98 %	1.480-1.488 0.969-0.980	
09.262 996	Phenethyl octanoate		3222 10884 5457-70-5	Liquid $C_{16}H_{24}O_2$ 248.37	Insoluble Miscible	295.5 NMR 98 %	1.479-1.486 0.973-0.977 (20°)	
09.407 998	2-Phenethyl 3-methylcrotonate		2869 246 42078-65-9	Liquid $C_{13}H_{16}O_2$ 204.27	Insoluble Miscible	285 NMR 97 %	1.514-1.520 1.011-1.019	
09.427 992	Phenethyl isobutyrate		2862 302 103-48-0	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble 1 mL in 3 Ml 80% ethanol	230 IR 98 %	1.485-1.490 0.987-0.990	
09.466 994	Phenethyl isovalerate		2871 461 140-26-1	Liquid $C_{13}H_{18}O_2$ 206.29	Insoluble 1 mL in 3 ml 80% ethanol	263 IR 97 %	1.482-1.487 0.973-0.976	
09.487 1028	2-Phenoxyethyl isobutyrate		2873 2089 103-60-6	Liquid $C_{12}H_{16}O_3$ 208.26	Insoluble 1 mL in 3 mL 70% alcohol	265 NMR 97 %	1.491-1.496 1.044-1.048	
09.496 997	Phenethyl 2-methylcrotonate		2870 2186 55719-85-2	Liquid $C_{13}H_{16}O_2$ 204.27	Insoluble Miscible	259 NMR 98 %	1.494-1.518 1.148-1.159	
09.538 993	Phenethyl 2-methylbutyrate		3632 10883 24817-51-4	Liquid $C_{13}H_{18}O_2$ 206.29	Insoluble Miscible	230 NMR 95 %	1.481-1.489 0.974-0.980	CASrn in Register refers to the racemate.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

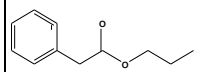
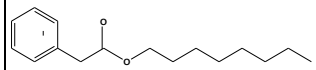
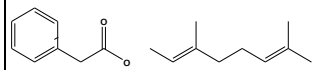
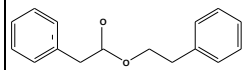
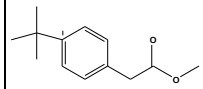
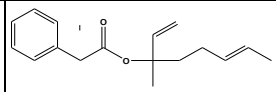
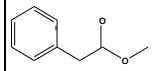
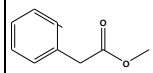
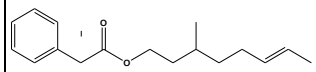
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.702 1010	Propyl phenylacetate		2955 229 4606-15-9	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	253 NMR 97 %	1.489-1.497 0.985-0.995 (15.5°)	
09.703 1017	Octyl phenylacetate		2812 230 122-45-2	Liquid $C_{16}H_{24}O_2$ 248.37	Insoluble Miscible	315 NMR 98 %	1.479-1.487 0.950-0.956	
09.704 1020	Geranyl phenylacetate		2516 231 102-22-7	Liquid $C_{18}H_{24}O_2$ 272.39		307-308 IR 97 %	1.501-1.512 0.971-0.978	CASrn in Register refers to (2E)-isomer. Register name to be changed to (2E)-Geranyl phenylacetate.
09.707 999	Phenethyl phenylacetate		2866 234 102-20-5	Solid $C_{16}H_{16}O_2$ 240.30	Insoluble 1 mL in 4 mL 90% ethanol	325 28 IR 98 %	1.545-1.551 1.079-1.082	
09.758 1025	Methyl p-tert-butylphenylacetate		2690 577 3549-23-3	Liquid $C_{13}H_{18}O_2$ 206.29	Insoluble Miscible	106 (3 hPa) NMR 97 %	1.494-1.504 0.995-1.003	
09.772 1019	Linalyl phenylacetate		3501 655 7143-69-3	Liquid $C_{18}H_{24}O_2$ 272.39	Insoluble Miscible	317 NMR 95 %	1.500-1.508 0.966-0.974	Racemate.
09.783 1008	Methyl phenylacetate		2733 2155 101-41-7	Liquid $C_9H_{10}O_2$ 150.18	Insoluble 1 mL in 6 mL 60% ethanol	215 IR 97 %	1.504-1.510 1.061-1.067	
09.784 1009	Ethyl phenylacetate		2452 2156 101-97-3	Liquid $C_{10}H_{12}O_2$ 164.20	Insoluble 1 mL in 3 mL 70% ethanol	228 IR 97 %	1.494-1.500 1.027-1.032	
09.785 1021	Citronellyl phenylacetate		2315 2157 139-70-8	Liquid $C_{18}H_{26}O_2$ 274.40	Insoluble Miscible	342 NMR 98 %	1.492-1.510 0.958-0.960	Racemate.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

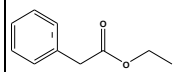
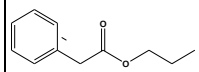
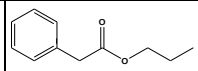
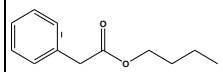
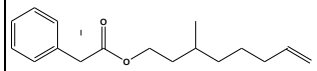
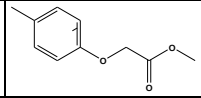
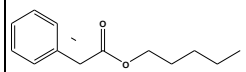
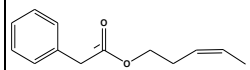
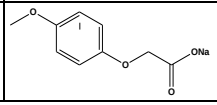
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.786 1011	Isopropyl phenylacetate		2956 2158 4861-85-2	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	238-253 NMR 97 %	1.483-1.491 1.006-1.012 (20°)	
09.787 1012	Butyl phenylacetate		2209 2159 122-43-0	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble 1 ml in 1 ml	258-260 IR 98 %	1.486-1.493 0.990-0.997	
09.788 1013	Isobutyl phenylacetate		2210 2160 102-13-6	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble 1 mL in 2 mL 80% ethanol	247 IR 98 %	1.484-1.488 0.984-0.988	
09.789 1014	3-Methylbutyl phenylacetate		2081 2161 102-19-2	Liquid $C_{13}H_{18}O_2$ 206.29	Insoluble 1 ml in 1 ml	268 IR 97%	1.483-1.490 0.975-0.981	CASrn in Register refers to 3-methylbutyl phenylacetate. According to JECFA: Min. assay value is" 97 (sum of n-amyl and isoamyl esters)" and the composition is "65 % n-amyl and 35 % 3-methylbutyl phenylacetate".
09.791 1018	Rhodinyl phenylacetate		2985 2163 10486-14-3	Liquid $C_{18}H_{26}O_2$ 274.40	Insoluble Miscible	340 NMR 95 %	1.494-1.505 0.965-0.972	CASrn in Register refers to to (3S)-enantiomer. Register name to be changed to (3S)-Rhodinyl phenylacetate.
09.797 1027	Ethyl (p-tolyloxy)acetate		3157 2243 67028-40-4	Liquid $C_{11}H_{14}O_2$ 178.22	Insoluble Miscible	120-121 NMR 98 %	1.499-1.506 1.075-1.080 (20°)	
09.804 1015	Hexyl phenylacetate		3457 10694 5421-17-0	Liquid $C_{14}H_{20}O_2$ 220.31	Insoluble Miscible	262 MS 97 %	1.480-1.490 0.970-0.977	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 42 Phenethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances (JECFA, 2002d)

FL-no JECFA-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)	EFSA comments
09.805 1016	Hex-3(cis)-enyl phenylacetate		3633 10682 42436-07-7	Liquid $C_{14}H_{18}O_2$ 218.30	Insoluble Miscible	299 NMR 97 %	1.497-1.504 0.996-1.004	According to JECFA: Min. assay value is "97 %" and "predominantly (>90 %) cis-isomer".
16.041 1029	Sodium 2-(4-methoxyphenoxy)propionate		3773 13794-15-5	Solid $C_{10}H_{11}O_4$,Na+ 218.19	Soluble Miscible	n.a. 190 IR 98 %	n.a. n.a.	Racemate.

