Toxicological profile for

Ethyl levulinate

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

Ethyl 4-oxopentanoate (PubChem).

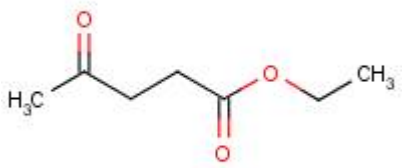
1.2. Synonyms

4-03-00-01562 (Beilstein Handbook Reference); AI3-00677; BRN 0507641; EINECS 208-728-2; Ethyl 3-acetylpropionate; Ethyl 4-ketovalerate; Ethyl 4-oxopentanoate; Ethyl 4-oxovalerate; Ethyl acetylpropanoate; Ethyl ketovalerate; Ethyl laevulinate; Ethyl levulate; Ethyl levulinate; Ethyl levulinate (natural); FEMA No. 2442; Levulinic acid, ethyl ester; NSC 24876; Pentanoic acid, 4-oxo-, ethyl ester; UNII-7BU24CSS2G (ChemIDplus).

1.3. Molecular formula

$C_7H_{12}O_3$

1.4. Structural Formula



1.5. Molecular weight (g/mol)

144.17

1.6. CAS registration number

539-88-8

1.7. Properties

1.7.1. Melting point

(°C): <25 (ChemIDplus; ChemSpider; EPISuite, 2017); -7.52 (estimated) (EPISuite, 2017); < -60 (GESTIS)

1.7.2. Boiling point

(°C): 203-205.8 (ChemIDplus; ChemSpider; EPISuite, 2017).

1.7.3. Solubility

4.57e+04 mg/L at 25°C (estimated) (ChemIDplus; EPISuite, 2017); “soluble in water” (PubChem)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 94 or 195 (ChemSpider)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): 425 (GESTIS)

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

0.208 mm Hg at 25°C (EPISuite, 2017).

1.7.11. log Kow

0.4 (ChemSpider); 0.299 (ChemSpider)

2. General information

2.1. Exposure

Cosmetics	Yes	Food	Yes (Burdock GA, 2010)
Environment	No evidence	Pharmaceuticals	No evidence
In tobacco naturally	No evidence	In the burned part	Yes

Estimated intake from use as a flavouring agent in Europe is 470 µg/person/day (EFSA, 2012).

Upper levels for use as a flavouring are 5 and 10 mg/kg in beverages and food, respectively (CoE, 2000).

Used as a perfuming ingredient in cosmetics in the EU. As taken from CosIng.

Reported as used in fragrance compounds (IFRA; US EPA InertFinder Database, 2022).

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.	Food Category	Usual	Max.
Alcoholic beverages	4.99	49.89	Gelatin, pudding	8.75	51.02
Baked Goods	9.28	48.50	Nonalcoholic beverages	2.53	11.28
Frozen dairy	9.19	48.53	Soft candy	7.54	28.76

Reported individual intake from use as a flavouring: 0.001370 mg/kg bw/day [about 96 µg/person/day].

As taken from Burdock, 2010

Ethyl levulinate (CAS RN 539-88-8) is used as a flavour enhancer in oral non-medicinal natural health products (Health Canada, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

COMPOUND	TWO STAGE HEATING		ONE STAGE HEATING	
	ABUNDANCE	AREA%	ABUNDANCE	AREA%
ethyl levulinate	3639662180	95.16	4076893014	94.89
butyl levulinate	142529823	3.73	176358254	4.11
Total ion chromatogram	3821174879	100	4290955085	100

2.3. Ingredient(s) from which it originates

“Usually obtained from direct esterification of levulinic acid with ethanol and H2SO4, in benzene or in toluene; also in presence of HCl in ethanol; other methods, starting with fructose, glucose and others are of less importance” (Burdock GA, 2010).

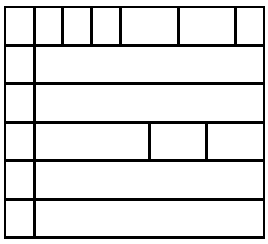
Reported to occur naturally in white bread, roasted onion, wheat bread, cognac, malt whiskey, grape wines, cocoa, bilberry wine, bourbon, vanilla and cherimoya.

As taken from Burdock, 2010.

Natural occurrence in cherimoya, onion, white bread, rum wine and cocoa (CoE, 2000).

Ethyl levulinate is reported to be found in onions, garlic, white bread, wine and cocoa (CIVO-TNO, 1977).

3. Status in legislation and other official guidance



It was given GRAS status by FEMA (Hall and Oser, 1965).

JECFA (2000) considered there to be no safety concern at the current levels of intake of ethyl levulinate when used as a flavouring agent. No precise estimate of daily intake was provided, but overall intake levels for all 47 flavouring substances being considered by the Committee at that time (including aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups) were estimated at 28 and 300 mg/person/day in Europe and the USA respectively.

A website reports that the US Government has approved the use of ethyl levulinate as a tobacco additive (Anon).

Summary of evaluations performed by the joint FAO/WHO expert committee on food additives Ethyl levulinate	
Coe no.:	373
Fema no.:	2442
Jecfa no.:	607
Chemical names:	Ethyl 4-oxovalerate
Synonyms:	Ethyl acetylpropanoate; ethyl gamma-ketovalerate; ethyl laevulate; ethyl 4-oxopentanoate
Functional class:	Flavouring agent
Latest evaluation:	1999
Adi:	Acceptable
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	Trs 896-jecfa 53/67
Specifications:	Compendium addendum 7/fnp 52 add.7/128
Tox monograph:	Fas 44-jecfa 53/229

As taken from JECFA, 2001. Evaluation of Ethyl Levulinate available at http://www.inchem.org/documents/jecfa/jeceval/jec_780.htm

Ethyl levulinate is included on the 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).

There is a REACH dossier for ethyl 4-oxovalerate (CAS RN 539-88-8) (ECHA, 2021a).

Ethyl 4-oxovalerate (CAS RN 539-88-8) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Ethyl levulinate is listed in the US EPA InertFinder Database (2022) as approved for fragrance use pesticide products.

Pentanoic acid, 4-oxo-, ethyl ester (CAS RN 539-88-8) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and 2020 CDR TSCA list.

The TSCA and 2020 CDR TSCA inventory are available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/advancedsearch/externalSearch.do?p_type=SRSITN&p_value=51755

Ethyl levulinate (CAS RN 539-88-8) is classified by the Environmental Protection Authority of New Zealand (NZ EPA CCID) and pentanoic acid, 4-oxo-, ethyl ester (CAS RN 539-88-8) is included on the New Zealand Inventory of Chemicals and does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006).

Ethyl 4-oxovalerate (CAS RN 539-88-8) 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 (European Commission, 2012).

Pentanoic acid, 4-oxo-, ethyl ester (Associated names: Ethyl levulinate), CAS 539-88-8 is listed on the Australian Inventory of Industrial Chemicals (AICIS, formerly NICNAS). As taken from AICIS, undated.***4. Metabolism/Pharmacokinetics***

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“Twenty-eight substances in this group [including ethyl levulinate] are esters or diesters, including one cyclic diester, which are expected to undergo hydrolysis to their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols or branched-chain hydroxy or keto alcohols) and acid components (alpha, beta-, or gamma-keto or hydroxy acids or simple aliphatic acids, diacids, or triacids), which would be further metabolized. Hydrolysis occurs in the intestinal tract, blood, and liver and in most tissues and is catalysed by carboxylesterases or esterases, the most important of which are the β-esterases” (JECFA, 2000).

“Small amounts of gamma-hydroxy and gamma-keto acids and related substances [including ethyl levulinate] are expected to be completely metabolized to carbon dioxide. With greater exposure, the ketone function may be reduced to the corresponding secondary alcohol (Bosron & Ting-Kai, 1980) and excreted as the glucuronic acid conjugate” (JECFA, 2000).

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

ORGANISM	TEST TYPE	ROUTE	REPORTED DOSE (NORMALIZED DOSE)	EFFECT	SOUREC
Rabbit	LD50	Skin	>5gm/kg (5000mg/kg)		Food and Chemical

					Toxicology. Vol.20, Pg. 679,1982
Rat	LD50	Oral	>5gm/kg (5000mg/kg)		Food and Chemical Toxicology. Vol. 20,Pg.679,1982.

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

Oral LD50 in rat and dermal LD50 in rabbit exceeded 5g/kg (Moreno, 1978).

5.2. Repeated dose toxicity

No data available to us at this time.

5.3. Reproduction toxicity

No data available to us at this time.

5.4. Mutagenicity

Ethyl levulinate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive:<80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a screening assay that assesses genotoxic stress through human-derived gene expression. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects on the target material. There are no studies assessing the mutagenic activity of ethyl levulinate; however, read-across can be made to methyl acetoacetate (CAS # 105-45-3; see Section V). The mutagenic activity of methyl acetoacetate has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with methyl acetoacetate in distilled water at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (ECHA, 2011). Under the conditions of the study, methyl acetoacetate was not mutagenic in the Ames test, and this can be extended to ethyl levulinate. There are no data assessing the clastogenic activity of ethyl levulinate; however, read-across can be made to methyl acetoacetate (CAS# 105-45-3; see Section V). The clastogenic activity of methyl acetoacetate was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with methyl acetoacetate in dimethyl sulfoxide (DMSO) at concentrations up to 1170µg/mL in the presence and absence of metabolic activation (S9) for 3h and in the absence of metabolic activation for 24h. Methyl acetoacetate did not induce binucleated cells with micronuclei when tested up to cytotoxic concentrations or the maximum concentration in either the presence or absence of an S9activationsystem (RIFM,2014). Under the conditions of the study, methyl acetoacetate was considered to be non-clastogenic in the in vitro micronucleus test, and this can be extended to ethyl levulinate. Based on the available data, methyl acetoacetate does not present a concern for genotoxic potential, and this can be extended to ethyl levulinate.

Read-across Justification:

Methyl acetoacetate (CAS 105-45-3) was used as a read-across analog for the target material ethyl levulinate (CAS 539-88-8) for genotoxicity and skin sensitization endpoints.

·The target substance and the read-across analog are structurally similar and belong to the class of keto esters.

·The target substance and the read-across analogs have a keto ester functionality.

·The key difference between the target substance and the read-across analog is that the read-across analog is a beta keto ester whereas the target substance is not. This structural difference is toxicologically insignificant.

·Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the keto ester functionality. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

·The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.

·According to the OECD QSAR Toolboxv3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.

·The target substance and the read-across analog have a protein binding alert for skin sensitization by OASIS v1.1. As described in the skin sensitization section above, based on existing data, read-across methyl acetoacetate does not present a safety concern for skin sensitization under the current, declared levels of use. Therefore, predictions are superseded by data

·The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

·The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

As taken from Api AM et al. 2019. Food Chem. Toxicol. 127(S1), S48-S54. Available at: http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/539-88-8.pdf

Global demand for alternative energy sources increases due to concerns regarding energy security and greenhouse gas emissions. However, little is known regarding the impacts of biofuels to the environment and human health even though the identification of such impacts is important to avoid biofuels leading to undesired effects. In this study mutagenicity and genotoxicity of the three biofuel candidates ethyl levulinate (EL), 2-methyltetrahydrofuran (2-MTHF) and 2-methylfuran (2-MF) were investigated in comparison to two petroleum-derived fuels and a biodiesel. None of the samples induced mutagenicity in the Ames fluctuation test. However, the Micronucleus assay revealed significant effects in Chinese hamster (Cricetulus griseus) V79 cells caused by the potential biofuels. 2-MF revealed the highest toxic potential with significant induction of micronuclei below 20.0 mg/L. EL and 2-MTHF induced micronuclei only at very high concentrations (>1000.0 mg/L). In regard to the genotoxic potential of 2-MF, its usage as biofuel should be critically discussed." As taken from Bluhm K et al. 2018. Environ. Toxicol. Pharmacol. 64, 131-138. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30391874

5.5. Cytotoxicity

Ethyl levulinate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive:<80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). As taken from Api AM et al. 2019. Food Chem. Toxicol. 127(S1), S48-S54. Available at: http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/539-88-8.pdf

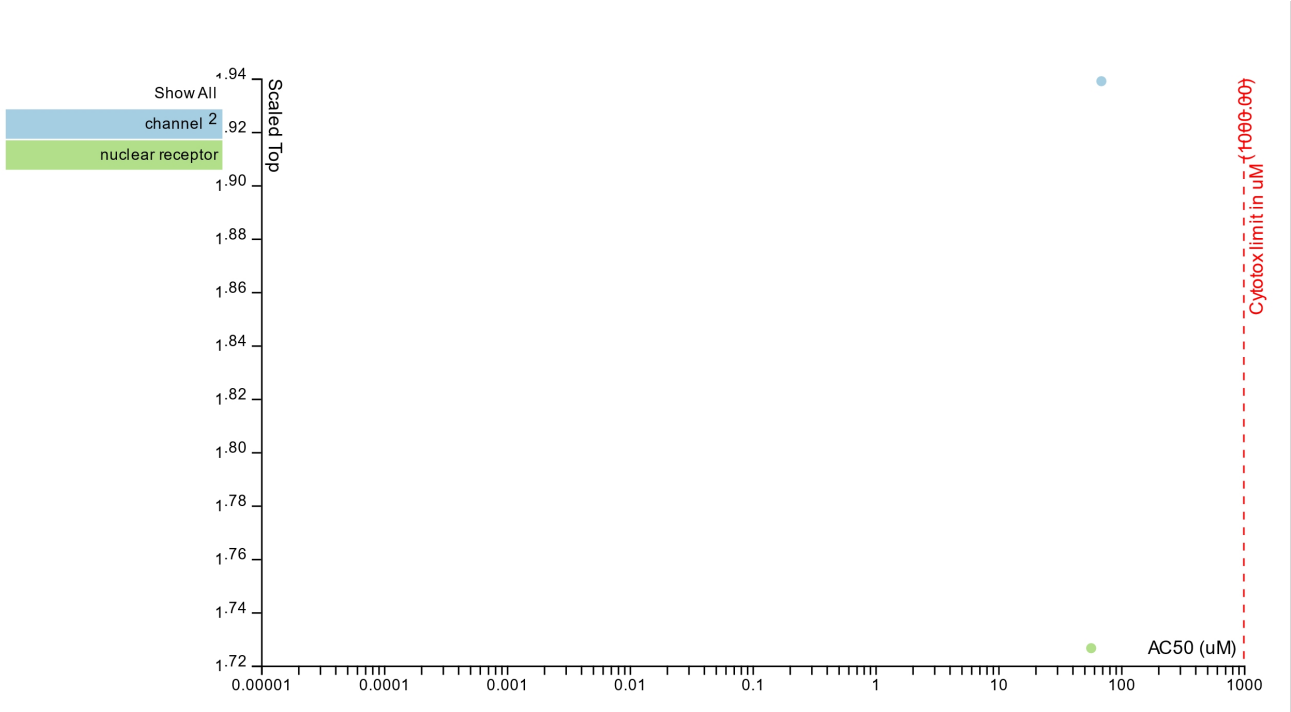
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated ethyl levulinate 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 ethyl levulinate is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

A 500 mg exposure during 24h on Rabbit adult skin produced only a mild irritant effect.

As taken from RTECS, 1997.

Slightly irritating to rabbit skin at full strength (Moreno 1978), not irritating to human skin at 4%, (Kligman, 1977).

Not sensitising to humans at 4% (Kligman, 1977).

Ethyl 4-oxovalerate (CAS RN 539-88-8) is a suspected skin sensitizer. The Toolbox profiler protein binding alerts for skin sensitization by OASIS v1.3 gives an alert for skin sensitization. The

CAESAR skin sensitization model in VEGA (Q)SAR platform predicts that the chemical is a sensitizer (moderate reliability).

As taken from ECHA, 2021b.

The reliability and applicability of this QSAR prediction as standalone source of toxicological information is limited and inappropriate for some complex endpoints like reprotoxicity or carcinogenicity. Nevertheless, for the toxicological assessment of this ingredient, this result was still taken into consideration and used within the WoE approach as a supportive tool, in combination with other sources of information when available, like experimental data or appropriate read-across.