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.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for 42 Phenetyl Alcohol, Aldehyde, Acid , and Related Acetals and Esters (JECFA, 2003a)

Table 2.1: Summary of Genotoxicity Data for 42 Phenethyl Alcohols, Aldehydes and Acids and Related Acetals and Esters (JECFA, 2003a)

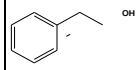
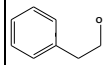
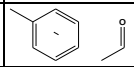
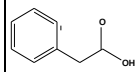
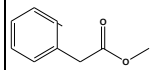
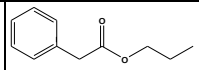
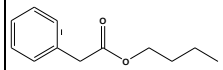
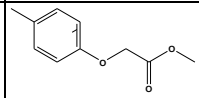
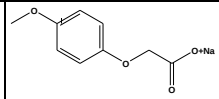
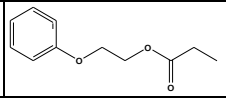
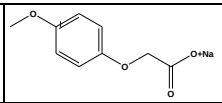
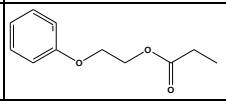
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
02.019	2-Phenylethan-1-ol		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 mmol/plate	Negative[a]	(Florin et al., 1980)
			Sister chromatid exchange	Human lymphocytes	Not specified	Negative	(Norppa & Vainio, 1983)
05.030	Phenylacetaldehyde		Reverse mutation	*S. typhimurium* TA98, TA100, TA104	Not specified	Negative[a]	(Kato et al., 1989)
			Mutation	*E. coli* WP2uvrA/pkM101	Not specified	Negative[a]	(Kato et al., 1989)
05.042	p-Tolylacetaldehyde		Reverse mutation	*S. typhimurium* TA100	0.1–1000 µg/plate	Negative	(Rapson et al., 1980)
			Mutation	*E. coli* PQ37	Not specified	Negative	(Ohshima et al., 1989)
08.038	Phenylacetic acid		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1000 mg	Negative[a]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	500 mg	Negative	(Heck et al., 1989)
			Mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	1500 mg	Negative[a]	(Heck et al., 1989)
09.784	Ethyl phenylacetate		Mutation	*B. subtilis* H17 (rec^+) and M45 (rec^-)	21 mg/disc	Negative	(Oda et al., 1979)
			Mutation	*B. subtilis* H17 (rec^+) and M45 (rec^-)	20 ml/disc	Positive[b]	(Yoo, 1986)
			Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	5 mg	Negative[a]	(Ishidate et al., 1984)
			Chromosomal abberation	Chinese hamster fibroblast cells	1 mg/ml	Negative[a]	(Ishidate et al., 1984)
			Mutation	*E. coli* WP2uvrA (trp^-)	200–1600 µg/plate	Negative[b]	(Yoo, 1986)

Table 2.1: Summary of Genotoxicity Data for 42 Phenethyl Alcohols, Aldehydes and Acids and Related Acetals and Esters (JECFA, 2003a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
09.788	Isobutyl phenylacetate		Reverse mutation	*S. typhimurium* TA97, TA102	0–0.1 mg/plate	Negative[a]	(Fujita et al., 1994)
09.789	3-Methylbutyl phenylacetate		Mutation	*B. subtilis* H17 (rec$^+$) and M45 (rec$^-$)	20 mg/disc	Positive[b]	(Oda et al., 1979)
			Mutation	*B. subtilis* H17 (rec$^+$) and M45 (rec$^-$)	20 ml/disc	Negative[b]	(Yoo, 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100	10 mg/plate	Negative[a]	(Oda et al., 1979)
					50 mg/plate	Lethal[a,b]	
09.797	Ethyl (p-tolyloxy)acetate		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	3600 µg/plate	Negative[a]	(Wild et al., 1983)
16.041	Sodium 2-(4-methoxyphenoxy)propionate		Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	5000 µg/plate	Negative[a]	(Varley, 1985)
09.487	2-Phenoxyethyl isobutyrate		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	3600 µg/plate	Negative[a]	(Wild et al., 1983)
In vivo							
16.041	Sodium 2-(4-methoxyphenoxy)propionate		Micronucleus formation	Mouse bone marrow cells	2000 mg/kg/bw	Negative[c]	(Asquith & Pickering, 1985)
09.487	2-Phenoxyethyl isobutyrate		Micronucleus formation	Mouse bone marrow cells	1900 mg/kg/bw	Negative[b]	(Wild et al., 1983)

[a] *With and without metabolic activation.*

[b] *Administered intraperitoneally.*

c Information reported in the table are not consistent with the text (see section 3.1 – in vivo*): however the results are considered negative – the substance could not be identified as the study report only containea a code number and not the substance name (unpublished data submitted from EFFA).*

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.14Rev1

Substances listed in brackets are JECFA evaluated supporting substances in FGE.14Rev1.

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.14Rev1

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Phenylethan-1-ol [02.019] (Phenethyl alcohol))	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (366 µg/plate)[8]	Negative[1]	(Florin et al., 1980)	Published non-GLP study. Limited report of study details. No results reported. Validity of the study cannot be evaluated.
	Ames reverse mutation assay	*S. typhimurium* TA100, TA1535, TA1538	0 - 99.6 µmol/plate (0 – 12200 µg/plate)[8]	Negative[2, 3]	(Zeiger & Pagano, 1984)	Spot-Test on inhibition of reversion induced by known mutagens. Published non-GLP study of acceptable quality. Limited report of study details and results. Overall, study and results are considered valid.
	Mutation Assay	*Saccharomyces saké* strain Kyokai no. 7	0.1, 0.15, 0.20% (1000, 1500, 2000 µg/ml)	Negative	(Kojima et al., 1976)	Published study in Japanese (summary and tables with results in English). Validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	0.1 - 10 mM (12.2 to 1220 µg/ml)[8]	Negative[4]	(Norppa & Vainio, 1983)	Published non-GLP study of acceptable quality.
2-(4-Hydroxyphenyl)ethan-1-ol [02.166]	Comet assay	PC human prostate cancer cells	0, 10, 50, 100, 250 µM (0, 1.4, 7, 14, 35 µg/ml)[9]	Negative[5]	(Quiles et al., 2002)	Published non-GLP study of acceptable quality. Study is considered valid.
(Phenylacetaldehyde [05.030])	Ames reverse mutation assay (preincubation)	*S. typhimurium* TA98, TA100, TA104 *E. coli* WP2*uvr*A/ pKM101	Not specified	Negative[1]	(Kato et al., 1989)	Only abstract reported. Validity of the study cannot be evaluated.
(Phenylacetic acid [08.038])	Ames reverse mutation assay	*S. typhimurium* TA98, A100, TA1535, TA1537, TA1538	1000 µg/plate[7]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated.
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg/ml[7]	Negative	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated.
	Forward mutation assay	Mouse lymphoma L5178Y TK+/- cells	1000, 1500 µg/ml[7]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated. It has to be noted, that there was some activity observed in the study even for GRAS substances (for which a negative result was found in the Ames test by the same authors), for which effects of nonphysiological medium conditions on the outcome of the study might be responsible for this. Therefore the validity of the study is questionable.
(Ethyl phenylacetate [09.784])	Ames reverse mutation assay	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	up to 5000 µg/plate[10]	Negative[1]	(Ishidate et al., 1984)	Published non-GLP study of acceptable quality.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	up to 1000 µg/ml[11]	Negative	(Ishidate et al., 1984)	Published non-GLP study of acceptable quality.
	Rec assay	*B. subtilis* H17 (rec +) and M45 (rec-)	21 µg/disk	Negative	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	20 µg/disk	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Mutation assay	*E. coli* WP2*uvr*A (trp-)	200 - 1600 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Isobutyl phenylacetate [09.788])	Ames reverse mutation assay	*S. typhimurium* TA97, TA102	1, 5, 10, 50 and 100 µg/plate	Negative[1]	(Fujita et al., 1994)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.14Rev1

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(3-Methylbutyl phenylacetate [09.789] (Isoamyl phenylacetate))	Ames reverse mutation assay	*S. typhimurium* TA98, TA100	10 µg/plate 50 µg/plate	Negative[1] Cytotoxic[1]	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	20 µg/disk	Positive	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	20 µg/disk	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(*p*-Tolyacetaldehyde [05.042])	Ames reverse mutation assay	*S. typhimurium* TA100	0.1, 1, 10, 100, 1000 µg/plate	Negative	(Rapson et al., 1980)	Published non-GLP study. Study design and results insufficiently reported. Validity of the study cannot be evaluated.
	SOS Chromtest	*E. coli* PQ37	Not specified	Negative[4]	(Ohshima et al., 1989)	Published non-GLP. p-Tolylacetaldehyde has not been analysed per se but after nitrosation (it is unclear to the rapporteur whether the substance has been assayed at all in the study). Due to limited report of experimental details and results the validity of the study cannot be evaluated.
(Isoeugenyl phenylacetate[6] [09.710])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	up to 3600 µg/plate[12]	Negative[1]	(Wild et al., 1983)	Published non-GLP study. No detailed results reported. However, as experimental details and evaluation criteria including results of positive controls are sufficiently reported the study is considered valid.

[1] *With and without S9 metabolic activation.*

[2] *With S9 metabolic activation.*

[3] *Toxic at concentrations from 91.3 µmol/plate.*

[4] *Without S9 metabolic activation.*

[5] *At the two highest dose levels evaluated 2-(4-hydroxyphenyl)ethan-1-ol reduced the DNA damage of H_2O_2 treated cells (by 23% at 100 µM and by 40%, at 250 µM).*

[6] *A phenyl acetate ester structurally related to the EFSA evaluated chemicals and JECFA evaluated chemicals, phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances JECFA (JECFA, 2004a).*

[7] *Highest inactive dose tested.*

[8] *Calculated based on molecular weight = 122.16.*

[9] *Calculated based on molecular weight = 138.17.*

[10] *Six different concentrations used (single concentrations not reported).*

[11] *Three different doses used (single doses not reported).*

[12] *Five different concentrations used (single concentrations not reported).*

Table 2.3: Genotoxicity Data (*in vivo*) EFSA / FGE.14Rev1

Substances listed in brackets are JECFA evaluated supporting substances in FGE.14Rev1.

Table 2.3: Summary of Genotoxicity Data (*in vivo)* EFSA / FGE.14Rev1

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Isoeugenyl phenylacetate[1] [09.710])	Micronucleus formation assay	Mouse bone marrow cells	i.p.	0, 564, 987 or 1410 mg/kg bw (two applications)	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
	Sex-linked recessive mutation	*D. melanogaster*	NR	25 mM	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. Study is considered valid.

NR=Not Reported

[1]*A phenyl acetate ester structurally related to the EFSA evaluated substances and JECFA-evaluated substances, phenethyl alcohol, aldehyde, acid and related acetals and esters and related substances JECFA (JECFA, 2004a).*

Table 2.4: Genotoxicity Data (*in vitro*) EFSA / FGE.202

Table 2.4: Genotoxicity Data (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments e)
Citral [05.020]	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	5–700 µg/plate	Negative[a]	(Gomes-Carneiro et al., 1998)	Valid. Published non-GLP study containing sufficient details. Result is considered as valid.
	Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 100 µg/plate	Negative[b]	(Ishidate et al., 1984)	Valid. According to current guidelines.The study is considered valid.
	Reverse mutation	*S. typhimurium* TA100	NR	Negative[a]	(Lutz et al., 1982)	Validity cannot be evaluated. One strain only; concentrations tested not specified; no re-run of the test; no other data on experimental results or design apart from a description of the test method.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–160 µg/plate	Negative[a]	(Zeiger et al., 1987) (NTP, 2003e)	Valid. Standard NTP study carried out according to US.EPA guidelines; result is considered valid.
	Mutation	E. coli WP2uvrA (trp -)	13–100 µg/plate	Negative	(Yoo, 1986)	Validity cannot be evaluated (study in Japanese).
	Sister chromatid exchange	Chinese hamster ovary cells	0.289–40.2 µg/ml	Positive[a]	(NTP, 2003e)	Valid. Standard NTP study carried out according to US EPA guidelines. Result is considered valid.
	Chromosomal aberration	Chinese hamster ovary cells	12.5–60.6 µg/ml	Negative[a]	(NTP, 2003e)	Valid. Standard NTP study carried out according to US.EPA guidelines. Result is considered valid.
	Chromosomal aberration	Chinese hamster fibroblast cells	Up to 30 µg/ml	Negative[c]	(Ishidate et al., 1984)	Limited validity (performed only in the presence of metabolic activation).
	Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)	The test system used is considered inappropriate; insufficient validity.
	Rec assay	*B. subtilis* M45 and H17	0.16, 0.32, 0.63 µl/disk (142, 284, 560 µg/disk)[d] 1.25, 2.5 µl/disk (1110, 2220 µg/disk)[d]	Negative Positive	(Kuroda et al., 1984a)	Validity cannot be evaluated. Article in Japanese; with limited information in tables and abstract. Assay of limited relevance.
	Rec assay	*B. subtilis* M45 and H17	<2.5 µl/disk (<2220 µg/disk)	Positive	(Yoo, 1986)	Validity cannot be evaluated (study in Japanese). Study of limited relevance.
	Induction of tumour suppressor protein p53 (DNA damage)	Mouse fibroblast cells (NTCT 929)	10–30 µg/ml	Positive	(Duerksen-Hughes et al., 1999)	The Induction of tumor suppressor protein p53 may be considered as indicator for genotoxicity. Result is considered valid, however, it has only limited relevance.
3-methyl-2-butenal [05.124]	Ames test (preincubation)	*S. typhimurium* TA98, TA100		Positive[a]	(BASF, 1991b)	Valid. Modified Ames test: Unpublished non-GLP study, carried out in accordance with the OECD guideline no 471. The study contains sufficient details and is considered valid.

NR not reported.

[a] *With and without metabolic activation.*

[b] *With metabolic activation.*

[c] *Without metabolic activation.*

[d] *Calculated using a density of 0.888 (Merck, 1997).*

e) Validity of genotoxicity studies:

1. *Valid.*
2. *Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).*
3. *Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).*
4. *Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).*

Table 2.5: Genotoxicity Data (*in vivo*) EFSA / FGE.202

Table 2.5: GENOTOXICITY DATA (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments a)
Citral [05.020]	Micronucleus formation	Mouse bone marrow erythrocytes	Three intraperitoneal injections given at 24-h intervals; male mice only	250, 500, or 750 mg/kg bw	Negative	(NTP, 2003e)	NTP study carried out according to US-EPA guideline. Result is considered as valid.
	Micronucleus formation	Mouse peripheral blood erythrocytes	Microencapsulated citral was administered in the diet for 14 weeks	745, 1840, 3915, or 8110 mg/kg bw per day (males) 790, 1820, 3870, or 7550 mg/kg bw per day (females)	Negative Negative	(NTP, 2003e)	NTP study carried out according to a non-standard guideline; result is considered of limited validity.
3-methyl-2-butenal [05.124]	UDS	Rat hepatocytes	Oral administration	350 and 700 mg/kg body weight	Negative	(BASF, 2001)	Unpublished GLP study, carried out in accordance with OECD guideline no 486. The study is considered valid.
	Micronucleus test	Mouse bone marrow erythrocytes	Oral administration	175, 350 and 750 mg/kg body weight	Negative	(BASF, 1992c)	Unpublished GLP study, carried out in accordance with OECD guideline (1991). The study is considered valid.

Validity of genotoxicity studies:

1. *Valid.*
2. *Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).*
3. *Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).*
4. *Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).*

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of 42 Phenylethyl Alcohol, Aldehyde, Acid, and Related Acetals and Esters (JECFA, 2003a)

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

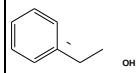
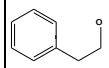
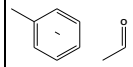
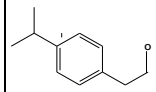
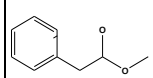
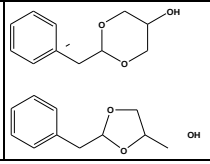
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.019 987	2-Phenylethan-1-ol		1200 330	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
05.030 1002	Phenylacetaldehyde		37 60	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
05.042 1023	p-Tolylacetaldehyde		5.5 3	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
05.044 1024	p-Isopropyl phenylacetaldehyde		0.061 0.01	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
06.006 1003	1,1-Dimethoxy-2-phenylethane		17 40	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
06.007 1004	Phenylacetaldehyde glyceryl acetal		0.12 1	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

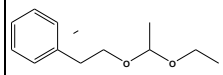
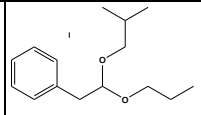
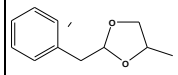
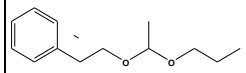
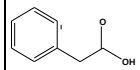
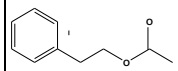
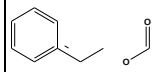
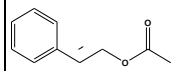
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.016 1000	1-Phenylethoxy-1-propoxy ethane		0.12 6	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
06.024 1006	1,1-Di-isobutoxy-2-phenylethane		27 0.4	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
06.027 1005	4,5-Dimethyl-2-benzyl-1,3-dioxolan		ND 1	Class I A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	No European production volumes available, preventing them to be evaluated using the Procedure. Stereoisomeric composition and composition of mixture to be specified.
06.036 1001	1-Butoxy-1-(2-phenylethoxy)ethane		0.012 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
08.038 1007	Phenylacetic acid		240 60	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.031 989	Phenethyl acetate		89 60	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.083 988	Phenethyl formate		2.1 30	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.137 990	Phenethyl propionate		0.97 3	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

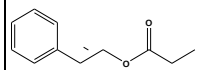
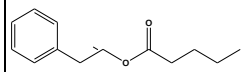
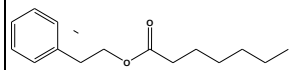
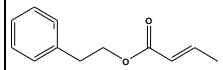
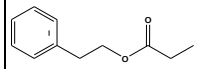
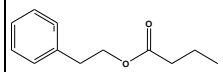
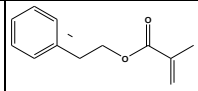
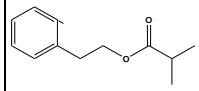
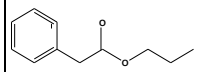
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.168 991	Phenethyl butyrate		28 30	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.261 995	2-Phenethyl hexanoate		12 2	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.262 996	Phenethyl octanoate		23 0.1	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.407 998	2-Phenethyl 3-methylcrotonate		1.3 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.427 992	Phenethyl isobutyrate		19 60	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.466 994	Phenethyl isovalerate		81 30	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.496 997	Phenethyl 2-methylcrotonate		0.24 1	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.538 993	Phenethyl 2-methylbutyrate		0.37 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.702 1010	Propyl phenylacetate		ND 0.3	Class I A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	No European production volumes available, preventing them to be evaluated using the Procedure.