Based on the existing data and read-across to methyl acetoacetate (CAS#105-45-3; see SectionV); ethyl levulinate does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structures of these materials indicate that they would not be expected to react with skin proteins directly (Toxtree 2.6.13; OECD toolbox v3.4). In a murine Local Lymph Node Assay (LLNA), read-across analog methyl acetoacetate was found to be negative up to 100%, which resulted in a Stimulation Index (SI) of 0.70 (https://echa.europa.eu/registrationdossier/-/registered-dossier/13864/7/5/2 ECHA, 2011). In human maximization tests, no reactions indicative of sensitization were observed with 4% ethyl levulinate (2760µg/cm2) (RIFM, 1977) or 8% read-across analog methyl acetoacetate (5520µg/cm2) (RIFM, 1976). Based on weight of evidence from structural analysis, human studies,and read-across analog methy lacetoacetate,ethyl levulinate does not present a concern for skin sensitization. As taken from Api AM et al. 2019. Food Chem. Toxicol. 127(S1), S48-S54. Available at: http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/539-88-8.pdf

“The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; ethyl levulinate is not expected to be phototoxic/photoallergenic.” As taken from Api AM et al. 2019. Food Chem. Toxicol. 127(S1), S48-S54. Available at: http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/539-88-8.pdf

5.8. All other relevant types of toxicity

High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated ethyl levulinate 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

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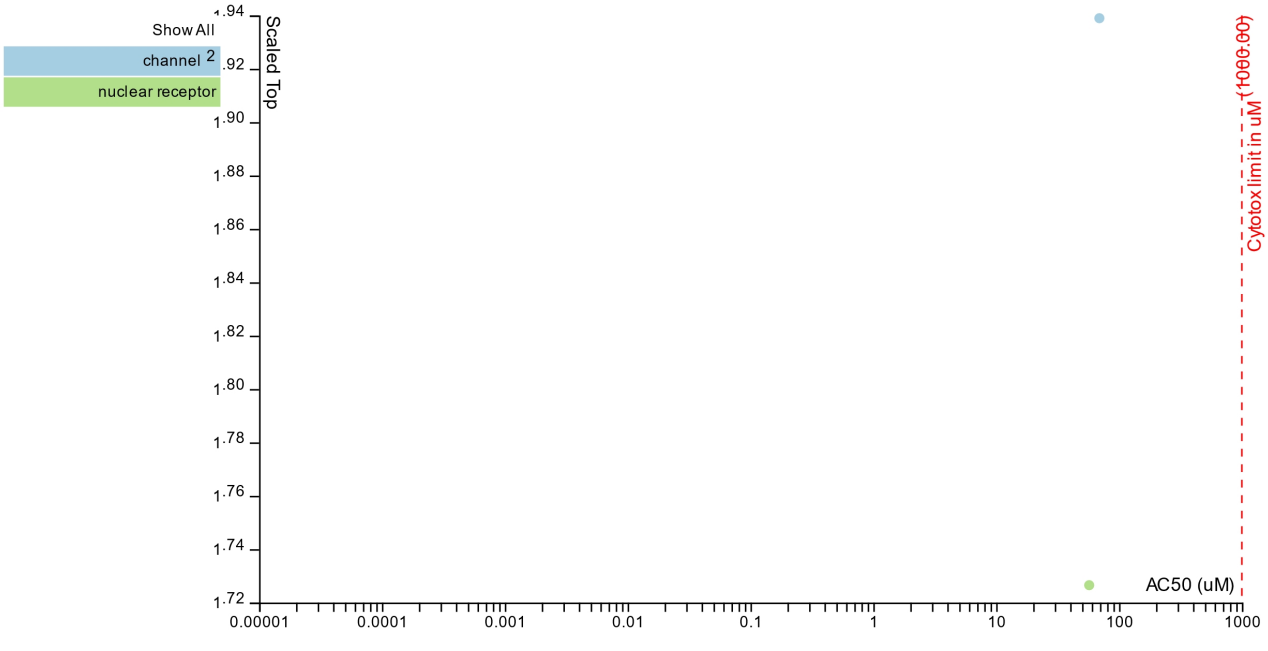

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

Showed anti-convulsant effects in mice at 300 mg/kg (Ledin, 1969).

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

No data available to us at this time.

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al. 1994 & 1998).

Tobacco smoke condensates from cigarettes containing ethyl levulinate 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 ethyl levulinate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	13 130	JTI KB Study Report(s)
In vitro genotoxicity	13	Renne et al. 2006
	13 130	JTI KB Study Report(s)
In vitro cytotoxicity	13 130	JTI KB Study Report(s)
Inhalation study	0.1	Gaworski et al. 1998
	13	Renne et al. 2006
	13 130	JTI KB Study Report(s)
Skin painting	0.1	Gaworski et al. 1999
	13 130	JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that pentanoic acid, 4-oxo-, ethyl ester (CAS RN 539-88-8) is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.9512
TOPKAT probability of biodegradation	1
EPI Predicted hydrolysis half-life (days)	1.80E+003
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	2.849

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

EPISuite provides the following data:

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

Bond Method :	2.08E-007 atm-m3/mole (2.11E-002 Pa-m3/mole)
Group Method:	2.94E-008 atm-m3/mole (2.98E-003 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.122E-006 atm-m3/mole (1.137E-001 Pa-m3/mole) VP: 0.27 mm Hg (source: MPBPVP) WS: 4.57E+004 mg/L (source: WSKOWWIN)

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

Log Kow used:	0.29 (KowWin est)
Log Kaw used:	-5.070 (HenryWin est
Log Koa (KOAWIN v1.10 estimate):	5.360
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.8599
Biowin2 (Non-Linear Model) :	0.9899
Biowin3 (Ultimate Survey Model):	2.9983 (weeks)
Biowin4 (Primary Survey Model) :	3.8465 (days)
Biowin5 (MITI Linear Model) :	0.8938
Biowin6 (MITI Non-Linear Model):	0.9512
Biowin7 (Anaerobic Linear Model):	0.5349
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	27.7 Pa (0.208 mm Hg)
Log Koa (Koawin est):	5.360
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.08E-007 5.62E-008

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	3.91E-006
Mackay model:	8.65E-006
Octanol/air (Koa) model:	4.5E-006

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

OVERALL OH Rate Constant =	3.7545 E-12 cm3/molecule-sec
Half-Life =	2.849 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	34.186 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 6.28E-006 (Junge-Pankow, Mackay avg) 4.5E-006 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	10 L/kg (MCI method)
Log Koc:	1.000 (MCI method)
Koc :	16.43 L/kg (Kow method)
Log Koc:	1.216 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Total Kb for pH > 8 at 25 deg C:	4.465E-002 L/mol-sec
Kb Half-Life at pH 8:	179.673 days

Kb Half-Life at pH 7:	4.919 years

(Total Kb applies only to esters, carbmates, alkyl halides)
Volatilization from Water: Henry LC: 2.94E-008 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	2.391E+004 hours (996.4 days)
Half-Life from Model Lake:	2.61E+005 hours (1.087E+004 days)

Removal In Wastewater Treatment:

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

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.55	68.4	1000
Water	31.6	360	1000
Soil	67.8	720	1000
Sediment	0.0712	3.24e+003	0

Persistence Time: 592 hr

10.2. Aquatic toxicity

According to the Ecological Categorization List from the Canadian Domestic Substances List, pentanoic acid, 4-oxo-, ethyl ester (CAS RN 539-88-8) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	117.3
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	117.3
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	179.362
Toxicity to fish (LC50 in mg/l) as predicted by Aster	180.228531
Toxicity to fish (LC50 in mg/l) as predicted by PNN	140.9624
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	4,120.792

Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.44E+003

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

“The increasing interest in the development of novel green solvents has led to the synthesis of benign alternative products with minimized environmental impacts. However, most of published studies on green solvents focus primarily on their physicochemical properties, with limited emphasis on absence of ecotoxicological assessment. In this study, we evaluated the acute ecotoxicity of four levulinates (levulinic acid, methyl levulinate, ethyl levulinate and butyl levulinate) on freshwater algae (Chlamydomonas reinhardtii), bacteria (Vibrio fischeri), daphnids (Daphnia magna) and earthworms (Eisenia foetida) using various dose-response tests. As a general trend, the toxicity of levulinate esters in aquatic exposure (assessed as the EC50) increased as a function of increasing alkyl chain length; accordingly, the most toxic compound for the aquatic organisms was butyl levulinate, followed by ethyl levulinate and methyl levulinate. The most toxic compound for E. foetida (terrestrial exposure) was methyl levulinate, followed by ethyl levulinate, butyl levulinate and levulinic acid; in this case, we observed an inverse relationship between toxicity and alkyl chain length. Based on both the lowest EC50 found in the aquatic media and the ratio between predicted environmental concentration and the predicted no-effect concentration, we have estimated the maximum allowable values in the environment for these chemicals to be 1.093 mg L(-1) for levulinic acid, 2.761 mg L(-1) for methyl levulinate, 0.982 mg L(-1) for ethyl levulinate and 0.151 mg L(-1) for butyl levulinate.” As taken from Lomba L et al. 2014. Ecotoxicology 23(8), 1484-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/?term=Ecotoxicity+studies+of+the+levulinate+ester+series

ECOSAR Version 1.11 reports the following aquatic toxicity data for CAS RN 539-88-8:

Values used to Generate ECOSAR Profile
Log Kow: 0.288 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 1.327E+005 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Fish	96-hr	LC50	192.711
Esters :	Daphnid	48-hr	LC50	481.197
Esters :	Green Algae	96-hr	EC50	268.610
Esters :	Fish		ChV	20.766
Esters :	Daphnid		ChV	538.365
Esters :	Green Algae		ChV	39.491
Esters :	Fish (SW)	96-hr	LC50	325.574
Esters :	Mysid	96-hr	LC50	744.493

Esters :	Fish (SW)		ChV	34.289
Esters :	Mysid (SW)		ChV	1.8e+006 *

Neutral Organic SAR :	Fish	96-hr	LC50	4085.145
(Baseline Toxicity) :	Daphnid	48-hr	LC50	1980.378
	Green Algae	96-hr	EC50	767.300
	Fish		ChV	331.375
	Daphnid		ChV	124.385
	Green Algae		ChV	141.308

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

“The demand for biofuels increases due to concerns regarding greenhouse gas emissions and depletion of fossil oil reserves. Many substances identified as potential biofuels are solvents or already used as flavors or fragrances. Although humans and the environment may be readily exposed little is known regarding their (eco)toxicological effects. In this study, the three potential biofuels ethyl levulinate (EL), 2-methyltetrahydrofuran (2-MTHF) and 2-methylfuran (2-MF) were investigated for their acute embryo toxicity and teratogenicity using the fish embryo toxicity (FET) test to identify unknown hazard potentials and to allow focusing further research on substances with low toxic potentials. In addition, two fossil fuels (diesel and gasoline) and an established biofuel (rapeseed oil methyl ester) were investigated as references. The FET test is widely accepted and used in (eco)toxicology. It was performed using the zebrafish Danio rerio, a model organism useful for the prediction of human teratogenicity. Testing revealed a higher acute toxicity for EL (LC50: 83mg/L) compared to 2-MTHF (LC50: 2980mg/L), 2-MF (LC50: 405mg/L) and water accommodated fractions of the reference fuels including gasoline (LC50: 244mg DOC/L). In addition, EL caused a statistically significant effect on head development resulting in elevated head lengths in zebrafish embryos. Results for EL reduce its likelihood of use as a biofuel since other substances with a lower toxic potential are available. The FET test applied at an early stage of development might be a useful tool to avoid further time and money requiring steps regarding research on unfavorable biofuels.” As taken from Bluhm K et al. 2016. Sci. Total Environ. 566-567, 786-95. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27243931

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

“The increasing interest in the development of novel green solvents has led to the synthesis of benign alternative products with minimized environmental impacts. However, most of published studies on green solvents focus primarily on their physicochemical properties, with limited emphasis on absence of ecotoxicological assessment. In this study, we evaluated the acute ecotoxicity of four levulinates (levulinic acid, methyl levulinate, ethyl levulinate and butyl levulinate) on freshwater algae (Chlamydomonas reinhardtii), bacteria (Vibrio fischeri), daphnids (Daphnia magna) and earthworms (Eisenia foetida) using various dose-response tests. As a general trend, the toxicity of levulinate esters in aquatic exposure (assessed as the EC50) increased as a function of increasing alkyl chain length; accordingly, the most toxic compound for the aquatic organisms

was butyl levulinate, followed by ethyl levulinate and methyl levulinate. The most toxic compound for E. foetida (terrestrial exposure) was methyl levulinate, followed by ethyl levulinate**,** butyl levulinate and levulinic acid; in this case, we observed an inverse relationship between toxicity and alkyl chain length. Based on both the lowest EC50 found in the aquatic media and the ratio between predicted environmental concentration and the predicted no-effect concentration, we have estimated the maximum allowable values in the environment for these chemicals to be 1.093 mg L(-1) for levulinic acid, 2.761 mg L(-1) for methyl levulinate, 0.982 mg L(-1) for ethyl levulinate and 0.151 mg L(-1) for butyl levulinate." As taken from Lomba L et al. 2014. Ecotoxicology 23(8), 1484-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/?term=Ecotoxicity+studies+of+the+levulinate+ester+series

ECOSAR Version 1.11 reports the following terrestrial toxicity data for CAS RN 539-88-8:

Values used to Generate ECOSAR Profile
Log Kow: 0.288 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 1.327E+005 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	6700.295

10.5. All other relevant types of ecotoxicity

Used in Pesticide: Kelevan, a reaction product of the insecticide Kepone (chlordecone) with ethyl levulinate, acts on insects primarily as a stomach poison. Kelevan is relatively not very toxic to warm-blooded animals. Its oral LD50 in corn oil or soybean oil is between 240 and 580 mg/kg in rats and dogs. In water suspension the value is several times greater. When added to the daily feed over the course of 90 days, Kelevan had a no-effect level of 300 ppm in rats, and between 20 and 100 ppm in dogs. The pattern of toxic effects in warm-blooded animals indicates that the central nervous system is the principal area of attack. The greater part of the 14C administered to rats as a single dose of Kelevan is excreted via the liver with the bile into the intestine, and only small proportions through the kidneys. After many repeated daily oral applictions (a total of 10 mg/kg of Kelevan in 8 weeks), no accumulation of Kelevan or its metabolite Kepone could be determined analytically in the body fat, liver, or brain of rats. No indications could be found of carcinogenic or reproduction-inhibiting effects in the toxicological investigations of Kelevan, its formulations, and its metabolite Kepone. Kelevan is degraded relatively rapidly in the soil. Its half-life was determined as 6 and 12 weeks in 2 different soils in laboratory tests. In soil samples from 0 to 7.5 cm depths taken from various districts of the German Federal Republic, a maximum of 0.06 ppm Kelevan + Kepone was found 1, 3, and 6 months after control of the potato beetle with 150 or 300 g of Kelvan/ha. Kepone is a degradation product of Kelevan in the soil. Kelevan acid" has been identified as another degradation product with an intact cage structure". The end products of the degradation of Kelevan and Kepone were found to be CO2 and HCl. (Author abstract by permission, abridged). Maier-Bode H . Residue Rev. 63: 45-76; 1976.(36 references) [PESTAB]

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01): Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)

Log Biotransformation Half-life (HL):	-2.1961 days (HL = 0.006366 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.029 (BCF = 0.9345)
Log BAF Arnot-Gobas method (upper trophic):	-0.029 (BAF = 0.9345)
log Kow used:	0.29 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List state that pentanoic acid, 4-oxo-, ethyl ester (CAS RN 539-88-8) is not bioaccumulative in the environment:

Log Kow predicted by KowWin	0.29
Log BAF T2MTL predicted by Gobas	0.0253407570699728
Log BCF 5% T2LTL predicted by Gobas	0.0205423292925737
Log BCF Max predicted by OASIS	1.09817480421873
Log BCF predicted by BCFWIN	0.5

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

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12. Other information

13. Last audited

March 2022

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

ETHYL LEVULINATE

COE No.:	373
FEMA No.:	2442
JECFA No.:	607
Chemical names:	ETHYL 4-OXOVALERATE
Synonyms:	ETHYL ACETYLPROPANOATE; ETHYL gamma-KETOVALERATE; ETHYL LAEVULATE; ETHYL 4-OXOPENTANOATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1999
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 896-JECFA 53/67
Specifications:	COMPENDIUM ADDENDUM 7/FNP 52 Add.7/128
Tox monograph:	FAS 44-JECFA 53/229

12 Nov 01

See Also:
Toxicological Abbreviations

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones (chemical group 9) 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 9 consists of primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones, of which 30 are currently authorised for use as flavours in food. The FEEDAP Panel was unable to perform an assessment of 2-oxopropanal because of issues related to the purity of the compound. The FEEDAP Panel concludes that lactic acid, succinic acid, fumaric acid, 4-oxovaleric acid, ethyl lactate, butyl lactate, butyl-O-butyryllactate, hex-3-enyl lactate, hexyl lactate, ethyl acetoacetate, ethyl 4-oxovalerate, diethylsuccinate and diethyl malonate are considered to be safe for all animal species at the use levels proposed when used as feed flavourings; octano-1,4-lactone, nonano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone are safe at 20 mg/kg complete feed; butyro-1,4-lactone, pentano-1,4-lactone, hexano-1,4-lactone, heptano-1,4-lactone, octano-1,5-lactone, nonano-1,5-lactone, decano-1,5-lactone and undecano-1,5-lactone at 5 mg/kg complete feed; dodecano-1,4-lactone, dodecano-1,5-lactone, tetradecano-1,5-lactone, and pentadecano-1,15-lactone at a maximum of 1.5 mg/kg complete feed for cattle, salmonids and non food producing animals and of 1 mg/kg complete feed for pigs and poultry. No safety concern was identified for the consumer from the use of compounds belonging to CG 9 up to the highest safe level in feedingstuffs for all animal species. All compounds should be considered as irritants to skin, eyes and respiratory tract, and as skin sensitizers. The compounds do not pose a risk to the environment when used at concentrations considered safe for the target species. Since all compounds are used in food as flavourings, no further demonstration of efficacy is necessary.