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

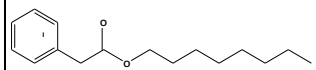
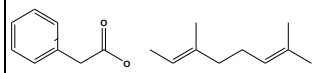
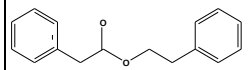
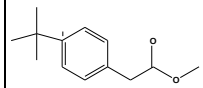
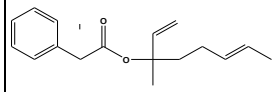
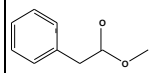
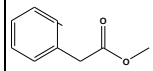
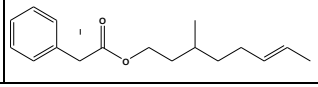
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.703 1017	Octyl phenylacetate		0.0037 0.006	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.704 1020	Geranyl phenylacetate		1.7 2	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	Register name to be changed to (2E)-Geranyl phenylacetate. No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.707 999	Phenethyl phenylacetate		33 80	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.758 1025	Methyl p-tert-butylphenylacetate		17 20	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.772 1019	Linalyl phenylacetate		0.073 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.783 1008	Methyl phenylacetate		ND 20	Class I A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	No European production volumes available, preventing them to be evaluated using the Procedure.
09.784 1009	Ethyl phenylacetate		110 20	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.785 1021	Citronellyl phenylacetate		1.2 2	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

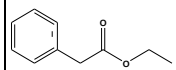
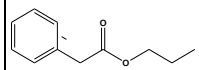
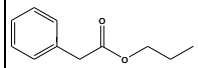
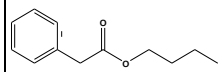
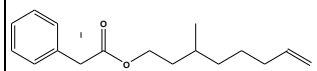
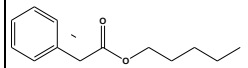
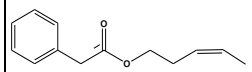
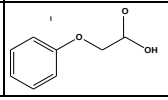
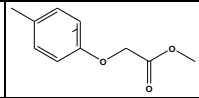
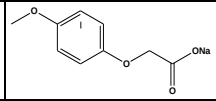
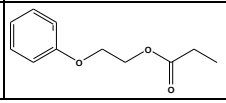
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.786 1011	Isopropyl phenylacetate		0.061 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.787 1012	Butyl phenylacetate		2.4 3	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.788 1013	Isobutyl phenylacetate		18 20	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.789 1014	3-Methylbutyl phenylacetate		28 30	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.791 1018	Rhodinyl phenylacetate		0.0012 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	Register name to be changed to (3S)-Rhodinyl phenylacetate. No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.804 1015	Hexyl phenylacetate		6.9 ND	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
09.805 1016	Hex-3(cis)-enyl phenylacetate		0.73 0.05	Class I A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	Composition of mixture to be specified.
08.049 1026	Phenoxyacetic acid		30 0.1	Class III A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of 41 JECFA Evaluated Substances (JECFA, 2003a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.797 1027	Ethyl (p-tolyloxy)acetate		0.12 ND	Class III A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
16.041 1029	Sodium 2-(4-methoxyphenoxy)propionate		ND 6	Class III A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	No European production volumes available, preventing them to be evaluated using the Procedure.
09.487 1028	2-Phenoxyethyl isobutyrate		1.7 110	Class III A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

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

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

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

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

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

ND: Not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.14Rev1 and One Phenoxyethyl Ester Evaluated in FGE.23Rev1)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

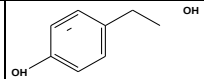
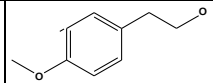
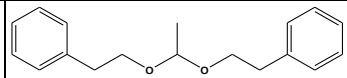
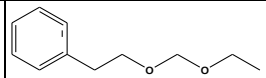
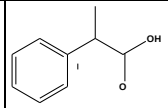
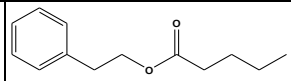
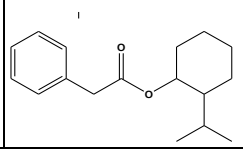
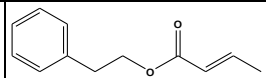
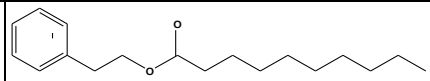
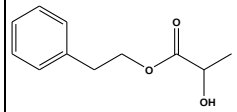
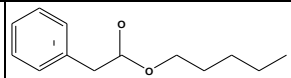
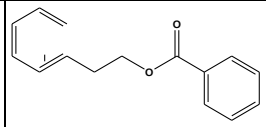
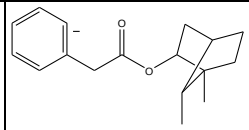
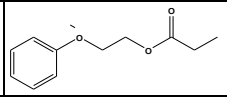
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.166	2-(4-Hydroxyphenyl)ethan-1-ol		0.12	Class I A3: Intake below threshold	4)	6)	
05.159	p-Methoxyphenylacetaldehyde		0.037	Class I A3: Intake below threshold	4)	6)	
06.078	1,1-Diphenethoxyethane	 2-Phenethyl structure shown	0.012	Class I A3: Intake below threshold	4)	6)	
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane		0.012	Class I A3: Intake below threshold	4)	6)	
08.108	2-Phenylpropionic acid		0.0012	Class I A3: Intake below threshold	4)	7)	
09.201	Phenethyl valerate	 2-Phenethyl structure shown	0.012	Class I A3: Intake below threshold	4)	6)	
09.620	Menthyl phenylacetate		1.5	Class I A3: Intake below threshold	4)	6)	

Table 3.2: 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
09.684	Phenethyl crotonate	 2-Phenethyl structure shown	0.73	Class I A3: Intake below threshold	4)	6)	
09.685	2-Phenethyl decanoate		0.037	Class I A3: Intake below threshold	4)	6)	
09.686	Phenethyl lactate	 2-Phenethyl structure shown	0.24	Class I A3: Intake below threshold	4)	6)	
09.761	Pentyl phenylacetate		1.9	Class I A3: Intake below threshold	4)	6)	
09.774	Phenethyl benzoate		33	Class I A3: Intake below threshold	4)	6)	
09.756	Isobornyl phenylacetate		0.012	Class II A3: Intake below threshold	4)	7)	
09.687	2-Phenoxyethyl butyrate		0.085	Class III A3: Intake below threshold	4)	6)	

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

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

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

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

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

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

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

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

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
ID	Identity
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE/NCE	Polychromatic eryhtrocyte/normochromatic erythrocyte ratio
(Q)SAR	(Quantitative) structure-activity relationship
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
SLRL	Sex-linked recessive lethal mutations
TAMDI	Theoretical Added Maximum Daily Intake

UDS	Unscheduled DNA synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation.

European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters (chemical group 15) when used as flavourings for all animal species [1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Chemical group 15 (CG 15) consists of phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters, of which 18 are currently authorised for use as flavours in food. For all 18 compounds belonging to CG 15 the maximum safe concentration was calculated and rounded to be 1 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals. Safe concentrations should be appropriately reduced if used in water for drinking. The absence of a margin of safety would not allow the simultaneous administration of the compounds under consideration in feed and water for drinking. No residues of safety concern derived from the compounds of CG 15 are expected in animal tissues or products when used up to 1–1.5 mg/kg feed. The FEEDAP Panel considered it prudent to treat all compounds under assessment as irritant to skin, eyes and respiratory tract, skin sensitiser and harmful if swallowed. At a concentration of 1–1.5 mg/kg complete feed, the compounds included in CG 15 are not expected to pose a risk for the environment. Since all 18 compounds are used in food as flavourings, and their function in feed is essentially the same as that in food, no further demonstration of efficacy was considered necessary.

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KEY WORDS

Sensory additives, flavourings, phenyl ethyl alcohols, phenyl acetic acids, related esters, phenoxyacetic acids and related esters, chemical group 15, 2-phenylethan-1-ol, phenylacetaldehyde, 1,1-dimethoxy-2-phenylethane, phenylacetic acid, phenethyl acetate, phenethyl formate, phenethyl butyrate, phenethyl octanoate, phenethyl isobutyrate, phenethyl isovalerate, phenethyl 2-methylbutyrate, phenethyl phenylacetate, phenethyl benzoate, methyl phenylacetate, ethyl phenylacetate, isobutyl phenylacetate, 3-methylbutyl phenylacetate, hexyl phenylacetate.

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[1] On request from the European Commission, Question No EFSA-Q-2010-00875, adopted on 7 March 2012. Revision 1 – 06/06/2012: minor editorial changes.

[2] Panel members: Gabriele Aquilina, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Joop de Knecht, Noël Albert Dierick, Mikolaj Antoni Gralak, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, Reinhard Kroker, Lubomir Leng, Secundino López Puente, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Giovanna Martelli, Miklós Mézes, Derek Renshaw, Maria Saarela, Kristen Sejrsen and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of and phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters (chemical group 15) for all animal species. All 18 additives, are currently authorised for use as flavours in food and have all been detected in plant materials or in processed foods, however the reports of their distribution vary greatly.

In the absence of valid toxicological data specific to the compounds belonging to CG 15, the threshold of toxicological concern (TTC) was used to derive safe feed concentrations for these Cramer Class I compounds. For all 18 compounds belonging to CG 15 the maximum safe concentration was calculated and rounded to be 1 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals. Safe concentrations should be appropriately reduced if used in water for drinking. The absence of a margin of safety would not allow the simultaneous administration of the compounds under consideration in feed and water for drinking.