[1] On request from the European Commission, Question No EFSA-Q-2010-01177, adopted on 17 October 2012.

[2] Panel members: Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Gerhard Flachowsky, Josep Gasa-Gasó, Mikolaj Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallac and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Feed Flavourings, including Georges Bories, Paul Brantom, Andrew Chesson, Joop de Knecht, Jürgen Gropp, Anne-Katrine Lundebye Haldorsen, and Guido Rychen, for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones (chemical group 9) when used as flavourings for all animal species. EFSA Journal 2012;10(10):2928. [24 pp.] doi:10.2903/j.efsa.2012.2928. Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

Sensory additives, flavourings, primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones, chemical group 9, 2-oxopropanal, lactic acid, 4-oxovaleric acid, succinic acid, fumaric acid, ethyl acetoacetate, ethyl lactate, butyl lactate, ethyl 4-oxovalerate, diethyl succinate, diethyl malonate, butyl O-butyryllactate, hex-3-enyl lactate, hexyl lactate, nonano-1,4-lactone, undecano-1,4-lactone, pentadecano-1,15-lactone, butyro-1,4-lactone, decano-1,5-lactone, dodecano-1,5-lactone, undecano-1,5-lactone, pentano-1,4-lactone, nonano-1,5-lactone, octano-1,5-lactone, tetradecano-1,5-lactone, decano-1,4-lactone, dodecano-1,4-lactone, heptano-1,4-lactone, hexano-1,4-lactone and octano-1,4-lactone

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 30 compounds (primary aliphatic saturated or unsaturated alcohols/aldehydes/ acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones belonging to chemical group 9) when used as flavourings for all animal species. All 30 compounds are currently authorised for use as flavours in food and have all been detected in plant materials, in fruits or in processed foods. However the reports of their distribution vary greatly.

The FEEDAP Panel was unable to perform an assessment of 2-oxopropanal because of issues related to the purity of the compound.

The FEEDAP Panel concludes that lactic acid, succinic acid, fumaric acid, 4-oxovaleric acid, ethyl lactate, butyl lactate, butyl-O-butyryllactate, hex-3-enyl lactate, hexyl lactate, ethyl acetoacetate, ethyl 4-oxovalerate, diethylsuccinate and diethyl malonate are considered to be safe for all animal species at the use levels proposed when used as feed flavourings; octano-1,4-lactone, nonano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone, and are safe at 20 mg/kg complete feed (with a margin of safety ranging from 1 to 3.5); butyro-1,4-lactone, pentano-1,4-lactone, hexano-1,4-lactone, heptano-1,4-lactone, octano-1,5-lactone, nonano-1,5-lactone, decano-1,5-lactone and undecano-1,5-lactone at 5 mg/kg complete feed (with a margin of safety ranging from 4 to 14); dodecano-1,4-lactone, dodecano-1,5-lactone, tetradecano-1,5-lactone, and pentadecano-1,15-lactone at a maximum of 1.5 mg/kg complete feed for cattle, salmonids and non food producing animals and of 1 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances.

No safety concern would arise for the consumer from the use of compounds belonging to CG 9 up to the highest safe level in feedingstuffs for all animal species.

The FEEDAP Panel considers it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitizers.

The compounds considered to be safe for the target species are extensively metabolised by the target species and excreted as innocuous metabolites and carbon dioxide. Therefore no environmental risk assessment is considered necessary.

Since all 29 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.

TABLE OF CONTENTS

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 30 substances listed in Table 1 belonging to chemical group 9 (primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones) 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 24 November 2011.

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 30 substances belonging to CG 9 except succinic acid and hexyl lactate have been previously assessed by JECFA (1999, 2000 and 2002). According to Regulation (EC) No 1565/2000,[8] 'Substances classified by JECFA as to present no safety concern at the current levels of intake with the exception of substances which have been accepted on the sole basis that their estimated intake is lower than the threshold of concern of 1.5 µg per person per day, as laid down in the reports of the 46th, 49th, 51st and 53rd JECFA meetings need not to be re-evaluated.' Sixteen substances evaluated by JECFA in 1999 and six substances evaluated in 2000, were not further evaluated by EFSA. 2-Oxopropanal, lactic acid, ethyl lactate, butyl lactate, butyl-O-butyryllactate and hex-3-enyl lactate have been assessed by both JECFA (2002) and EFSA (2009b) as food flavourings. Succinic acid and hexyl lactate have been assessed by EFSA only (2009a).

[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-2010-0097.
[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.
[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.

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.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Chemical defined flavourings from Chemical Group 9: 2-Oxopropanal 4-Oxovaleric acid Butyl lactate Butyl-O-butyryllactate Butyro-1,4-lactone Decano-1,4-lactone Decano-1,5-lactone Diethyl malonate Diethyl succinate Dodecano-1,4-lactone Dodecano-1,5-lactone Ethyl 4-oxovalerate Ethyl acetoacetate Ethyl lactate Fumaric acid Heptano-1,4-lactone Hex-3-enyl lactate Hexano-1,4-lactone Hexyl lactate Lactic acid Nonano-1,4-lactone Nonano-1,5-lactone Octano-1,4-lactone Octano-1,5-lactone Pentadecano-1,15-lactone Pentano-1,4-lactone Succinic acid Tetradecano-1,5-lactone Undecano-1,4-lactone Undecano-1,5-lactone
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)
2-Oxopropanal (CAS No 78-98-8)	$C_3H_4O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
4-Oxovaleric acid (CAS No 123-76-2)	$C_5H_8O_3$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Butyl lactate (CAS No 138-22-7)	$C_7H_{14}O_3$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Butyl-O-butyryllactate (CAS No 7492-70-8)	$C_{11}H_{20}O_4$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Butyro-1,4-lactone (CAS No 96-48-0)	$C_4H_6O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Decano-1,4-lactone (CAS No 706-14-9)	$C_{10}H_{18}O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Decano-1,5-lactone (CAS No 705-86-2)	$C_{10}H_{18}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)

Diethyl malonate (CAS No 105-53-3)	$C_7H_{12}O_4$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Diethyl succinate (CAS No 123-25-1)	$C_8H_{14}O_4$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Dodecano-1,4-lactone (CAS No 2305-05-7)	$C_{12}H_{22}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Dodecano-1,5-lactone (CAS No 713-95-1)	$C_{12}H_{22}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Ethyl 4-oxovalerate (CAS No 539-88-8)	$C_7H_{12}O_3$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Ethyl acetoacetate (CAS No 141-97-9)	$C_6H_{10}O_3$	97.5%	Gas Chromatography – Mass Spectrometry (GC-MS)
Ethyl lactate (CAS No 97-64-3)	$C_5H_{10}O_3$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Fumaric acid (CAS No 110-17-8)	$C_4H_4O_4$	99.5%	Gas Chromatography – Mass Spectrometry (GC-MS)
Heptano-1,4-lactone (CAS No 105-21-5)	$C_7H_{12}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Hex-3-enyl lactate (CAS No 61931-81-5)	$C_9H_{16}O_3$	96%	Gas Chromatography – Mass Spectrometry (GC-MS)
Hexano-1,4-lactone (CAS No 695-06-7)	$C_6H_{10}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Hexyl lactate (CAS No 20279-51-0)	$C_9H_{18}O_3$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Lactic acid (CAS No 598-82-3, 50-21-5, 79-33-4)	$C_3H_6O_3$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Nonano-1,4-lactone (CAS No 104-61-0)	$C_9H_{16}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Nonano-1,5-lactone (CAS No 3301-94-8)	$C_9H_{16}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Octano-1,4-lactone (CAS No 104-50-7)	$C_8H_{14}O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Octano-1,5-lactone (CAS No 698-76-0)	$C_8H_{14}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Pentadecano-1,15-lactone (CAS No 106-02-5)	$C_{15}H_{28}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Pentano-1,4-lactone (CAS No 108-29-2)	$C_5H_8O_2$	95%	Gas Chromatography – Mass Spectrometry (GC-MS)
Succinic acid (CAS No 110-15-6)	$C_4H_6O_4$	99%	Gas Chromatography – Mass Spectrometry (GC-MS)
Tetradecano-1,5-lactone (CAS No 2721-22-4)	$C_{14}H_{26}O_2$	97%	Gas Chromatography – Mass Spectrometry (GC-MS)
Undecano-1,4-lactone (CAS No 104-67-6)	$C_{11}H_{20}O_2$	98%	Gas Chromatography – Mass Spectrometry (GC-MS)
Undecano-1,5-lactone (CAS No 710-04-3)	$C_{11}H_{20}O_2$	98%	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) 9 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[9] as 'primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones'. The present application concerns 30 compounds, which can be assigned to this CG. The flavours included in this assessment have all been detected in plant materials, fruits or in processed foods (e.g., cheese, wine, and cider)*;* however, the reports of their distribution vary greatly.

The 30 compounds belonging to CG 9 except succinic acid and hexyl lactate have been previously assessed by JECFA (1999, 2000 and 2002). According to Regulation (EC) No 1565/2000,[9] 'Substances classified by JECFA as to present no safety concern at the current levels of intake with the exception of substances which have been accepted on the sole basis that their estimated intake is lower than the threshold of concern of 1.5 µg per person per day, as laid down in the reports of the 46th, 49th, 51st and 53rd JECFA meetings need not to be re-evaluated.' Sixteen substances evaluated by JECFA in 1999 and six substances evaluated in 2000 were not further evaluated by EFSA. 2-Oxopropanal, lactic acid, ethyl lactate, butyl lactate, butyl-O-butyryllactate and hex-3-enyl lactate have been assessed by both JECFA (2002) and EFSA (2009b) as food flavourings. Succinic acid and hexyl lactate have been assessed by EFSA only (2009a). The 30 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.

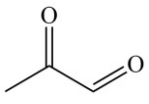
Regulation (EC) No 429/2008[10] allows substances already approved for use in human food to be assessed with a more limited procedure than for other feed additives. However, the use of this procedure is always subject to the condition that food safety assessment is relevant to the use in feed.

2. Characterisation

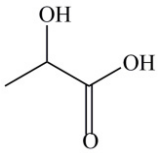
2.1. Characterisation of the flavouring additives

The molecular structures of the additives under application are shown in Figure 1 and their physico-chemical characteristics are summarised in Table 2.

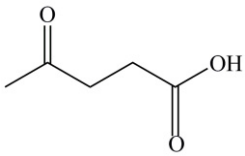
2-Oxopropanal (07.001)



Lactic acid (08.004)



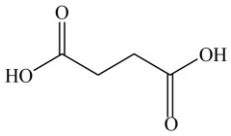
4-Oxovaleric acid (08.023)



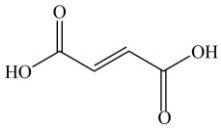
[9] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

[10] 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.

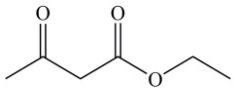
Succinic acid (08.024)



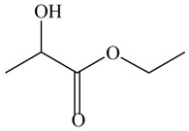
Fumaric acid (08.025)



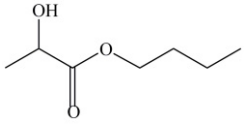
Ethyl acetoacetate (09.402)



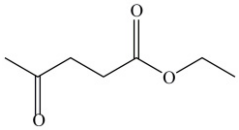
Ethyl lactate (09.433)



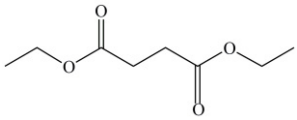
Butyl lactate (09.434)



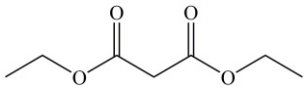
Ethyl 4-oxovalerate (09.435)



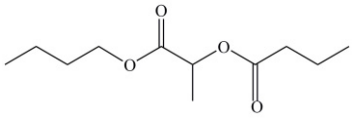
Diethyl succinate (09.444)



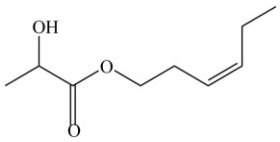
Diethyl malonate (09.490)



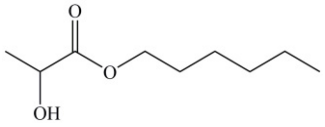
Butyl-O-butyryllactate (09.491)



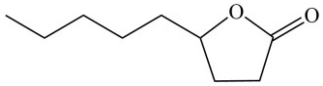
Hex-3-enyl lactate (09.545)



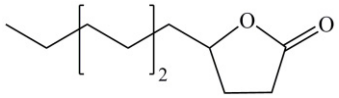
Hexyl lactate (09.580)



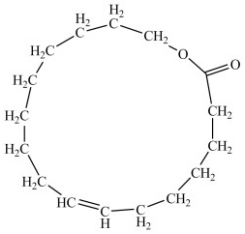
Nonano-1,4-lactone (10.001)



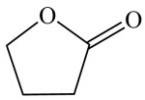
Undecano-1,4-lactone (10.002)



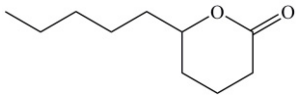
Pentadecano-1,15-lactone (10.005)



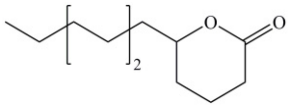
Butyro-1,4-lactone (10.006)



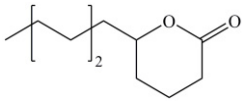
Decano-1,5-lactone (10.007)



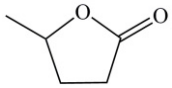
Dodecano-1,5-lactone (10.008)



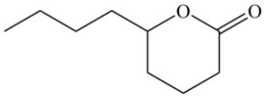
Undecano-1,5-lactone (10.011)



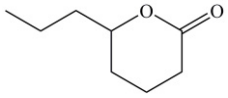
Pentano-1,4-lactone (10.013)



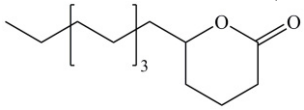
Nonano-1,5-lactone (10.014)



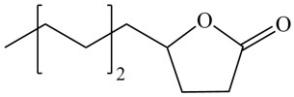
Octano-1,5-lactone (10.015)



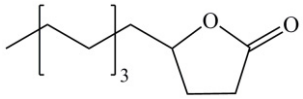
Tetradecano-1,5-lactone (10.016)



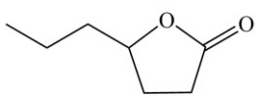
Decano-1,4-lactone (10.017)



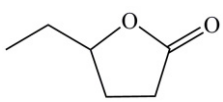
Dodecano-1,4-lactone (10.019)



Heptano-1,4-lactone (10.020)