No residues of safety concern derived from the compounds of CG 15 are expected in animal tissues or products when used up to 1–1.5 mg/kg feed.

The FEEDAP Panel considered it prudent to treat all compounds under assessment as irritant to skin, eyes and respiratory tract, skin sensitiser and harmful if swallowed.

At a concentration of 1–1.5 mg/kg complete feed, the compounds included in CG 15 are not expected to pose a risk for the environment.

Since all 18 compounds are used in food as flavourings, and their function in feed is essentially the same as that in food, no further demonstration of efficacy was considered necessary.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7; in addition, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[5] for authorisation of the eighteen substances listed in Table 1, to be used as feed additives for all animal species (category: sensory additives; functional group: flavourings) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 1 July 2010.

The additives are listed as food and feed flavourings in the register of Flavouring substances (CD 217/1999)[7] and in the European Union Register of Feed Additives, respectively. They have not been previously assessed by EFSA for this purpose.

The eighteen substances belonging to CG 15 have been previously assessed by JECFA (2002) and EFSA (2009a and 2009b) as food flavourings.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the active substances listed in Table 1, when used under the conditions described in Table 1.

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[5] Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG), Avenue Louise 130A, B-1050, Brussels, Belgium.

[6] EFSA Dossier reference: FAD-2010-0027.

[7] Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. OJ L 84, 27.3.1999, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Chemical defined flavourings from Chemical Group 15: 1,1-Dimethoxy-2-phenylethane 2-Phenylethan-1-ol 3-Methylbutyl phenylacetate Ethyl phenylacetate Hexyl phenylacetate Isobutyl phenylacetate Methyl phenylacetate Phenethyl 2-methylbutyrate Phenethyl acetate Phenethyl benzoate Phenethyl butyrate Phenethyl formate Phenethyl isobutyrate Phenethyl isovalerate Phenethyl octanoate Phenethyl phenylacetate Phenylacetaldehyde Phenylacetic acid
Registration number/EC No/No (if appropriate)	-
Category(ies) of additive	2. Sensory additives
Functional group(s) of additive	b) flavouring compounds

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
1,1-Dimethoxy-2-phenylethane (CAS-Nr 101-48-4)	$C_{10}H_{14}O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
2-Phenylethan-1-ol (CAS-Nr 60-12-8)	$C_8H_{10}O$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
3-Methylbutyl phenylacetate (CAS-Nr 102-19-2)	$C_{13}H_{18}O_2$	97 %	Gas Chromatography – Mass Spectrometry (GC-MS)
Ethyl phenylacetate (CAS-Nr 101-97-3)	$C_{10}H_{12}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Hexyl phenylacetate (CAS-Nr 5421-17-0)	$C_{14}H_{20}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Isobutyl phenylacetate (CAS-Nr 102-13-6)	$C_{12}H_{16}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Methyl phenylacetate (CAS-Nr 101-41-7)	$C_9H_{10}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl 2-methylbutyrate (CAS-Nr 24817-51-4)	$C_{13}H_{18}O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl acetate (CAS-Nr 103-45-7)	$C_{10}H_{12}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl benzoate (CAS-Nr 94-47-3)	$C_{15}H_{14}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl butyrate (CAS-Nr 103-52-6)	$C_{12}H_{16}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl formate (CAS-Nr 104-62-1)	$C_9H_{10}O_2$	96%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl isobutyrate (CAS-Nr 103-48-0)	$C_{12}H_{16}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)

Phenethyl isovalerate (CAS-Nr 140-26-1)	$C_{13}H_{18}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl octanoate (CAS-Nr 5457-70-5)	$C_{16}H_{24}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenethyl phenylacetate (CAS-Nr 102-20-5)	$C_{16}H_{16}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenylacetaldehyde (CAS-Nr 122-78-1)	C_8H_8O	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Phenylacetic acid (CAS-Nr 103-82-2)	$C_8H_8O_2$	99%	Gas Chromatography – Mass Spectrometry (GC-MS)

Trade name (if appropriate)	-
Name of the holder of authorisation (if appropriate)	-

Conditions of use

Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
All species and categories	-	-	-	-

Other provisions and additional requirements for the labelling

Specific conditions or restrictions for use (if appropriate)	-
Specific conditions or restrictions for handling (if appropriate)	All feedingstuffs and water for drinking, as part of a premixture only
Post-market monitoring (if appropriate)	-
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)

Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

The Chemical Group (CG) 15 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[8] as 'phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters'. The present application concerns 18 compounds, which can be assigned to this CG. The flavours included in this assessment have all been detected in plant materials or in processed foods; however, the reports of their distribution vary greatly. Some are evidently widely distributed (e.g. 2-phenylethan-1-ol, phenyl acetaldehyde) while for others there appears only a single reference to their occurrence in food (e.g. 1,1 dimethoxy-2-phenylethane, iso-butyl phenylacetate).

All compounds except phenethyl benzoate have been assessed by JECFA (2002) and were considered safe for use in food without limit. No Acceptable Daily Intake (ADI) values were specified. Subsequently, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) considered the same compounds for use as food flavourings in Flavouring Group Evaluations 14 and 53 and reached similar conclusions (EFSA, 2009a, 2009b). All 18 compounds are currently listed in the European Union database of flavouring substances and as such authorised for use in food.

A consortium of companies (FFAC) supplying flavours to the feed industry has requested authorisation for the use of the substances listed in Table 2 as additives to feed and water for drinking (category: sensory additives, flavouring compounds) for use in all animal species.

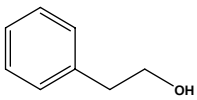
Regulation (EC) No 429/2008[9] allows substances already approved for use in human food to be assessed with a more limited procedure than for other feed additives. However, the use of this procedure is always subject to the condition that food safety assessment is relevant to the use in feed.

2. Characterisation

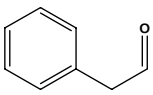
2.1. Characterisation of the flavouring additives

The molecular structures of the additives under application are summarized in Figure 1, their physico-chemical characteristics in Table 2.

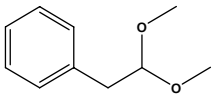
2-Phenylethan-1-ol (02.019)



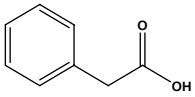
Phenylacetaldehyde (05.030)



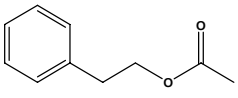
1,1-Dimethoxy-2-phenylethane (06.006)



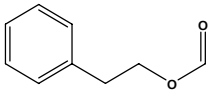
Phenylacetic acid (08.038)



Phenethyl acetate (09.031)



Phenethyl formate (09.083)



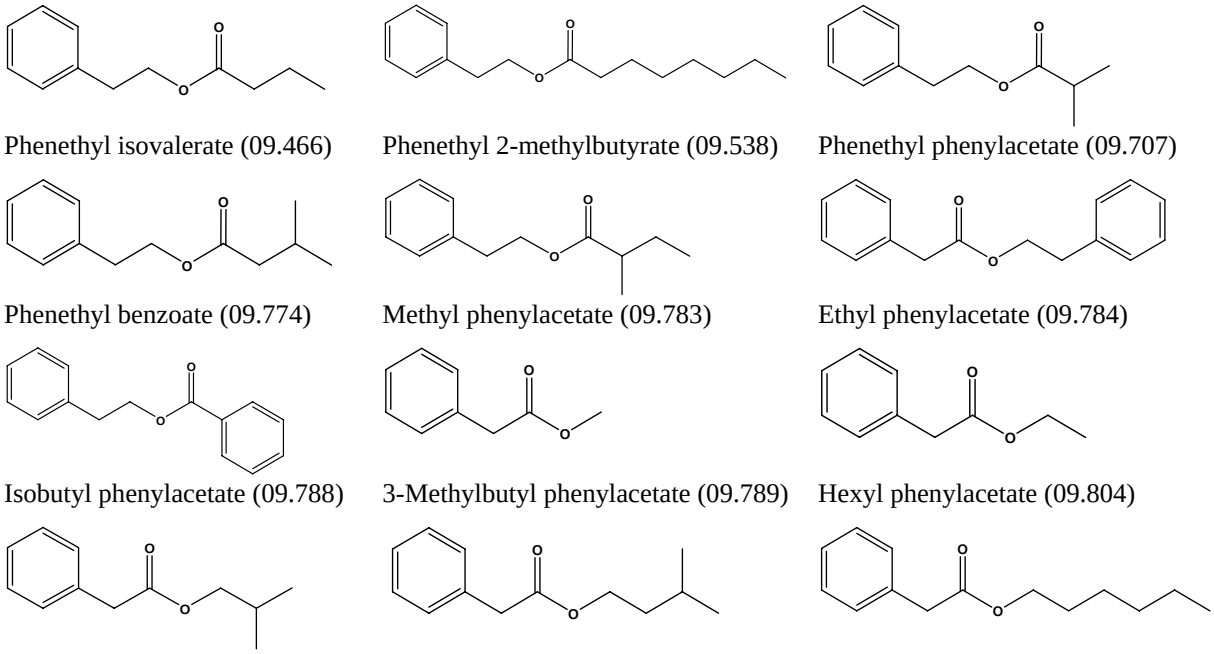
Phenethyl butyrate (09.168)

Phenethyl octanoate (09.262)

Phenethyl isobutyrate (09.427)

[8] Commission Regulation (EC) 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 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1-65.



Phenethyl isovalerate (09.466) | Phenethyl 2-methylbutyrate (09.538) | Phenethyl phenylacetate (09.707)

Phenethyl benzoate (09.774) | Methyl phenylacetate (09.783) | Ethyl phenylacetate (09.784)

Isobutyl phenylacetate (09.788) | 3-Methylbutyl phenylacetate (09.789) | Hexyl phenylacetate (09.804)

Figure 1: Molecular structures and FLAVIS numbers of flavourings from CG 15

Table 2: Physico-chemical properties of the chemically defined flavourings from CG 15 under application

EU Register name	CAS No.	Flavis No.	Molecular formula	Molecular weight	Physical status	Log K_{ow}
2-Phenylethan-1-ol	60-12-8	02.019	$C_8H_{10}O$	122.17	Liquid	1.36
Phenylacetaldehyde	122-78-1	05.030	C_8H_8O	120.15	Oily liquid	1.78
1,1-Dimethoxy-2-phenylethane	101-48-4	06.006	$C_{10}H_{14}O_2$	166.22	Liquid	2.07
Phenylacetic acid	103-82-2	08.038	$C_8H_8O_2$	136.15	Solid	1.41
Phenethyl acetate	103-45-7	09.031	$C_{10}H_{12}O_2$	164.20	Liquid	2.3
Phenethyl formate	104-62-1	09.083	$C_9H_{10}O_2$	150.18	Liquid	1.96
Phenethyl butyrate	103-52-6	09.168	$C_{12}H_{16}O_2$	192.26	Liquid	3.36
Phenethyl octanoate	5457-70-5	09.262	$C_{16}H_{24}O_2$	248.37	Oily liquid	5.49
Phenethyl isobutyrate	103-48-0	09.427	$C_{12}H_{16}O_2$	192.26	Liquid	3.13
Phenethyl isovalerate	140-26-1	09.466	$C_{13}H_{18}O_2$	206.29	Liquid	3.71
Phenethyl 2-methylbutyrate	24817-51-4	09.538	$C_{13}H_{18}O_2$	206.29	Liquid	3.71
Phenethyl phenylacetate	102-20-5	09.707	$C_{16}H_{16}O_2$	240.30	Liquid/solid	4.28
Phenethyl benzoate	94-47-3	09.774	$C_{15}H_{14}O_2$	226.28	Liquid	4.01
Methyl phenylacetate	101-41-7	09.783	$C_9H_{10}O_2$	150.18	Liquid	1.83
Ethyl phenylacetate	101-97-3	09.784	$C_{10}H_{12}O_2$	164.20	Liquid	2.28
Isobutyl phenylacetate	102-13-6	09.788	$C_{12}H_{16}O_2$	192.26	Liquid	3.48
3-Methylbutyl phenylacetate	102-19-2	09.789	$C_{13}H_{18}O_2$	206.29	Liquid	3.87
Hexyl phenylacetate	5421-17-0	09.804	$C_{14}H_{20}O_2$	220.31	Oily liquid	4.62

All 18 substances are produced by chemical synthesis. Typically several routes of synthesis are available and described in the dossier.[10]

Data was provided on the batch to batch variation in five batches of each additive (with the exception of hexyl phenylacetate where four batches were available).[11] The content of the active substance exceeded the JECFA specifications (Combined Compendium of Food Additives Specifications; JECFA, 2006) for all compounds (Table 3).

Table 3: Purity of the chemically defined flavourings from CG 15 under application

EU Register name	JECFA specification %	Assay %	
		Average	Range
2-Phenylethan-1-ol	> 98	99.7	99.2–99.8
Phenylacetaldehyde	> 95	99.3	98.0–99.8
1,1-Dimethoxy-2-phenylethane	> 95	99.6	98.3–100
Phenylacetic acid	> 99	99.7	99.2–100
Phenethyl acetate	> 98	99.5	99.2–99.9
Phenethyl formate	> 96	98.6	97.9–99.8
Phenethyl butyrate	> 97	99.8	99.5–100
Phenethyl octanoate	> 98	99.3	98.9–100
Phenethyl isobutyrate	> 98	99.2	98.4–100
Phenethyl isovalerate	> 97	99.4	99.2–99.6
Phenethyl 2-methylbutyrate	> 95	99.5	99.1–99.7
Phenethyl phenylacetate	> 98	99.2	98.0–99.9
Phenethyl benzoate	> 98	99.6	99.4–99.6
Methyl phenylacetate	> 97	99.9	99.9-100
Ethyl phenylacetate	> 97	99.9	99.9–100
Isobutyl phenylacetate	> 98	99.7	99.1–99.9
3-Methylbutyl phenylacetate	> 97[(a)]	99.8	99.3–100
Hexyl phenylacetate	> 97	99.6	99.4–99.8

(a): sum of n-amyl and isoamyl esters

Potential contaminants are considered as part of the product specification and are monitored as part of the HACCP procedure applied by all consortium members. The parameters considered include residual solvents, heavy metals and other undesirable substances.

2.2. Stability and homogeneity

A shelf life of at least 12 to 38 months is given for the 18 chemicals when stored in closed containers under recommended conditions (in a cool and dry place).[12] This assessment is made on the basis of compliance with the original specification after storage.

Although no data is required for the stability of volatile additives in premixes and feed, use in water for drinking introduces other issues relating to product stability, such as degradation due to microbial activity. As no data on the short term stability of the additive in water for drinking were provided, the FEEDAP Panel is not in the position to comment on this route of administration.

2.3. Conditions of use

The applicant proposes the use of the 18 additives in feed or water for drinking for all animal species without withdrawal. In each case the applicant proposes a normal use level and a high use level of five times the normal level in feed as shown in Table 4.

[10] Technical dossiers/Section II.
[11] Technical dossiers/Section II/Annex 2.1 and Supplementary Information June 2011.
[12] Technical dossier/Supplementary information June 2011.

No specific proposals are made for doses to be used in water for drinking. However the FEEDAP Panel notes that 13 out of the18 compounds are described as insoluble in water, and four compounds are considered slightly soluble.

Table 4: Proposed dose for feed use

EU Register name	Feed concentration (mg/kg)	
	Normal	High
2-Phenylethan-1-ol	5	25
Phenylacetaldehyde	1	5
1,1-Dimethoxy-2-phenylethane	1	5
Phenylacetic acid	5	25
Phenethyl acetate	1	5
Phenethyl formate	1	5
Phenethyl butyrate	1	5
Phenethyl octanoate	1	5
Phenethyl isobutyrate	1	5
Phenethyl isovalerate	5	25
Phenethyl 2-methylbutyrate	1	5
Phenethyl phenylacetate	1	5
Phenethyl benzoate	1	5
Methyl phenylacetate	1	5
Ethyl phenylacetate	1	5
Isobutyl phenylacetate	1	5
3-Methylbutyl phenylacetate	1	5
Hexyl phenylacetate	1	5

2.4. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of 2-phenylethan-1-ol, phenylacetaldehyde, 1,1-dimethoxy-2-phenylethane, phenylacetic acid, phenethyl acetate, phenethyl formate, phenethyl butyrate, phenethyl octanoate, phenethyl isobutyrate, phenethyl isovalerate, phenethyl 2-methylbutyrate, phenethyl phenylacetate, phenethyl benzoate, methyl phenylacetate, ethyl phenylacetate, isobutyl phenylacetate, 3-methylbutyl phenylacetate, and hexyl phenylacetate in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

The assessment of safety is based on the high use level proposed by the applicant (25 mg/kg complete feed for 2-phenylethanol, phenylacetic acid and phenethyl isovalerate and 5 mg/kg complete feed for the other additives, see Table 4).

3.1. Safety for the target species

The first approach to the safety assessment for target species takes account of the applied use levels in animal feed relative to the maximum reported exposure of humans on the basis of the metabolic body weight. The data for human exposure in the EU (EFSA, 2009a and 2009b) range between 0.37 and 1200 µg/person/day. Table 5 summarises the result of the comparison with human exposure for representative target animals. The body weight of target animals is taken from the default values shown in Table 7.

Table 5: Comparison of exposure of humans and target animals to the flavourings under application

Flavouring	Use level in feed (mg/kg)	Human exposure µg/mbw ($kg^{0.75}$)/day*	Target animal exposure µg/mbw ($kg^{0.75}$)/day		
			Salmon	Piglet	Dairy cow
2-Phenylethan-1-ol	25	55.74	588	2632	3885
Phenylacetaldehyde	5	1.72	118	526	777
1,1-Dimethoxy-2-phenylethane	5	0.79	118	526	777
Phenylacetic acid	25	11.13	588	2632	3885
Phenethyl acetate	5	4.13	118	526	777
Phenethyl formate	5	0.10	118	526	777
Phenethyl butyrate	5	1.30	118	526	777
Phenethyl octanoate	5	1.07	118	526	777
Phenethyl isobutyrate	5	0.88	118	526	777
Phenethyl isovalerate	25	3.76	588	2632	3885
Phenethyl 2-methylbutyrate	5	0.02	118	526	777
Phenethyl phenylacetate	5	1.53	118	526	777
Phenethyl benzoate	5	1.53	118	526	777
Methyl phenylacetate	5	4.40**	118	526	777
Ethyl phenylacetate	5	5.10	118	526	777
Isobutyl phenylacetate	5	0.83	118	526	777
3-Methylbutyl phenylacetate	5	1.30	118	526	777
Hexyl phenylacetate	5	0.32	118	526	777

* mbw = metabolic body weight ($kg^{0.75}$) for a 60 kg person = 21.6; ** EU figures not available (exposure based on US intake figures)

The data in Table 5 clearly indicate that the intake by the target animals greatly exceeds that of humans, resulting from use in food for all 18 compounds. As a consequence, safety for the target species at the feed concentration applied cannot be derived from the risk assessment for food use.