Hexano-1,4-lactone (10.021)



Octano-1,4-lactone (10.022)

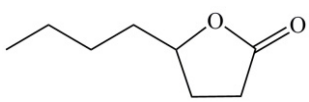


Figure 1: Molecular structures and FLAVIS numbers of flavourings of CG 9

Table 2: Chemically defined flavourings of CG 9 under application

EU Register name	CAS No.	Flavis No.	Molecular formula	Molecular weight	Physical status	Log K_{ow}
2-Oxopropanal	78-98-8	07.001	$C_3H_4O_2$	72.06	Liquid	-1.5
Lactic acid	598-82-3[#]	08.004	$C_3H_6O_3$	90.08	Solid	-0.72
4-Oxovaleric acid	123-76-2	08.023	$C_5H_8O_3$	116.12	Liquid	-0.49
Succinic acid	110-15-6	08.024	$C_4H_6O_4$	118.09	Solid	-0.59
Fumaric acid	110-17-8	08.025	$C_4H_4O_4$	116.07	Solid	0.46
Ethyl acetoacetate	141-97-9	09.402	$C_6H_{10}O_3$	130.14	Liquid	0.25
Ethyl lactate	97-64-3	09.433	$C_5H_{10}O_3$	118.13	Liquid	-0.18
Butyl lactate	138-22-7	09.434	$C_7H_{14}O_3$	149.19	Liquid	0.80
Ethyl 4-oxovalerate	539-88-8	09.435	$C_7H_{12}O_3$	144.17	Liquid	0.29
Diethyl succinate	123-25-1	09.444	$C_8H_{14}O_4$	174.2	Liquid	1.2
Diethyl malonate	105-53-3	09.490	$C_7H_{12}O_4$	160.17	Liquid	0.96
Butyl-O-butyryllactate	7492-70-8	09.491	$C_{11}H_{20}O_4$	216.28	Liquid	2.79*
Hex-3-enyl lactate	61931-81-5	09.545	$C_9H_{16}O_3$	172.22	Liquid	1.57*
Hexyl lactate	20279-51-0	09.580	$C_9H_{18}O_3$	174.24	Liquid	3.32
Nonano-1,4-lactone	104-61-0	10.001	$C_9H_{16}O_2$	156.22	Liquid	2.21*
Undecano-1,4-lactone	104-67-6	10.002	$C_{11}H_{20}O_2$	184.28	Liquid	3.06
Pentadecano-1,15-lactone	106-02-5	10.004	$C_{15}H_{28}O_2$	240.39	Solid	6.15
Butyro-1,4-lactone	96-48-0	10.006	$C_4H_6O_2$	86.09	Liquid	-0.64
Decano-1,5-lactone	705-86-2	10.007	$C_{10}H_{18}O_2$	170.25	Liquid	2.57*
Dodecano-1,5-lactone	713-95-1	10.008	$C_{12}H_{22}O_2$	198.31	Liquid	3.55*
Undecano-1,5-lactone	701-04-3	10.011	$C_{11}H_{20}O_2$	184.28	Liquid	3.06*
Pentano-1,4-lactone	108-29-2	10.013	$C_5H_8O_2$	100.12	Liquid	-0.13*
Nonano-1,5-lactone	3301-94-8	10.014	$C_9H_{16}O_2$	156.22	Liquid	2.08*
Octano-1,5-lactone	698-76-0	10.015	$C_8H_{14}O_2$	142.2	Liquid	1.59*
Tetradecano-1,5-lactone	2721-22-4	10.016	$C_{14}H_{26}O_2$	226.4	Liquid	4.53
Decano-1,4-lactone	706-14-9	10.017	$C_{10}H_{18}O_2$	170.25	Liquid	2.72
Dodecano-1,4-lactone	2305-05-7	10.019	$C_{12}H_{22}O_2$	198.31	Liquid	3.46*
Heptano-1,4-lactone	105-21-5	10.020	$C_7H_{12}O_2$	128.17	Liquid	1.19*
Hexano-1,4-lactone	695-06-7	10.021	$C_6H_{10}O_2$	114.14	Liquid	0.60
Octano-1,4-lactone	104-50-7	10.022	$C_8H_{14}O_2$	142.2	Liquid	1.72*

[#] Three CAS numbers have been used to identify lactic acid, two refer to DL-lactic acid (50-21-5 and CAS no 598-82-3) and one to the isomer L-lactic acid (79-33-4). They are all relevant to the assessment of this compound

* Generated from Epi-Suite 4.01

All 30 substances are produced by chemical synthesis. Typically several routes of synthesis are available and described in the dossier.[11]

Data was provided on the batch to batch variation in five batches of each additive (with the exception of oxopropanal, fumaric acid and tetradecano-1,5-lactone (one batch was available) and hexyl lactate (four batches)).[12] Except for 2-oxopropanal, the content of the active substance exceeded the JECFA specifications (Combined Compendium of Food Additives Specifications; JECFA, 2006) for all compounds (Table 3). 2-Oxopropanal is specified to contain a minimum of 40 % in aqueous solution. This description does not allow the setting of a specification or the extrapolation of consumer safety assessments of 2-oxopropanal to this product. Consequently, this additive is excluded from further consideration.

[11] Technical dossiers/Section II.

[12] Technical dossiers/Section II/Annex 2.1 and Supplementary Information June 2011.

Table 3: Identification of the substances and data on purity

EU Register name	JECFA specification %	Assay %	
		Average	Range
2-Oxopropanal	> 95[a]	45.3[b]	45.3
Lactic acid	> 95	96.3	95.0–97.0
4-Oxovaleric acid	> 97	99.6	99.1–100
Succinic acid	> 99	99.4	99.1–99.7
Fumaric acid	> 99.5	99.8[b]	99.8
Ethyl acetoacetate	> 97.5	99.7	99.2–99.9
Ethyl lactate	> 97	99.6	98.0–100
Butyl lactate	> 95	99.4	99.0–99.8
Ethyl 4-oxovalerate	> 98	98.6	98.1–100
Diethyl succinate	> 98	99.7	99.4–100
Diethyl malonate	> 97	99.8	99.5–100
Butyl-O-butyryllactate	> 95	99.6	99.4–99.8
Hex-3-enyl lactate	> 96	98.5	98.2–98.7
Hexyl lactate	> 95	98.7[c]	98.4–98.6
Nonano-1,4-lactone	> 98	99.3	98.8–99.7
Undecano-1,4-lactone	> 98	99.1	98.5–99.5
Pentadecano-1,15-lactone	> 98	99.2	98.8–99.6
Butyro-1,4-lactone	> 98	98.8	98.1–100
Decano-1,5-lactone	> 98	98.8	98.0–99.3
Dodecano-1,5-lactone	> 98	99.0	98.4–99.7
Undecano-1,5-lactone	> 98	98.2	98.0–98.5
Pentano-1,4-lactone	> 95	99.7	98.7–100
Nonano-1,5-lactone	> 98	98.3	98.0–98.6
Octano-1,5-lactone	> 98	99.5	98.5–99.9
Tetradecano-1,5-lactone	> 97	98.6[b]	98.6
Decano-1,4-lactone	> 95	99.5	98.6–99.8
Dodecano-1,4-lactone	> 97	98.8	98.4–99.3
Heptano-1,4-lactone	> 98	99.6	98.9–99.9
Hexano-1,4-lactone	> 98	99.5	98.7–99.8
Octano-1,4-lactone	> 95	99.5	99.3–99.9

[a] Only available as 40 % aqueous solution; [b] One batch available; [c] average of four batches

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

The minimum shelf life for all compounds is 12 months with the majority stable for a longer period when stored in closed containers under recommended conditions (in a cool and dry place). 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.

The FEEDAP Panel notes that 13 out of 29 compounds in CG 9 have low water solubility (Log K_{ow} > 2) which makes it difficult to assess the safety in water for drinking. 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 29 additives in feed or water for drinking for all animal species without withdrawal. In all cases the applicant proposes a normal use level in feed and a high use level of five times the normal levels:

- a normal use level of 25 mg/kg and a high use level of 125 mg/kg for ethyl lactate and nonano-1,4-lactone
- a normal use level of 5 mg/kg and a high use level of 25 mg/kg for octano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone
- a normal use level of 1 mg/kg and a high use level of 5 mg/kg for the remaining compounds

No specific proposals are made for levels to be used in water for drinking.

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 chemically defined flavourings from Group 9 – Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones 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 (125 mg/kg complete feed for ethyl lactate and nonano-1,4-lactone, 25 mg/kg complete feed for octano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone, and 5 mg/kg complete feed for the other additives).

3.1. Safety for the target species

Lactic acid, succinic acid and fumaric acid are normal components of cell metabolism and are considered to be safe up to the highest proposed use level. Esters of these acids (ethyl lactate, butyl lactate, butyl-O-butyryllactate, hex-3-enyl lactate and hexyl lactate, diethylsuccinate and diethyl malonate) are generally rapidly hydrolysed to the corresponding acids and alcohols and thus raise no concerns when used as flavours in animal feed at the proposed levels. Ethyl acetoacetate and ethyl 4-oxovalerate are hydrolysed to their respective acids and alcohols, which are either normal components of cell metabolism or readily degraded by similar pathways (see section 3.2.1). The use of these esters as flavours is thus considered to be safe without restrictions for all animal species.

No further consideration on the safety for the target species of these compounds is deemed necessary.

For the remaining 16 compounds 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,b) range between 73 and 1600 µg/person/day, corresponding to 3.4 and 74.2 µg/mbw ($kg^{0.75}$)/day. Table 5 summarises the result of the comparison for representative target animals at the maximum proposed dose level in complete feed with human exposure. The body weight of target animals is taken from the default values shown in Table 5.

Table 4: Comparison of exposure of humans and target animals to 16 of 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
Nonano-1,4-lactone	125	46.4	2941	13158	19425
Undecano-1,4-lactone	25	10.8	588	2632	3885
Pentadecano-1,15-lactone	5	3.4	118	526	777
Butyro-1,4-lactone	5	5.1	118	526	777
Decano-1,5-lactone	5	334	118	526	777
Dodecano-1,5-lactone	5	269	118	526	777
Undecano-1,5-lactone	5	13.9	118	526	777
Pentano-1,4-lactone	5	5.6	118	526	777
Nonano-1,5-lactone	5	6.0	118	526	777
Octano-1,5-lactone	5	10.7	118	526	777
Tetradecano-1,5-lactone	5	5.1	118	526	777
Decano-1,4-lactone	25	74.2	588	2632	3885
Dodecano-1,4-lactone	5	8.8	118	526	777
Heptano-1,4-lactone	5	7.9	118	526	777
Hexano-1,4-lactone	5	7.4	118	526	777
Octano-1,4-lactone	25	19.9	588	2632	3885

* mbw = metabolic body weight (kg 0.75) for a 60 kg person = 21.6

The data in Table 4 clearly indicate that the intake by the target animals usually exceeds that of humans, resulting from use in food for all 16 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 can be derived from the lowest No Observed Adverse Effect Level (NOAEL) when suitable data is available. Toxicological data could be found for some of the 16 lactone compounds. The studies have been recently reviewed by EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF; EFSA, 2012a).

A NOAEL of 112 mg/kg bw per day was derived for butyro-1,4-lactone (or γ-butyrolactone) from a two-year carcinogenicity study in rats (50M/50F, doses: 0, 112, 225 mg/kg bw per day for male rats; 0, 225, 450 mg/kg bw per day for female rats; administration route: gavage) and mice (50M/50F, doses: 0, 262, 525 mg/kg bw per day; administration route: gavage) (NTP, 1992). The NOAEL of 112 mg/kg is based on a reduced survival of male rats at the dose of 225 mg/kg bw per day. No histopathological abnormalities could be observed at any dose level. There was no evidence of carcinogenic activity of butyro-1,4-lactone at the doses tested in male and female rats and female mice. There was equivocal evidence of carcinogenicity of butyro-1,4-lactone in male $B6C3F_1$ mice based on marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia at the low dose group (262 mg/kg bw per day) but not at the high dose group 525 mg/kg bw per day). This effect was not considered of relevance to other species in the report of the National Toxicology Program (NTP, 1992) and the FEEDAP Panel agrees with these conclusions.

On the basis of the structural and metabolic similarity (see section 3.2.2), the FEEDAP Panel considered the 16 lactone compounds as a single group and a group NOAEL approach was taken. The NOAEL derived from the study with butyro-1,4-lactone is considered to be relevant to each member of this group and is not in conflict with data for any individual substances (EFSA, 2012a). Following the EFSA Guidance for sensory additives (EFSA, 2012b), a safety factor of 100 to account for intra-species variation (2), inter-species variation (10) and uncertainty about the toxicity of untested compounds (5) was applied to this value and, thus, the maximum safe intake and the maximum safe feed concentration for different target species was derived for the following compounds belonging to

CG 9: butyro-1,4-lactone, pentano-1,4-lactone, hexano-1,4-lactone, heptano-1,4-lactone, octano-1,4-lactone, nonano-1,4-lactone, decano-1,4-lactone, undecano-1,4-lactone, octano-1,5-lactone, nonano-1,5-lactone, decano-1,5-lactone and undecano-1,5-lactone. The results of the calculations are shown in Table 5.

Table 5: Derived maximum safe concentration in feed for different target animals for 12 compounds belonging to CG 2 (see the list in the paragraph above)

Target animal	Default settings		Maximum safe intake/feed concentration	
	BW (kg)	FI (g/d)	Intake (mg/d)	mg/kg feed
Salmonids	2	40	2	56
Veal calves (milk replacer)	100	2000	112	56
Cattle for fattening	400	8000	448	56
Pigs for fattening	100	3000	112	37
Sows	200	6000	224	37
Dairy Cows	650	20000	728	36
Turkeys for fattening	12	400	13	34
Piglets	20	1000	22	22
Chickens for fattening	2	120	2	19
Laying hens	2	120	2	19
Dogs	15	250	17	67
Cats	3	60	3	56

Because of the higher lipophylicity (LogKow > 3.5) and the potential difference in toxicokinetics dodecano-1,4-lactone, dodecano-1,5-lactone, tetradecano-1,5-lactone and pentadecano-1,15-lactone were excluded from the group NOAEL approach. For these Cramer Class I compounds the threshold of toxicological concern (TTC) approach was adopted to derive the maximum safe feed concentration. The calculated safe use level for these compounds is 1.5 mg/kg complete feed for cattle, salmonids and non food producing animals and 1 mg/kg complete feed for pigs and poultry.

3.1.1. Conclusions on the safety for target species

Lactic acid, succinic acid and fumaric acid are normal components of cell metabolism and are considered to be safe up to the highest proposed use level. Esters of these acids (ethyl lactate, butyl lactate, butyl-O-butyryllactate, hex-3-enyl lactate and hexyl lactate, diethylsuccinate and diethyl malonate) are generally rapidly hydrolysed to the corresponding acids and alcohols and thus raise no concerns when used as flavours in animal feed at the proposed levels. Ethyl acetoacetate and ethyl 4-oxovalerate are hydrolysed to their respective acids and alcohols which are either normal components of cell metabolism or readily degraded by similar pathways (see section 3.2.1).

For the remaining 16 lactones, the FEEDAP Panel concludes that the use of the following is safe for all animal species:

- octano-1,4-lactone, nonano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone at 20 mg/kg complete feed with a margin of safety ranging from 1 to 3.5

- butyro-1,4-lactone, pentano-1,4-lactone, hexano-1,4-lactone, heptano-1,4-lactone, octano-1,5-lactone, nonano-1,5-lactone, decano-1,5-lactone and undecano-1,5-lactone at 5 mg/kg complete feed with a margin of safety ranging from 4 to 14

- dodecano-1,4-lactone, dodecano-1,5-lactone, tetradecano-1,5-lactone, and pentadecano-1,15-lactone at a maximum of 1.5 mg/kg complete feed for cattle, salmonids and non food producing animals and of 1 mg/kg complete feed for pigs and poultry. The absence of a

margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances

The total dose from all sources should not exceed that obtained when given in feed alone. Consequently the concentrations used when substances are administered in water for drinking should be proportionally reduced. The exact ratio for inclusion when used in both feed and water for drinking is beyond the scope of data available to the FEEDAP Panel.

3.2. Safety for the consumer

The safety for the consumer of CG 9 compounds when used as food flavours has already been assessed by JECFA (1999, 2000 and 2002) and/or EFSA (2009a, 2009b). An Acceptable Daily Intake (ADI) of 1.25 mg/kg bw was established for nonano-1,4-lactone and undecano-1,4-lactone. All 29 compounds are currently authorised as food additives without limitations.

As the intake of all 29 compounds by target animals exceeds that of humans resulting from use in food by one to three orders of magnitude, the metabolic fate and potential transfer of significant amounts of residues in edible tissues and products has to be considered.

3.2.1. Esters, acids and aldehydes containing additional oxygenated functional groups

According to JECFA (2000 and 2002), studies on the absorption, metabolism and elimination of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters with additional oxygenated functional groups show that these substances are readily hydrolysed and absorbed and are completely metabolized. Many of these substances or their metabolites are endogenous in humans. Mono-esters (ethyl acetoacetate, ethyl 4-oxovalerate and aliphatic esters of lactic acid) and di-esters (diethyl succinate and diethyl malonate) are expected to undergo hydrolysis in humans to yield their corresponding alcohol and acid components (i.e. β- or γ-keto or α-hydroxy acids; or diacids), which would be further metabolised and excreted through the common pathways of detoxication of aliphatic alcohols and carboxylic acids. JECFA stated also that the presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters.

Hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases or esterases (Heymann, 1980), the most important of which are the β-esterases (Heymann, 1980; Anders, 1989). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). Mammalian carboxylesterases represent a multigene family and play an important role in the hydrolytic biotransformation of a vast number of structurally diverse drugs (Satoh and Hosokawa 1998). Carboxylesterase activity also plays a significant role in detoxification processes in fish (Di Giulio and Hinton 2008; Tocher, 2003) as well as in birds (Beasley, 1999). The most probable metabolic reactions of the hydrolysis products are: oxidation of alcohols to aldehydes and acids; conjugations of alcohols and acids to glucuronides and sulphates; β-oxidation of carboxylic acids; ω-oxidation of carboxylic acids.

β-Keto acids and derivatives like acetoacetic acid undergo decarboxylation. Along with α-keto and α-hydroxyacids (lactic acid), they yield breakdown products, which are incorporated into normal biochemical pathways. The γ-keto-acids and related substances (4-oxovaleric acid) may undergo complete or partial β-oxidation to yield metabolites that are eliminated in the urine. Simple aliphatic di-carboxylic acids (succinic acid and fumaric acid) and their precursors (2-oxopropanal) are metabolised in the fatty acid β-oxidation pathway or tricarboxylic acid cycle (EFSA, 2009a).

3.2.2. Lactones

In its evaluation, JECFA (1999) recognized that lactones are formed by acid-catalysed intramolecular cyclisation of γ- or δ-hydroxycarboxylic acids. In an aqueous environment, a pH-dependent

equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic and neutral media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured. Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclization are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones with low molecular weight being uncharged may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including β-, γ-, δ- and ω-lactones, lactones fused to alicyclic rings such as 2-(2- hydroxycyclopent-4-enyl)ethanoic acid γ-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993). Members of the serum paraoxonase (PON) family have also been identified in other vertebrates than humans and mammals, where they exhibit a wide range of physiologically important hydrolytic activities (Estin et al., 2009; Tucker and Halver 1986).

JECFA stated also that the hydroxycarboxylic acid obtained from lactone hydrolysis enters the fatty acid pathway and undergoes α-oxidation (linear saturated 4- or 6-hydroxycarboxylic acids formed from γ- or ε-lactones) or β-oxidation (linear saturated 5-hydroxycarboxylic acids formed from delta-lactones) and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by 2-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then metabolised in the citric acid cycle (Voet and Voet, 1990). In rats and dogs, $^{14}CO_1$-gamma-decalactone and $^{14}CO_1$-gammadodecalactone are metabolised in a manner similar to $^{14}CO_1$-lauric acid, with approximately 75 % of the labeled ^{14}CO being eliminated as carbon dioxide within 48 hours (Fassett, 1961).

Information on hydrolysis of aliphatic lactones is mainly derived from studies on butyro-1,4-lactone (γ-butyrolactone), which has been extensively studied in animals and humans. The majority of ^{14}C-labeled 4-hydroxybutanoate administered by intravenous injection to rats was recovered as $^{14}CO_2$ within 2.5 hours (Roth and Giarman, 1965). Oxidation of γ-butyrolactone to succinate by alcohol dehydrogenase and succinic semialdehyde dehydrogenase occurs primarily in the liver (Jakoby and Scott, 1959); succinate then participates in the citric acid cycle (Walkenstein et al., 1964; Möhler et al., 1976; Lee, 1977; Doherty and Roth, 1978). However, this pathway accounts for only a limited proportion of the metabolised compound. The main biotransformation route through which γ-butyrolactone is metabolised is β-oxidation as indicated by the presence of (S)-3,4-dihydroxybutyric acid, glycolic acid and 3-oxobutyric acid in the urine of human volunteers given orally 1.0 g γ-butyrolactone (Lee, 1977); other intermediates derived from β-oxidation have been previously detected in samples of human urine (Walkenstein et al., 1964).

3.2.3. Conclusions on the safety for the consumer

Primary aliphatic saturated or unsaturated alcohols, aldehydes, acids, acetals and esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones are rapidly converted to innocuous substances. Mammals, birds and fish share a similar metabolic capacity to handle these compounds. Consequently, no safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds.

3.3. Safety for the user

No experimental data on the safety for the user was provided. In the material safety data sheets[13] hazards for skin and eye contact and respiratory exposure are recognised for 18 (lactic acid, 4-oxovaleric acid, succinic acid, fumaric acid, ethyl acetoacetate, ethyl lactate, butyl lactate, diethyl succinate, diethyl malonate, butyl O-butyryllactate, hex-3-enyl lactate, undecano-1,4-lactone, butyro-1,4-lactone, dodecano-1,5-lactone, undecano-1,5-lactone, dodecano-1,4-lactone, heptano-1,4-lactone, and octano-1,4-lactone) out of the 29 compounds. Seventeen compounds are classified as irritating to eyes and/or skin. For three compounds (lactic acid, succinic acid and 2-ethyl lactate) the risk of serious damage to eyes is reported. Eleven compounds (lactic acid, succinic acid, ethyl lactate, butyl lactate, diethyl succinate, butyl O-butyryllactate, hex-3-enyl lactate, undecano-1,4-lactone, dodecano-1,5-lactone, undecano-1,5-lactone and dodecano-1,4-lactone) 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, and as skin sensitizers.

3.4. Safety for the environment

The compounds considered to be safe for the target species are extensively metabolised by the target species and excreted as innocuous metabolites and carbon dioxide. Therefore no environmental risk is foreseen.

4. Efficacy

Since all 29 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

The FEEDAP Panel was unable to perform an assessment of 2-oxopropanal because of issues related to the purity of the compound.

The following compounds are considered to be safe for all animal species at the use levels proposed when used as feed flavourings:

- lactic acid, succinic acid, fumaric acid, 4-oxovaleric acid, ethyl lactate, butyl lactate, butyl-O-butyryllactate, hex-3-enyl lactate, hexyl lactate, ethyl acetoacetate, ethyl 4-oxovalerate, diethylsuccinate and diethyl malonate

For the remaining 16 lactones, the FEEDAP Panel concludes that the use of the following is safe for all animal species:

- octano-1,4-lactone, nonano-1,4-lactone, decano-1,4-lactone and undecano-1,4-lactone at 20 mg/kg complete feed with a margin of safety ranging from 1 to 3.5

- butyro-1,4-lactone, pentano-1,4-lactone, hexano-1,4-lactone, heptano-1,4-lactone, octano-1,5-lactone, nonano-1,5-lactone, decano-1,5-lactone and undecano-1,5-lactone at 5 mg/kg complete feed with a margin of safety ranging from 4 to 14

[13] Technical dossier/Section II/Annex II.3.

– dodecano-1,4-lactone, dodecano-1,5-lactone, tetradecano-1,5-lactone and pentadecano-1,15-lactone at a maximum of 1.5 mg/kg complete feed for cattle, salmonids and non food producing animals and of 1 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances.

No safety concern would arise for the consumer from the use of compounds belonging to CG 9 up to the highest safe level in feedingstuffs for all animal species.

The FEEDAP Panel considers it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitizers.

The compounds considered to be safe for the target species are extensively metabolised by the target species and excreted as innocuous metabolites and carbon dioxide. Therefore no environmental risk is foreseen.

Since all 29 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 09 - Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones (CDG 9) for all animal species and categories. October 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

2. Chemically defined flavourings from Flavouring Group 09 - Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones (CDG 9) for all animal species and categories. Supplementary information. June 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

3. Chemically defined flavourings from Flavouring Group 09 - Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones (CDG 9) for all animal species and categories. Supplementary information. April 2012. 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 9 (CDG 9 Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones).

5. Comments from Member States received through the ScienceNet.

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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 09 (CDG09, Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones)[14]

The *Chemically Defined Flavourings - Group 09 (Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group including aliphatic lactones*), in this application comprises thirty 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 99.5%.

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* or in *water*.

For the identification of volatile chemically defined flavouring compounds *CDG 09* 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 EURL. The Applicant provided the typical chromatogram for the *CDG 09* 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 *CDG 09* 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 *CDG 09* in the *mixture of flavouring compounds*.

Based on the satisfactory experimental evidence provided, the EURL 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 EURL is

[14] The full report is available on the EURL website http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0097.pdf

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*.

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 EURL evaluation report, available from the EURL website.

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

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

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

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

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

MATERIALS AND METHODS

Cigarette Design

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

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

TABLE 1
Blend composition of prototype cigarettes

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

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

Cigarette Performance

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

In Vitro Study Design

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

TABLE 2
Ingredients added to test cigarettes in study 1

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

(Continued on next page)

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

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

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

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

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

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

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

Inhalation Toxicity Study Design

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

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

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

Smoke Generation and Exposure System

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

TABLE 3
Ingredients added to study 2 test cigarettes

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

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

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

Exposure Atmosphere Characterization

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

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

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

Animals and Animal Care

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

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

Body Weight and Clinical Observations

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

Respiratory Function Measurements

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

Carboxyhemoglobin and Plasma Nicotine Determinations

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

Clinical Pathology

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

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

Necropsy and Tissue Collection

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

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

Histopathology

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

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

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

Statistical Methods

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

RESULTS

Cigarette Performance

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

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

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

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

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

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

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

Note. Cig, cigarette.