As an alternative the maximum feed concentration which can be considered as safe for the target animal is derived from the lowest No Observed Adverse Effect Level (NOAEL) found. Toxicological data could only be found for three out of 18 compounds, none of which was considered suitable to derive a NOAEL either for the individual compounds or the group (reviewed in EFSA, 2009a).

In the absence of any valid toxicological data specific to the compounds belonging to CG 15, the threshold of toxicological concern (TTC) was used to derive maximum safe feed concentrations for these Cramer Class I compounds. This provides values of 1.0 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals.

3.1.1. Conclusions on the safety for target species

For all 18 compounds belonging to CG 15 the maximum safe concentration was calculated and rounded to be 1 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals.

Safe concentrations should be appropriately reduced if used in water for drinking. The absence of a margin of safety would not allow the simultaneous administration of the compounds under consideration in feed and water for drinking.

3.2. Safety for the consumer

The safety for the consumer of all 18 compounds used as food flavours has already been assessed by the CEF Panel (EFSA, 2009a, 2009b) and JECFA (2002). All 18 compounds are currently authorised as food additives without limitations.

As the intake of all 18 compounds by target animals exceeds by one to three orders of magnitude that of humans resulting from use in food, the potential transfer of significant amounts of residues in edible tissues and products has to be considered.

In its evaluation of this chemical group, JECFA identified that phenethyl and phenylacetate esters and phenylacetaldehyde acetals are rapidly hydrolysed *in vivo* to yield 2-phenylethan-1-ol, phenylacetic acid and phenylacetaldehyde, respectively. Phenylacetaldehyde and 2-phenylethan-1-ol are both oxidised to phenylacetic acid, which is conjugated and excreted primarily in the urine. Therefore, most of the flavouring agents in this group will be hydrolysed and/or oxidised to yield phenylacetic acid that is excreted either free or in conjugated form (JECFA, 2002).

The enzymes involved in the metabolism of the CG 15 compounds to phenylacetic acid, i.e. alcohol dehydrogenases, aldehyde dehydrogenases and esterases, can be found in mammals, birds and fish. Phenylacetic acid is a metabolite of aromatic amino acids and an endogenous product of mammals, fish (Di Giulio and Hinton, 2008) and birds (Pan and Fouts, 1978). The types of conjugates formed from phenylacetic acid are both dose-dependent and species-specific. The major metabolic pathways for phenylacetic acid are conjugation with glucuronic acid, glutamine (man), glycine (rat, rabbit, cat and dog), taurine (dogfish, winter flounder and pigeon), ornithine (laying hen), or elimination as the free acid (EFSA 2009a).

Phenethyl benzoate is expected to be hydrolysed to the corresponding carboxylic acid and alcohol (benzoic acid and 2-phenylethan-1-ol), benzoic acid being metabolised via common pathways already described (EFSA 2009a).

3.2.1. Conclusions on the safety for the consumer

Published data demonstrate that the chemicals of CG 15 are rapidly absorbed, distributed, metabolised and excreted. Mammals, birds and fish share a similar metabolic capacity to handle these compounds and produce hydrophilic metabolites with low affinity for tissues which are excreted efficiently in the urine. The similarity of metabolic fate of these substances in the target animal and laboratory animals ensures that the consumer is exposed to the same residues as the laboratory animals used in toxicological studies. No ADI has been specified for these compounds. No residues of safety concern derived from the compounds of CG 15 when used up to the highest safe level in feed are to be expected in animal tissues or products.

3.3. Safety for the user

No experimental data on the safety for the user was provided. Potential hazards for skin and eyes contact and respiratory exposure are recognised (material safety data sheets) for eight out of the 18 compounds. Six compounds (2-phenylethan-1-ol, phenylacetic acid, isobutyl phenylacetate, phenethyl acetate, phenethyl phenylacetate and 3-methylbutyl phenylacetate) are classified as irritating to eyes and skin. Phenyl acetaldehyde may cause skin sensitisation, and 2-phenyl-1-ethanol is considered harmful in contact with skin and if swallowed. Two compounds (phenylacetic acid and isobutyl phenylacetate) are identified as 'irritating to the respiratory system'. For the remaining substances, no hazards are identified, probably because the substances have not yet been tested.

The FEEDAP Panel considers it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, skin sensitisers and harmful if swallowed.

3.4. Safety for the environment

As indicated in section 3.2, all the flavouring agents in CG 15 are expected to be metabolised in the target species to yield phenylacetic acid that is excreted either free or in conjugated form. The

available experimental data indicate that phenylacetic acid will rapidly degrade in the environment. The K_{OC} values are between 26–31, which suggest a very high mobility in soil (HSDB).

Based on a dose of 1–1.5 mg/kg considered safe for all target species, the predicted environmental concentration (PEC) soil will not be higher than 6 µg/kg, assuming that manure will be ploughed into arable soil or will be applied in several spreading events onto grassland. Using the screening method the maximum PEC surface water will be 3 µg/L. Considering the rapid degradability in soil, the groundwater concentration is not expected to exceed 0.1 µg/L.

The acute toxicity of phenylacetic acid to the algae *Scenedesmus subspicatus*, and the crustaceans *Daphnia magna* and *Thamnocephalus platyurus* is 177, 140 and 277 mg/L, respectively (Šepič et al., 2003). Based on these data the PNEC for aquatic species is 140 µg/L applying an assessment factor of 1000. No data are available for terrestrial non-target species. Using the equilibrium approach concentrations < 90 µg/kg would be safe for terrestrial species. Therefore, at a concentration of 1–1.5 mg/kg complete feed, the compounds included in CG 15 are not expected to pose a risk for the environment.

4. Efficacy

Since all 18 compounds are used in food as flavourings, and their function in feed is essentially the same as that in food no further demonstration of efficacy is necessary.

CONCLUSIONS

For all 18 compounds belonging to CG 15 the maximum safe concentration was calculated and rounded to be 1 mg/kg feed for poultry and pigs and 1.5 mg/kg feed for cattle, salmonids and non food-producing animals. Safe concentrations should be appropriately reduced if used in water for drinking. The absence of a margin of safety would not allow the simultaneous administration of the compounds under consideration in feed and water for drinking.

No residues of safety concern derived from the compounds of CG 15 are expected in animal tissues or products when used up to 1-1.5 mg/kg feed.

The FEEDAP Panel considers it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, skin sensitisers and harmful if swallowed.

At a concentration of 1–1.5 mg/kg complete feed, the compounds included in CG 15 are not expected to pose a risk for the environment.

Since all 18 compounds are used in food as flavourings, and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

DOCUMENTATION PROVIDED TO EFSA

1. Chemically defined flavourings from Flavouring Group 15 - Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters (CDG 15). May 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

2. Chemically defined flavourings from Flavouring Group 15 - Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters (CDG 15). Supplementary information. June 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

3. Chemically defined flavourings from Flavouring Group 15 - Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters (CDG 15). Supplementary information. September 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – Group 15 (CDG15 Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters).

5. Comments from Member States received through the ScienceNet.

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Šepič E, Bricelj M and Leskovšec H, 2003. Toxicity of fluoranthene and its biodegradation metabolites to aquatic organisms. Chemosphere, 52, 1125-1133.

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Chemically Defined Flavourings – Group 15 (CDG15 Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters)[13]

The Chemically Defined Flavourings - Group 15 (CDG15 Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters), in this application comprises 18 substances, for which authorisation as feed additives is sought under the category "sensory additives", functional group 2(b) "flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

In the current application submitted according to Article 4(1) and Article 10 (2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. The flavouring compounds of interest have a purity ranging from 95% to 99%.

Mixtures of flavouring compounds are intended to be incorporated only into feedingstuffs or drinking water. The Applicant suggested no minimum or maximum levels for the different flavouring compounds in feedingstuffs.

For the identification of volatile chemically defined flavouring compounds CDG15 in the feed additive, the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method, using Retention Time Locking (RTL), which allows a close match of retention times on GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant maintained two FLAVOR2 databases/libraries (for retention times and for MS spectra) containing data for more than 409 flavouring compounds. These libraries were provided to the CRL. The Applicant provided the typical chromatogram for the CDG15 of interest.