Exposure Atmosphere Characterization

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

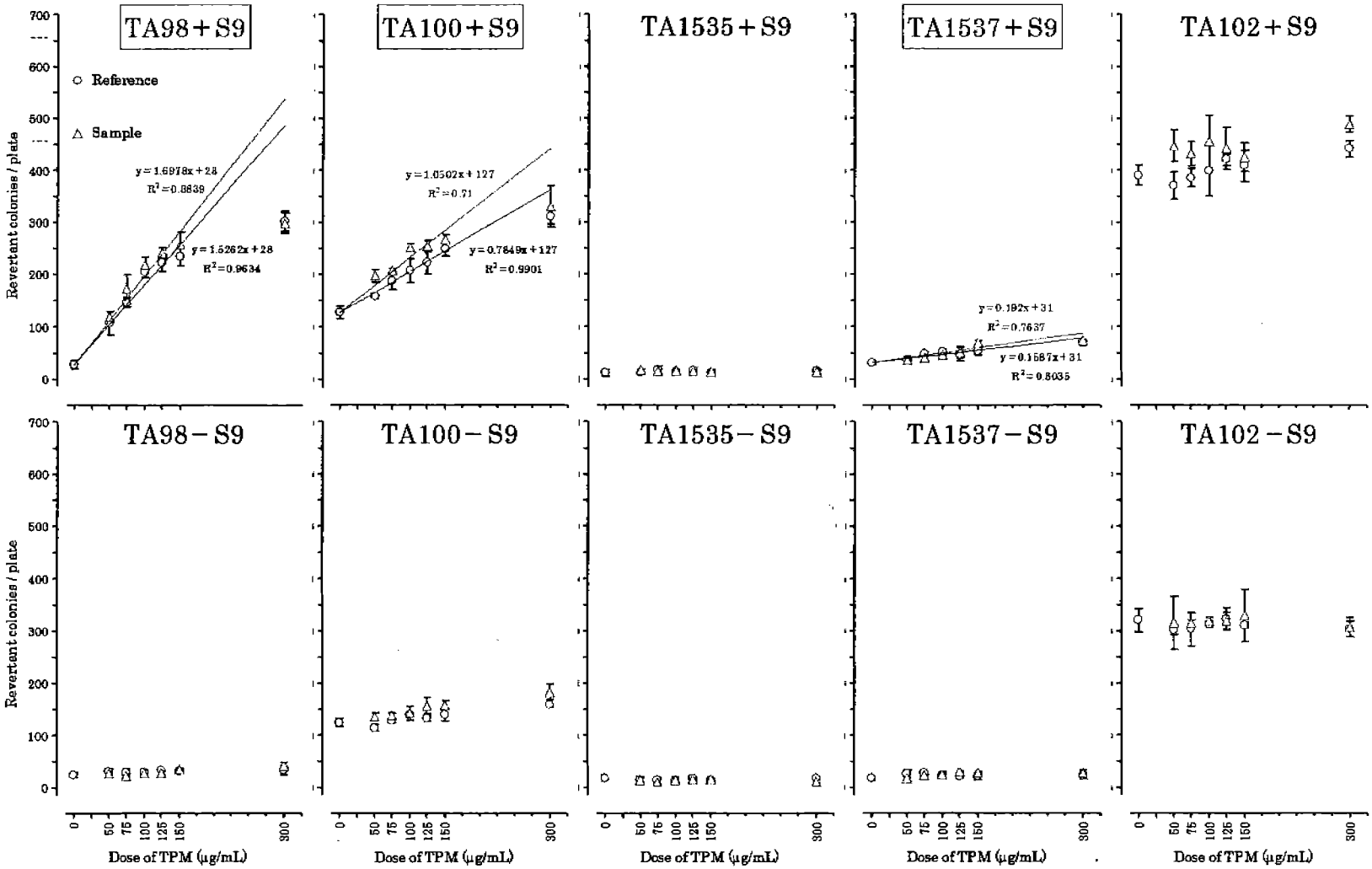
Body Weights and Clinical Observations

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

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

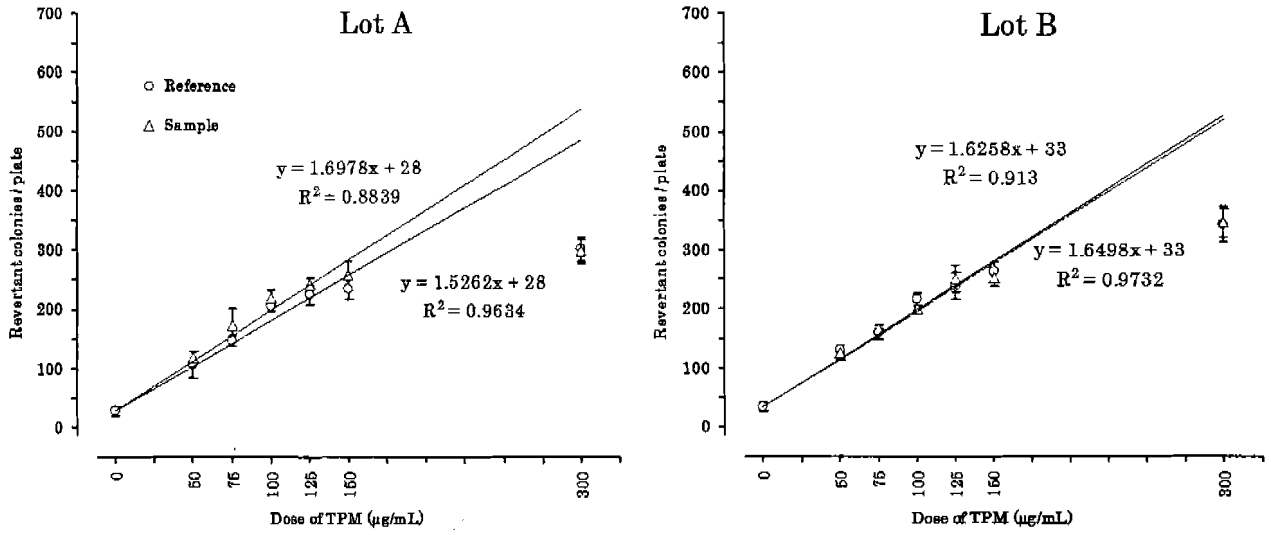
Organ Weights

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



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

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



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

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

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

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

Respiratory Physiology

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

Clinical Pathology

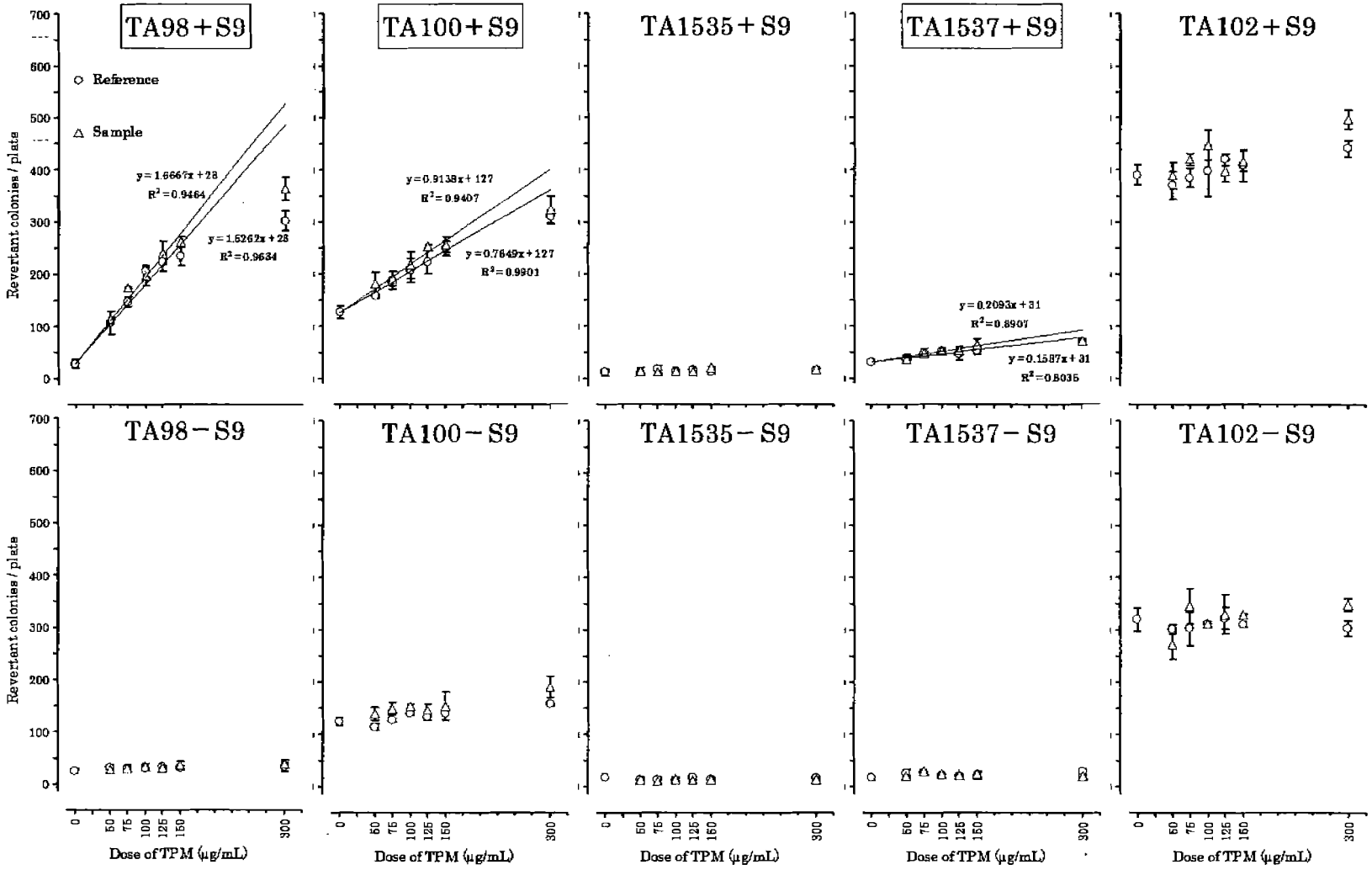
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2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

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

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

Pathology

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



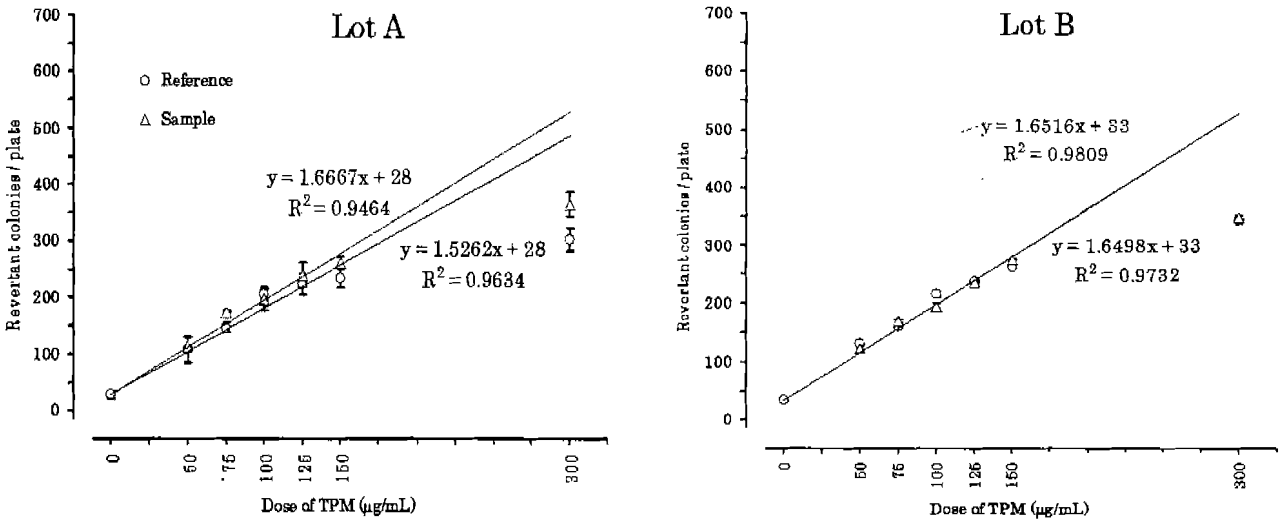
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

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

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

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



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

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

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

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

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

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

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

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

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

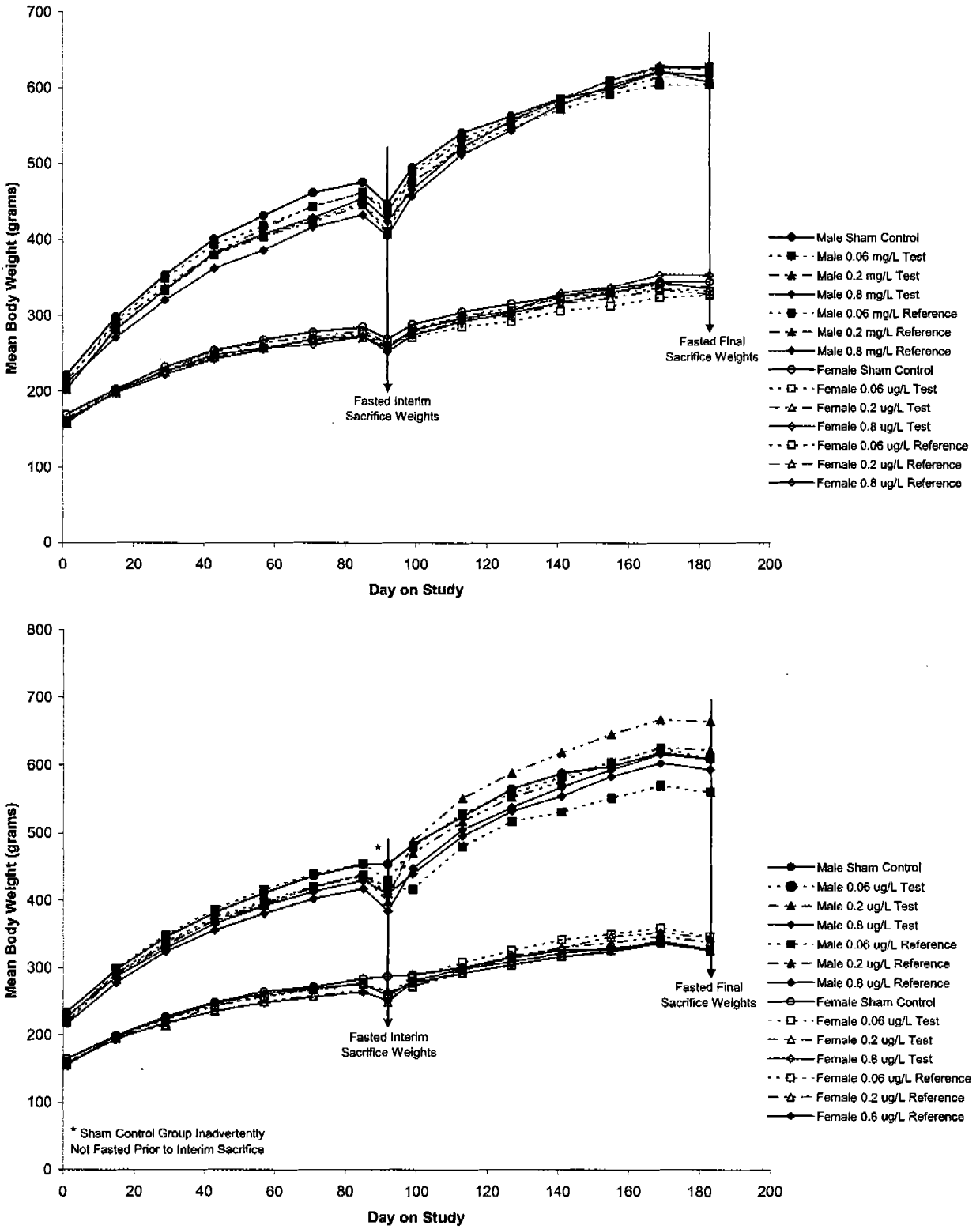


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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

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

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

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

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

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

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

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

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

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

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

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

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

Evaluation of Cell Proliferation Rates

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64 in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 10, 2010.

Dedication

Dr Paul M. Kuznesof

It was with great sadness that the Committee noted the passing of Dr Paul M. Kuznesof. Paul served on the Committee at the thirty-fifth meeting and from the forty-first until its sixty-ninth meeting in 2008, acting as FAO rapporteur on eight occasions and as Chairperson/Vice-Chairperson of five meetings. He brought wisdom, dedication and good humour to the work of the Committee. A measure of his commitment is indicated by the fact that he continued to prepare working papers for the seventy-first meeting of the Committee even though his illness ultimately prevented his attendance. His cheerful personality and valuable contribution to the Committee will be greatly missed.

In recognition of his services, the Committee dedicated this report to the memory of Paul.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 8 to 17 June 2010. The meeting was opened by Dr Asamoa-Baah, Deputy Director-General of the World Health Organization (WHO), on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Asamoa-Baah noted the long history of the Committee, illustrating the importance of its work. He also noted that this activity was undertaken jointly with FAO from the beginning and is one of the examples of excellent collaboration between these two United Nations organizations. Dr Asamoa-Baah emphasized that the two organizations are cognizant of the important contribution by experts in providing their time and expertise to the programme. He expressed his sincere appreciation to the experts for taking time from their very busy daily work schedules to prepare for and participate in these expert meetings. Dr Asamoa-Baah then informed the Committee about the recent World Health Assembly at which food safety was discussed. The large interest expressed in this topic reflects the global nature of and the increasing importance given to food safety by Member States. He also noted the increasing need by countries to have access to objective and clear advice on food safety matters.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-third meeting had completed declaration of interest forms and that no conflicts had been identified. The following declared interests and potential conflicts were discussed by the Committee. Professor Glenn Sipes serves on a scientific expert panel of the Research Institute of Fragrance Materials; Dr Josef Schlatter, Professor Gary Williams, Dr Barbara Petersen and Professor Andrew Renwick have consulted on steviol glycosides or related compounds and did not contribute to the discussions on these compounds, although these discussions related only to revisions of specifications. Professor Renwick consulted for several food manufacturers, but none of the consultancies were related to any of the compounds on the agenda (exception mentioned above).

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been 72 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-second meeting (Annex 1, reference *199*).

The tasks before the Committee were:

- to elaborate further principles for evaluating the safety of food additives, flavouring agents and contaminants in food (section 2);
- to review and prepare specifications for certain food additives (section 3 and Annex 2);
- to undertake toxicological evaluations of certain flavouring agents (section 4 and Annex 2);
- to undertake toxicological evaluations of certain contaminants in food (section 5 and Annex 2).

2.1 Modification of the agenda

When discussing the food additive sucrose esters of fatty acids produced from vinyl esters, the Committee decided to name this food additive sucrose monoesters of lauric, palmitic or stearic acid and to prepare a separate specifications monograph, as the impurities differed from those considered in the existing specifications of sucrose esters of fatty acids.

The revision of the specifications monographs of β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester and β-carotene (synthetic) was deferred to a future meeting, pending submission of data requested.

The food additive titanium dioxide was added to the agenda for revision of the specifications. Seven flavouring agents (Nos 2070–2076) were proposed for evaluation as additions to the previously evaluated group of saturated aliphatic acyclic secondary alcohols, acetals and related esters. However, only four of the seven flavouring agents (Nos 2070 and 2072–2074) are in

accordance with the group name. As all seven flavouring agents fit better into the previously evaluated group of aliphatic secondary alcohols, ketones and related esters, all substances were evaluated as additions to this group, and the group name was extended to include the acetals.

Flavour No. 2043, 2-aminoacetophenone, was on the agenda to be evaluated in the group of aromatic substituted secondary alcohols, ketones and related esters. Although the compound fulfils some of the structural requirements for this group, the main toxicologically relevant structural feature is the amino group; hence, the compound was not evaluated and should be evaluated in the future in the group of aliphatic and aromatic amines and amides.

Flavour No. 2069, (±)-2-phenyl-4-methyl-2-hexenal, was on the agenda to be evaluated in the group of benzyl derivatives. However, as it does not meet the structural requirements for this group, the compound was not evaluated at this meeting.

2.2 Report from the Forty-second Session of the Codex Committee on Food Additives (CCFA) and the Fourth Session of the Codex Committee on Contaminants in Foods (CCCF)

The Chairperson of the CCFA, Dr Junshi Chen, informed the Committee about the principal achievements and outputs of the Forty-second Session of CCFA. CCFA had forwarded 123 food additive provisions to the Codex Alimentarius Commission for adoption. In addition, amendments to the International Numbering System for Food Additives (*2*) and to names and descriptors of some food categories of the Codex General Standard for Food Additives (*3*)—namely, food categories 06.0, 06.2 and 06.2.1—were proposed for adoption. As well, 28 new and revised specifications for the identity and purity of food additives, prepared by the seventy-first meeting of the Committee, were proposed for adoption as Codex specifications. CCFA finalized work on the Guidelines on Substances Used as Processing Aids (*4*), which were forwarded to the Commission for adoption.

CCFA also took action as a result of various changes in acceptable daily intake (ADI) status and other toxicological recommendations arising from the seventy-first meeting of the Committee and agreed on a list of priority compounds to be evaluated by JECFA.

Ms Annamaria Bruno of the Codex Secretariat informed the Committee about the principal achievements and outputs of the Fourth Session of CCCF. CCCF considered the conclusions of the assessments of the seventy-second meeting of the Committee. CCCF agreed to initiate new work on maximum limits for deoxynivalenol (DON) in cereals and cereal products. With regard to acrylamide, CCCF agreed to encourage the use of the Code of Practice for

the Reduction of Acrylamide in Foods; to recommend further research on mitigation measures and their impact on acrylamide production; and to reconsider work on acrylamide in the future to allow sufficient time for the implementation of the Code of Practice.

CCCF agreed to develop discussion papers on arsenic in rice and on furan and agreed on a priority list of substances for evaluation by JECFA.

2.3 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in the new publication, Environmental Health Criteria, No. 240, *Principles and methods for the risk assessment of chemicals in food*, published in 2009 (*5*).

2.4 Food additive specifications

2.4.1 *HPLC methods for subsidiary dyes and isomers in food colours*

The Committee at its current meeting noted the need for high-performance liquid chromatographic (HPLC) methods for the separation and quantification of subsidiary dyes and isomers in food colours to replace the paper chromatographic method in Volume 4 of the *Combined compendium of food additive specifications* (Annex 1, reference *180*). Producers of food colours, industries and organizations are encouraged to notify the FAO JECFA Secretariat of appropriate methods.

2.4.2 *Withdrawal of specifications*

2.4.2.1 *Annatto extract (oil-processed bixin)*

During its sixty-seventh meeting (Annex 1, reference *184*), the Committee prepared tentative specifications for annatto extract (oil-processed bixin) and requested information on chemical characterization of the non-colouring matter compounds. The Committee also decided that the tentative specifications would be withdrawn if sufficient information was not received before the end of 2008. As this information had not been received, the Committee decided to withdraw the existing tentative specifications.

2.5 Update on the activities of GEMS/Food

The Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) is composed of 1) a

network of about 140 national contact points submitting data to WHO, 2) a database on chemical occurrence and exposure, 3) the GEMS/Food consumption cluster diets, 4) a training course on total diet studies (TDSs) for capacity building and 5) the monitoring of human milk for persistent organic pollutants (POPs). In order to improve both the networking and the GEMS/Food database, the following changes are proposed by WHO:

- *Modification of the status for data providers:* Currently, data collection for GEMS/Food is performed by an informal network of institutions. In order to improve overall network collaboration, the institutions submitting data will be encouraged to obtain official status as National Institutions recognized by WHO (National GEMS/Food Centres or NGCs). This process has begun with about 50 institutions around the world, which will then be able to develop multilateral collaborations with other data providers as well as with the WHO GEMS/Food Collaborating Centres, which also deal with methodological developments and training.
- *Update of the information technology system for data submission:* The submission of data to the GEMS/Food database is currently done electronically via software (OPAL) installed locally at each of the National Institutions. Because of the difficulties in updating such a system, a web-based system (OPAL-web) will be developed. The NGCs can then upload XML or Excel files directly into the GEMS/Food database via the WHO web site.
- *Development of a common food classification system for data exchange:* The GEMS/Food database is based on the Codex Classification of Foods and Animal Feed, which includes mainly primary food products. This classification often does not fit the purpose of preparing dietary exposure assessments, which include processed foodstuffs. The key issue will be to determine the adequate level of specificity for each category. It has been noted that the European Food Safety Authority (EFSA) is currently undertaking a revision of food groupings and codings, with which the GEMS/Food groups should be harmonized as appropriate.

WHO has recently set up two working groups to consider occurrence data and food consumption data, respectively. The conclusions and recommendations of these working groups will be used to improve GEMS/Food with regard to data submission, storage and interchange.

The Committee also recommends improving web access to the GEMS/Food database and allowing data extraction.

2.6 Possible improvements in dietary exposure assessment as a consequence of increased data submissions

JECFA evaluated the safety of cadmium at its sixteenth and several subsequent meetings (e.g. at its fifty-fifth meeting in 2000; Annex 1, reference *149*). In 2000, the international estimates of dietary exposure were based on the combination of the five GEMS/Food regional diets with a set of about 6000 analytical results on cadmium concentrations. At the current meeting, the evaluation was based on more than 150 000 analytical results on cadmium concentrations and on national dietary exposures using individual food consumption surveys. In general, the increased data availability illustrated by the above cadmium example enables the preparation of improved dietary exposure assessments and allows a stochastic approach to or stochastic modelling of dietary exposures instead of point estimates. This shift would imply that, in general:

- the handling of censored data (i.e. below the limit of detection [LOD] or limit of quantification [LOQ]), which can have a major impact on exposure estimates, needs additional consideration;
- the collection of food consumption data from individuals, including children, needs to be one of the objectives of GEMS/Food. This would be in addition to the collection of data for the consumption cluster diets;
- data collected should include information on the data source, the purpose of data collection and the representativeness of the analysed samples. Information should also be given on analytical techniques and sample preparation;
- the kinetics of elimination for chemicals with a long half-life in the human body is part of the process of establishing health-based guidance values and needs to be integrated as well in the dietary exposure assessment;
- guidelines on the application of stochastic modelling by the Committee should be developed, as well as software allowing this modelling. A stochastic approach to combine data on food consumption with data on food composition needs to be implemented.

2.7 Further consideration of combined intakes of flavouring agents

At the sixty-eighth meeting (Annex 1, reference *187*), the Committee decided that the safety assessment of possible combined intakes of flavouring agents should be based on the combined exposure to a common metabolite (on a molecular weight basis) or to a homologous series. For each common metabolite or homologous series, the intake estimates for about four or five flavouring agents with the highest intakes are summed. Following the introduction

of the single portion exposure technique (SPET) for dietary exposure assessment of flavouring agents, the Committee concluded at the sixty-ninth meeting (Annex 1, reference *190*) that the maximized survey-derived intake (MSDI) values should be used for calculating the combined intake.

The calculated combined intake is compared with the threshold of concern for the structural class of the common metabolite or the highest structural class relevant to the homologous series. When considering the combined intake for additional flavouring agents evaluated at the present meeting, the Committee recognized the amount of work required to develop data on combined intake and recommended that screening assessments should be used to determine whether such data are necessary. The Committee recommends that the following screening assessments should be used:

1. Many of the MSDIs for additional groups of flavouring agents are very low. Evaluation of combined intake is not necessary if the highest MSDI value in the additional group is less than 20 μg/day, because the combined intake for the highest four or five intakes would not exceed the lowest threshold of concern (90 μg/day for structural class III).

2. When an additional group contains compounds with low MSDIs compared with flavouring agents in the same group evaluated previously, consideration of combined intake is not necessary because it can be concluded that the additional flavouring agents would not contribute significantly to the combined intake of the flavouring group.

3. If the highest MSDI value in an additional group of flavouring agents is greater than 20 μg/day, then identification of a common metabolite or homologous series should be undertaken, but calculation of the combined intake would not be necessary if the highest MSDI is less than 20% of the relevant threshold of concern, because the combined intake for the highest four or five intakes would not exceed the relevant threshold of concern.

3. Specific food additives (other than flavouring agents)

The Committee revised the specifications for seven food additives. Information on the specifications is summarized in Annex 2. Details of information required for certain substances are given in Annex 3.

3.1 Revision of specifications

3.1.1 *Activated carbon*

The Committee at its thirty-seventh meeting (Annex 1, reference *94*) prepared specifications for activated carbon and included test methods for the determination of alcohol-soluble substances and higher aromatic compounds. At its current meeting, the Committee recognized that these methods were in need of revision. The specifications were revised accordingly.

3.1.2 *Cassia gum*

The seventy-first meeting of the Committee (Annex 1, reference *196*) prepared tentative specifications for cassia gum. In order to be able to remove the tentative status, the Committee requested a suitable method for the determination of anthraquinones at a level of less than 0.5 mg/kg in cassia gum. An HPLC method for the determination of anthraquinones was submitted. The Committee revised the specifications and removed the tentative designation.

3.1.3 *Indigotine*

The Committee was informed of an error in the current specifications for indigotine, under method of assay, for the determination of isomer content by HPLC. The Committee revised the existing specifications by introducing an HPLC method for the determination of the main component, its isomer and subsidiary colouring matter. The paper chromatographic method for subsidiary colouring matter was removed.

3.1.4 *Steviol glycosides*

The Committee was requested to add two new steviol glycosides, rebaudiosides D and F, to the seven named steviol glycosides in the existing specifications. The specifications were revised to include the new steviol glycosides as requested, and the method of assay was revised accordingly.

3.1.5 *Sucrose esters of fatty acids*

The Committee revised the existing method of assay for sucrose esters of fatty acids to correspond with the method of assay used for sucrose oligoesters type I and type II.

3.1.6 *Sucrose monoesters of lauric, palmitic or stearic acid*

The Committee was requested to consider the inclusion of sucrose esters of fatty acids manufactured by the reaction of sucrose with vinyl esters of lauric, palmitic or stearic acid within the existing specifications monograph for sucrose esters of fatty acids. However, the Committee noted that the new sucrose esters were different from those covered by the existing specifications monograph for sucrose esters of fatty acids in terms of starting materials, manufacturing process, composition and potential impurities. The Committee therefore decided that it was more appropriate to establish new specifications for the new sucrose esters under the name "sucrose monoesters of lauric, palmitic or stearic acid".

When establishing these new specifications, the Committee considered the toxicology of the potential impurities resulting from the use of the new sucrose esters, based on a proposed limit of 10 mg/kg for vinyl laurate, vinyl palmitate and vinyl stearate, a proposed limit of 1 mg/kg for acetaldehyde and a worst-case maximum level of 20 mg/kg for *p*-methoxyphenol in the new sucrose esters.

The proposed limit of 10 mg/kg for the vinyl esters would result in an estimated dietary exposure of 0.0026 mg/day (0.001% of the dietary exposure of the corresponding sucrose monoester, i.e. 260 mg/day). The vinyl esters would be hydrolysed in the intestine to release vinyl alcohol, which would immediately tautomerize to acetaldehyde in amounts of less than 0.001 mg/day. The amounts of acetaldehyde formed (equivalent to less than 0.000 02 mg/kg body weight [bw] per day) are not a safety concern, as there is a margin of exposure of more than 1 million between this value and the no-observed-adverse-effect level (NOAEL) of 125 mg/kg bw per day for acetaldehyde in a 28-day toxicity study in rats (*6*).

The proposed limit of 1 mg/kg for acetaldehyde would result in an estimated dietary exposure of 0.000 26 mg/day (0.0001% of that of the corresponding sucrose monoester). This amount of acetaldehyde is equivalent to 0.000 004 mg/kg bw per day and is not a safety concern, as there is a margin of exposure of more than 10 million between this value and the NOAEL of 125 mg/kg bw per day for acetaldehyde in a 28-day toxicity study in rats (*6*).

The levels of *p*-methoxyphenol reported in batches of sucrose esters of lauric, palmitic and stearic acids (<0.0001%) would give an estimated dietary exposure of <0.000 26 mg/day. A *p*-methoxyphenol concentration of 20 mg/kg is used in the vinyl esters. Even if all of the *p*-methoxyphenol were to be present in the final product, the estimated dietary exposure would be less than 0.005 mg/day. Such amounts of *p*-methoxyphenol are not a safety concern because of its simple structure, its low potential for toxicity and its predicted rapid urinary excretion following metabolism by conjugation with glucuronic acid and sulfate. As a result, the Committee decided that a limit for *p*-methoxyphenol was not necessary.

When considering sucrose oligoesters type I and II at the seventy-first meeting (Annex 1, reference *196*), the Committee noted that type I sucrose oligoesters contained 80–100% monoesters to triesters. These esters were included within the group ADI of 0–30 mg/kg bw for sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II, and this group ADI would also apply to sucrose monoesters of lauric, palmitic or stearic acid. The Committee prepared new specifications, including an assay for the total content of sucrose esters, the content of monoesters, as well as limits and analytical methods for vinyl laurate, vinyl palmitate, vinyl stearate and acetaldehyde.

The specifications were made tentative pending the submission of a test method capable of distinguishing sucrose monoesters of lauric, palmitic or stearic acid from sucrose esters of fatty acids. The tentative specifications will be withdrawn if the requested data are not received by the end of 2011.

3.1.7 *Titanium dioxide*

The Committee at its seventy-first meeting (Annex 1, reference *196*) revised the specifications for titanium dioxide. At its current meeting, the Committee noted that the assay method was in need of a minor revision. The specifications were revised accordingly.

4. Flavouring agents

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

Assignment to structural class

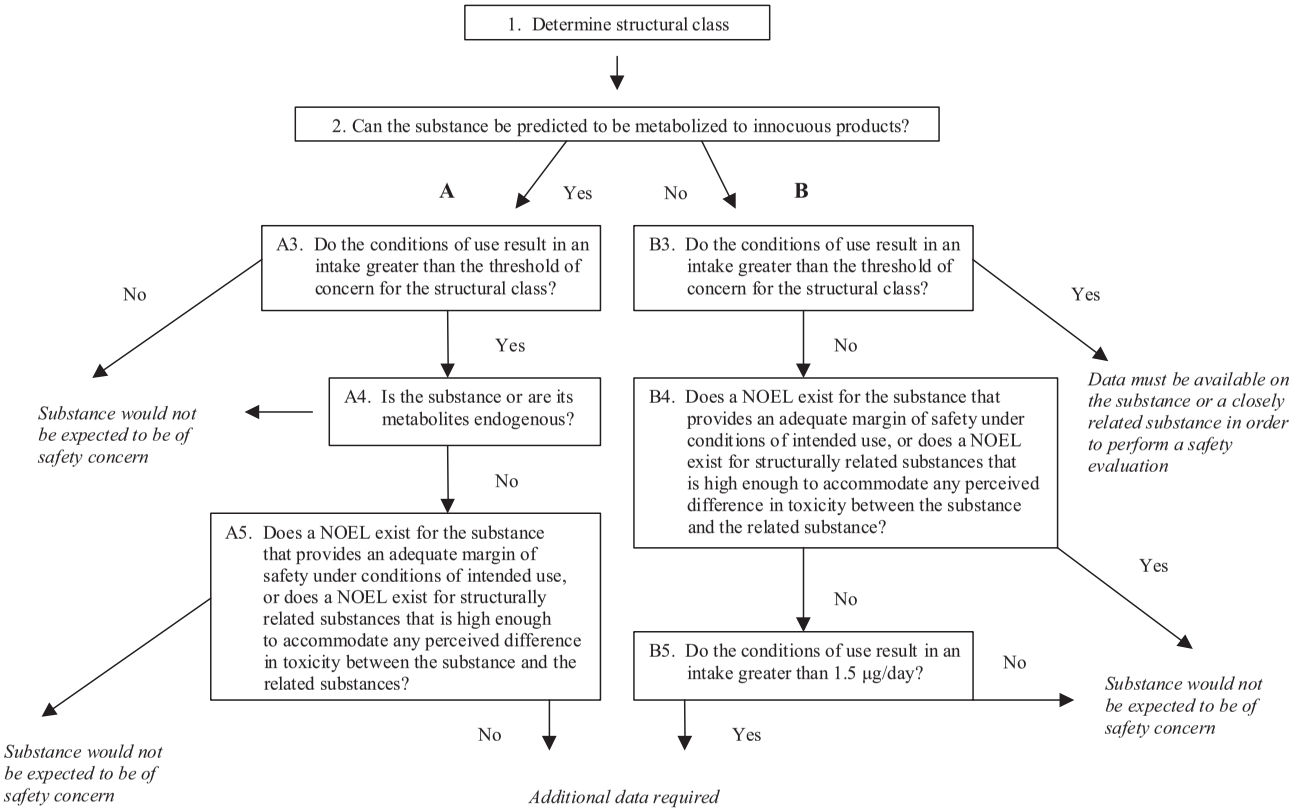
Twelve groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned 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 that 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 (Annex 1, reference *122*):

- *Innocuous metabolic products* are defined as products that are known or readily predicted to be harmless to humans at the estimated dietary exposure to the flavouring agent.

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



- *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 dietary exposure to 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.

Assessment of dietary exposure

Maximized survey-derived intake (MSDI)

Estimates of the dietary exposure to flavouring agents by populations are based on annual volumes of production. These data were derived from surveys in Europe, Japan and the USA. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products when compiling these data. When using these production volumes to estimate dietary exposures, a correction factor of 0.8 is applied to account for under-reporting.

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

The population of consumers was assumed to be 32×10^6 in Europe, 13×10^6 in Japan and 28×10^6 in the USA.

Single portion exposure technique (SPET)

The SPET was developed by the Committee at its sixty-seventh meeting (Annex 1, reference *184*) to account for presumed patterns of consumer behaviour with respect to food consumption and the possible uneven distribution of dietary exposures among consumers of foods containing flavouring agents. It is based on reported use levels supplied by the industry. This single portion–derived estimate was designed to account for individuals' brand loyalty to food products and for niche products that would be expected to be consumed by only a small proportion of the population. Its use in the Procedure was endorsed at the sixty-ninth meeting of the Committee (Annex 1, reference *190*) to render the safety assessment more robust, replacing the sole use of MSDI estimates with the higher of the highest MSDI or the SPET estimate as the exposure estimate in the decision-tree. The Committee also agreed that it would not be necessary to re-evaluate flavouring agents that had already been assessed previously using the Procedure.