In order to demonstrate the transferability of the proposed analytical method (relevant for the method verification), the Applicant prepared a model mixture of flavouring compounds on a solid carrier to be identified by two independent expert laboratories. This mixture contained twenty chemically defined flavourings belonging to twenty different chemical groups to represent the whole spectrum of compounds in use as feed flavourings with respect to their volatility and polarity. Both laboratories properly identified all the flavouring compounds in all the formulations. Since the substances of CDG15 are within the volatility and polarity range of the model mixture tested, the Applicant concluded that the proposed analytical method is suitable to determine qualitatively the presence of the substances from CDG15 in the mixture of flavouring compounds.

Based on the satisfactory experimental evidence provided, the CRL recommends for official control for the qualitative identification in the feed additive of the individual (or mixture of) flavouring compounds of interest listed in Table 1 (*) the GC-MS-RTL (Agilent specific) method submitted by the Applicant.

As no experimental data were provided by the Applicant for the identification of the active substance(s) in feedingstuffs and water, no methods could be evaluated. Therefore the CRL is unable to recommend a method for the official control to identify the active substance(s) of interest listed in Table 1 (*) in feedingstuffs or water.

[13] The full report is available on the EURL website http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0027.pdf

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

(*)Full list provided in CRL evaluation report, available from the CRL website.

V201549 PHENYL ACETIC ACID

Safety Data Sheet

OSHA Hazard Communication Standard 29 CFR 1910.1200 (HazCom 2012)

Issue date: 04/30/2015 Revision date: 09/27/2022

SECTION 1: Identification

1.1. Identification

Substance name : V201549 PHENYL ACETIC ACID

CAS-No. : 103-82-2

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use of the substance/mixture : Industrial uses: Uses of substances as such or in preparations* at industrial sites

1.3. Details of the supplier of the safety data sheet

Berje
700 Blair Road
Carteret, NJ 07008 - USA
T 973-748-8980 - F 973-680-9618
berje@berjeinc.com - www.berjeinc.com

1.4. Emergency telephone number

Emergency number : 1-800-424-9300 24 Hours Emergency Chemtrec. ERR - CCN2624

SECTION 2: Hazard(s) identification

2.1. Classification of the substance or mixture

GHS-US classification

Eye Irrit. 2 H319 - Causes serious eye irritation.

Full text of H-statements: see section 16

2.2. Label elements

GHS US labelling

Hazard pictograms (GHS US) :



GHS07

Signal word (GHS US) : Warning

Hazard statements (GHS US) : H319 - Causes serious eye irritation.

Precautionary statements (GHS US) : P264 - Wash hands thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 - If eye irritation persists: Get medical advice/attention.

2.3. Other hazards

2.4. Unknown acute toxicity (GHS US)

SECTION 3: Composition/information on ingredients

3.1. Substances

Name	Product identifier	%	GHS-US classification
V201549 PHENYL ACETIC ACID (Main constituent)	(CAS-No.) 103-82-2	100	Eye Irrit. 2, H319

Full text of H- and EUH-statements: see section 16

3.2. Mixtures

Not applicable

SECTION 4: First aid measures

4.1. Description of first aid measures

First-aid measures after inhalation : Remove person to fresh air and keep comfortable for breathing. Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. Remove victim to fresh air. Respiratory problems: consult a doctor/medical service.

First-aid measures after skin contact : If irritation persists, consult a doctor. Do not apply (chemical) neutralizing agents. Wash with plenty of water/…. Wash contaminated clothing before reuse. If skin irritation occurs: Get medical advice/attention. Specific treatment (see supplemental first aid instruction on this label). Wash skin with plenty of water.

First-aid measures after eye contact : Rinse immediately with plenty of water for 15 minutes. If eye irritation persists: Get medical advice/attention. Do not apply (chemical) neutralizing agents. Contact ophthalmologist immediately. Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

First-aid measures after ingestion : Call a poison center or a doctor if you feel unwell.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/effects after skin contact : Causes skin irritation. Tingling/irritation of the skin.

Symptoms/effects after eye contact : Causes serious eye irritation. Irritation of the eye tissue. Eye irritation.

4.3. Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media : Water spray. Dry powder. Foam.

5.2. Special hazards arising from the substance or mixture

Reactivity : The product is non-reactive under normal conditions of use, storage and transport.

5.3. Advice for firefighters

Protection during firefighting : Do not attempt to take action without suitable protective equipment. Self-contained breathing apparatus. Complete protective clothing.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

6.1.1. For non-emergency personnel

Emergency procedures : Ventilate spillage area. Avoid contact with skin and eyes.

6.1.2. For emergency responders

Protective equipment : Do not attempt to take action without suitable protective equipment. For further information refer to section 8: "Exposure controls/personal protection".

6.2. Environmental precautions

Avoid release to the environment.

6.3. Methods and material for containment and cleaning up

Methods for cleaning up : Mechanically recover the product.

Other information : Dispose of materials or solid residues at an authorized site.

6.4. Reference to other sections

For further information refer to section 13.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling : Ensure good ventilation of the work station. Avoid contact with skin and eyes. Wear personal protective equipment.

Hygiene measures : Wash hands thoroughly after handling. Do not eat, drink or smoke when using this product. Always wash hands after handling the product.

7.2. Conditions for safe storage, including any incompatibilities

Storage conditions : Store in a well-ventilated place. Keep cool.

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

8.2. Exposure controls

Appropriate engineering controls	:	Ensure good ventilation of the work station.
Hand protection	:	Protective gloves.
Eye protection	:	Safety glasses.
Skin and body protection	:	Protective clothing. Wear suitable protective clothing. Dustproof clothing.
Respiratory protection	:	In case of insufficient ventilation, wear suitable respiratory equipment.
Environmental exposure controls	:	Avoid release to the environment.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	:	Solid
Colour	:	No data available
Odour	:	No data available
Odour threshold	:	No data available
pH	:	No data available
Melting point	:	No data available
Freezing point	:	Not applicable
Boiling point	:	No data available
Flash point	:	> 200 °F (Closed cup)
Relative evaporation rate (butylacetate=1)	:	No data available
Flammability (solid, gas)	:	No data available
Explosive limits	:	Not applicable
Explosive properties	:	No data available
Oxidising properties	:	No data available
Vapour pressure	:	0.004 mm Hg 25°C
Relative density	:	(25 °C)
Relative vapour density at 20 °C	:	No data available
Solubility	:	No data available
Partition coefficient n-octanol/water (Log Pow)	:	No data available
Auto-ignition temperature	:	Not applicable
Decomposition temperature	:	No data available
Viscosity	:	No data available
Viscosity, kinematic	:	Not applicable
Viscosity, dynamic	:	No data available

9.2. Other information

SECTION 10: Stability and reactivity

10.1. Reactivity

The product is non-reactive under normal conditions of use, storage and transport.

10.2. Chemical stability

Stable under normal conditions.

10.3. Possibility of hazardous reactions

No dangerous reactions known under normal conditions of use.

10.4. Conditions to avoid

None under recommended storage and handling conditions (see section 7).

10.5. Incompatible materials

No additional information available

10.6. Hazardous decomposition products

Under normal conditions of storage and use, hazardous decomposition products should not be produced.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity	:	Not classified
Skin corrosion/irritation	:	Not classified
Serious eye damage/irritation	:	Causes serious eye irritation.
Respiratory or skin sensitisation	:	Not classified
Germ cell mutagenicity	:	Not classified
Carcinogenicity	:	Not classified
Reproductive toxicity	:	Not classified
STOT-single exposure	:	Not classified
STOT-repeated exposure	:	Not classified
Aspiration hazard	:	Not classified
Symptoms/effects after skin contact	:	Causes skin irritation. Tingling/irritation of the skin.
Symptoms/effects after eye contact	:	Causes serious eye irritation. Irritation of the eye tissue. Eye irritation.

SECTION 12: Ecological information

12.1. Toxicity

Ecology - general : The product is not considered harmful to aquatic organisms nor to cause long-term adverse effects in the environment.

12.2. Persistence and degradability

No additional information available

12.3. Bioaccumulative potential

No additional information available

12.4. Mobility in soil

No additional information available

12.5. Other adverse effects

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Waste treatment methods : Dispose of contents/container in accordance with licensed collector's sorting instructions.

SECTION 14: Transport information

Department of Transportation (DOT)

In accordance with DOT

Not regulated for transport

Transportation of Dangerous Goods

Transport by sea

Air transport

SECTION 15: Regulatory information

15.1. US Federal regulations

V201549 PHENYL ACETIC ACID (103-82-2)
Listed on the United States TSCA (Toxic Substances Control Act) inventory

15.2. International regulations

CANADA

EU-Regulations

National regulations

No additional information available

15.3. US State regulations

No additional information available

SECTION 16: Other information

Revision date : 09/27/2022

Full text of H-statements:

H319	Causes serious eye irritation.

Hazard Rating

Health : 1 Slight Hazard - Irritation or minor reversible injury possible

Flammability : 1 Slight Hazard - Materials that must be preheated before ignition will occur. Includes liquids, solids and semi solids having a flash point above 200 F. (Class IIIB)

Physical : 0 Minimal Hazard - Materials that are normally stable, even under fire conditions, and will NOT react with water, polymerize, decompose, condense, or self-react. Non-Explosives.

Personal protection : B

B - Safety glasses, Gloves

SDS US (Berje) 5.2

This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.