The SPET provides an estimate of dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day. The SPET combines an average (or usual) added use level provided by the flavour industry with a standard portion size from 75 predefined food

categories as described by the Committee at its sixty-seventh meeting. The standard portion is taken to represent the mean food consumption for consumers of these food categories. Among all the food categories with a reported use level, the calculated dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate:

SPET (μg/day) = standard portion size of food category *i* (g/day) × use level for food category *i* (μg/g)

The highest result is used in the evaluation.

The use level data provided by industry for each flavouring agent evaluated at this meeting and used in the SPET calculations are available on the WHO JECFA web site at http://www.who.int/ipcs/publications/jecfa/en/.

4.1.1 *Alicyclic ketones, secondary alcohols and related esters: additional compounds*

The Committee evaluated 12 additional flavouring agents that are members of a group entitled alicyclic ketones, secondary alcohols and related esters. The additional flavouring agents included one saturated alicyclic ketone (No. 2050), two unsaturated alicyclic ketones (Nos 2049 and 2052), one alicyclic diether (No. 2051), one alicyclic secondary ester (No. 2053), one alicyclic α-hydroxy ketone (No. 2054), two unsaturated alicyclic keto-esters (Nos 2055 and 2056), one tri-unsaturated alicyclic ketone (No. 2057), one di-unsaturated alicyclic keto-hydroxy-diol (No. 2058) and two di-unsaturated bicyclic keto-ethers (Nos 2059 and 2060). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has been evaluated previously.

The Committee previously evaluated 25 other members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*). The Committee concluded that all 25 flavouring agents in that group were of no safety concern based on estimated dietary exposures.

Four of the 12 flavouring agents (Nos 2052, 2054, 2057 and 2058) in this group have been reported to occur naturally and can be found in honey, black teas, green and roasted mate, tomatoes and tomato juice, starfruit, clams, coffee, hazelnuts and grapefruit juice.

Assessment of dietary exposure

The total annual volumes of production of the 12 alicyclic ketones, secondary alcohols and related esters are approximately 0.4 kg in the USA and 18 kg in Japan. Approximately 55% of the total annual volume of production in Japan

is accounted for by one substance in this group—namely, cyclotene butyrate (No. 2056).

The estimated dietary exposures for each flavouring agent, calculated either as the MSDI or using the SPET, are reported in Table 1. The estimated daily dietary exposure is greatest for (–)-8,9-dehydrotheaspirone (No. 2059) (4000 μg, the SPET value obtained from milk [dairy] and other fermented milk products). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 600 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

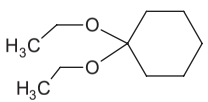
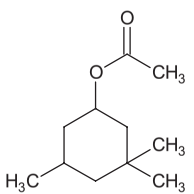
The esters in this group (Nos 2053 and 2055–2056) and the ketal (No. 2051) are predicted to be hydrolysed to their corresponding alcohols and carboxylic acids by carboxylesterases found in abundance in hepatocytes. The resulting alicyclic secondary alcohols can be interconverted enzymatically with the corresponding ketone in vivo. The principal detoxication pathway involves reduction of the ketone to yield the corresponding secondary alcohol, which is conjugated with glucuronic acid and excreted mainly in the urine. Side-chain oxidation, glutathione conjugation of α,β-unsaturated ketones and hydrogenation of endocyclic or exocyclic double bonds are other elimination pathways involved. Polar oxygenated metabolites are excreted primarily in the urine, either unchanged or as conjugates.

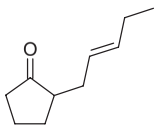
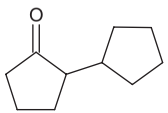
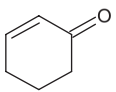
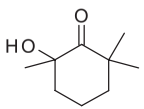
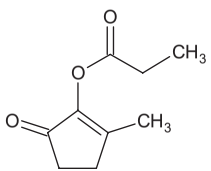
The alicyclic ketones in this group (Nos 2049–2050, 2052 and 2054–2060) are likely to be reduced to the corresponding secondary alcohol and excreted primarily as the glucuronic acid conjugate. If a double bond is present, it may be reduced to the corresponding dihydro- derivative. For metabolites excreted into the bile, reduction of the double bond may occur, mediated by the gut microflora. Endocyclic double bonds (Nos 2052 and 2055–2060) are more prone to reduction compared with exocyclic double bonds (Nos 2049 and 2057–2058). In addition to reductive pathways, alicyclic ketones containing an alkyl or alicyclic side-chain (Nos 2049, 2050 and 2054–2060) may undergo oxidation of the side-chain to form polyoxygenated metabolites, which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

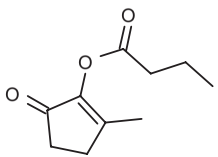
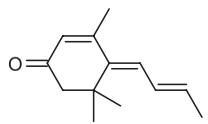
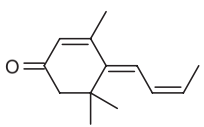
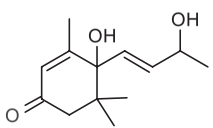
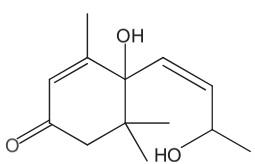
Application of the Procedure for the Safety Evaluation of Flavouring Agents

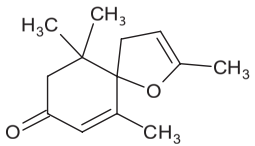
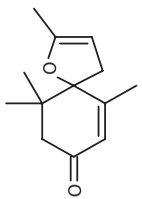
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned two flavouring agents (Nos 2051 and 2053) to structural class I, eight flavouring agents (Nos 2049, 2050, 2052 and 2054–2058) to structural class II and two flavouring agents (Nos 2059 and 2060) to structural class III.

Table 1
Summary of the results of the safety evaluations of alicyclic ketones, secondary alcohols and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I						
Cyclohexanone diethyl ketal	2051	1670-47-9 	A3. No, SPET: 400	NR	Note 1	No safety concern
3,3,5-Trimethylcyclohexyl acetate	2053	67859-96-5 	A3. No, SPET: 150	NR	Note 1	No safety concern

Structural class II						
2-(*trans*-2-Pentenyl) cyclopentanone	2049	51608-18-5 	A3. No, SPET: 450	NR	Note 2	No safety concern
2-Cyclopentylcyclopentanone	2050	4884-24-6 	A3. No, SPET: 400	NR	Note 2	No safety concern
2-Cyclohexenone	2052	930-68-7 	A3. No, SPET: 200	NR	Note 3	No safety concern
2,6,6-Trimethyl-2-hydroxycyclohexanone	2054	7500-42-7 	A3. No, SPET: 300	NR	Note 4	No safety concern
Cyclotene propionate	2055	87-55-8 	A3. No, SPET: 300	NR	Note 1	No safety concern

Cyclotene butyrate	2056	68227-51-0 	A3. No, SPET: 200	NR		Note 1	No safety concern
4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (mixture of isomers)	2057	13215-88-8  	A3. No, SPET: 300	NR		Notes 2 and 3	No safety concern
4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (mixture of isomers)	2058	24427-77-8  	A3. No, SPET: 300	NR		Notes 2 and 3	No safety concern

Structural class III						
(−)-8,9-Dehydrotheaspirone	2059	85248-56-2 	B3. Yes, SPET: 4000	The NOAEL of 60 mg/kg bw per day in a 28-day oral study in rats for the structural analogue No. 2060 is 900 (based on the SPET) and >1 million (based on the MSDI) times the estimated daily dietary exposure to No. 2059 when used as a flavouring agent.	Notes 2 and 3	No safety concern
(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	2060	80722-28-7 	B3. Yes, SPET: 600	The NOAEL of 60 mg/kg bw per day in a 28-day oral study in rats for No. 2060 is at least 6000 times its estimated daily dietary exposure when used as a flavouring agent.	Notes 2 and 3	No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure.

[a] Twenty-five flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *161*).

[b] *Step 1:* Two flavouring agents in this group (Nos 2051 and 2053) are in structural class I. Eight flavouring agents in this group (Nos 2049, 2050, 2052 and 2054–2058) are in structural class II. Two flavouring agents in this group (Nos 2059 and 2060) are in structural class III.

[c] *Step 2:* Ten agents in this group (Nos 2049–2058) are expected to be metabolized to innocuous products. Two agents (Nos 2059 and 2060) are not expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. Metabolized by hydrolysis of ester, glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid and/or reduction of the ketone, resulting from ketal hydrolysis, to an alcohol, which would be conjugated and excreted.
2. Metabolized by reduction of the ketone and alkyl side-chain oxidation and excretion.
3. Metabolized by reduction of the ketone functional group, followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone.
4. Metabolized by reduction of the ketone, followed by glucuronic acid conjugation of the corresponding alcohol.

Step 2. Ten flavouring agents in this group (Nos 2049–2058) are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. Two of the flavouring agents in this group (Nos 2059 and 2060) cannot be predicted to be metabolized to innocuous products. The evaluation of these two flavouring agents therefore proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily intakes of the two flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/day for class I). The highest estimated daily intakes of the eight flavouring agents in structural class II are below the threshold of concern (i.e. 540 μg/day for class II). The safety of these 10 flavouring agents raises no concern at current estimated dietary exposures.

Step B3. The highest estimated daily intakes of the two flavouring agents in structural class III (Nos 2059 and 2060) are above the threshold of concern (i.e. 90 μg/day for class III). Accordingly, additional data are necessary for the evaluation of these flavouring agents.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

Additional data were evaluated for (–)-8,9-dehydrotheaspirone (No. 2059) and (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060), as the estimated intakes exceeded the threshold of concern for structural class III (90 μg/day).

A NOAEL of 60 mg/kg bw per day for (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) was identified in a 28-day oral study. In this study, doses of 12, 60 or 300 mg/kg bw per day were administered by gavage to rats (10 of each sex per dose). No changes attributable to No. 2060 were reported for body weight, food or water consumption, haematological examination or urinalyses. Some behavioural/motor effects were observed at 300 mg/kg bw per day. Changes in serum enzyme activities and cholesterol and triglyceride levels were reported at the end of the study in those rats treated with the 300 mg/kg bw per day dose. An increase in liver weight was reported for females only at 60 mg/kg bw per day. This change was considered non-adverse and led to the designation of 60 mg/kg bw per day as the NOAEL. This NOAEL provides a margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2060 (SPET = 600 μg/day) when used as a flavouring agent.

(–)-8,9-Dehydrotheaspirone (No. 2059) is a close structural analogue of (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060), and toxicological studies on that compound can be used for the evaluation of No. 2059. The NOAEL of 60 mg/kg bw per day provides a margin of safety

of 900 in relation to the highest estimated dietary exposure to No. 2059 (SPET = 4000 μg/day) when used as a flavouring agent. The Committee noted that the margin of safety of 900 between the SPET estimate for No. 2059 and the NOAEL for No. 2060 is lower than the value of 1000, which was proposed as an adequate margin of safety for flavouring agents on the B-side of the decision-tree at the forty-fourth meeting of the Committee (Annex 1, reference *116*). The value of 1000 was based on the comparison of the NOAEL with the MSDI. The Committee noted that the margin of safety for No. 2059 based on the MSDI of 0.02 μg/day and the NOAEL of 60 mg/kg bw per day for No. 2060 exceeds 1 million and concluded that the values of 900 (based on the SPET) and greater than 1 million (based on the MSDI) provided an adequate margin of safety.

The Committee therefore concluded that both (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) and (–)-8,9-dehydrotheaspirone (No. 2059) would not pose safety concerns at current estimated dietary exposures.

Table 1 summarizes the evaluations of the 12 alicyclic ketones, secondary alcohols and related esters (Nos 2049–2060) in this group of flavouring agents.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Flavouring agents in this group with the highest intakes that have the common metabolite cyclohexanol are Nos 1093, 1094–1097 and 2051 in structural class I and No. 1100 in structural class II. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 10.2, 7.3 and 1.1 μg/day, respectively, which would not exceed either threshold of concern (i.e. 1800 μg/day for class I and 540 μg/day for class II).

Flavouring agents in this group with the highest intakes that have the common metabolite cyclohexanol or a cyclohexenol derivative are Nos 1099 and 2053 in structural class I, Nos 1098, 1108, 1109, 1111–1113, 2052 and 2054 in structural class II and No. 2059 in structural class III. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 22.5, 4.9 and 0.3 μg/day, respectively, which would not exceed any of the thresholds of concern (i.e. 1800 μg/day for class I, 540 μg/day for class II and 90 μg/day for class III).

Flavouring agents in this group with the highest intakes that have a cyclopentanol derivative as the common metabolite are Nos 1101, 1106 and 1114–1117 in structural class I and Nos 2049, 2050, 2055 and 2056 in structural class II. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 31, 21.2 and 2.2 μg/day, respectively, which would not exceed either threshold of concern (i.e. 1800 μg/day for class I and 540 μg/day for class II).

The overall evaluation of the data indicates that combined intakes would not raise concern about safety at current estimated dietary exposures.

Consideration of secondary components

Two flavouring agents in this group (Nos 2053 and 2055) have minimum assay values of less than 95%. The secondary component of 3,3,5-trimethylcyclohexyl acetate (No. 2053) is 3,3,5-trimethylcyclohexanol (No. 1099). The secondary component of cyclotene propionate (No. 2055) is cyclotene (No. 418). Nos 1099 and 418 were evaluated at the fifty-ninth and fifty-fifth meetings of the Committee (Annex 1, references *149* and *160*), respectively, and were found to be of no safety concern. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the previous evaluation of flavouring agents in this group, studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *160*).

The Committee concluded that these 12 flavouring agents, which are additions to the group of alicyclic ketones, secondary alcohols and related esters evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.2 *Alicyclic primary alcohols, aldehydes, acids and related esters: additional compounds*

The Committee evaluated 11 additional flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters, which was evaluated previously. The additional flavouring agents included three saturated and unsaturated primary alcohols (Nos 1903–1905), four aldehydes

(Nos 1900, 1902, 1906 and 1908), two acids (Nos 1899 and 1907), one acetal (No. 1901) and one related ester (No. 1898). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 26 other members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*). The Committee concluded that all 26 flavouring agents in this group were of no safety concern at estimated dietary exposures.

Three of the 11 flavouring agents in this group are natural components of foods (Nos 1898, 1905 and 1906). Methyl dihydrojasmonate (No. 1898), for example, has been detected in tea, 1,3-*p*-menthadien-7-al (No. 1906) in cumin seed and honey and *p*-menthan-7-ol (No. 1905) in cherries, citrus fruits, berries, dill and grape brandy.

Assessment of dietary exposure

The total annual volumes of production of the 11 flavouring agents in this group are approximately 6321 kg in Europe, 15 388 kg in the USA and 93 kg in Japan. Methyl dihydrojasmonate (No. 1898) contributes the most to the total annual production volumes in Europe, Japan and the USA (100%, 94% and 100%, respectively).

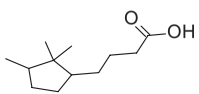
The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 2. The highest daily dietary exposure is estimated for *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899) (3000 µg, the SPET value obtained from non-alcoholic beverages), followed by methyl dihydrojasmonate (No. 1898) (1875 µg, the MSDI). For all but one of the other flavouring agents, the estimated daily dietary exposures were higher using the SPET and were in the range of 0.01–240 µg.

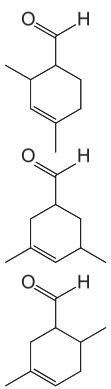
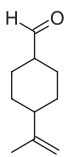
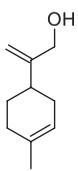
Absorption, distribution, metabolism and elimination

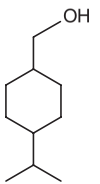
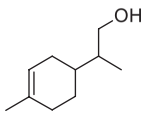
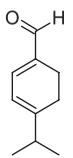
Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters has previously been described in the report of the fifty-ninth meeting of the Committee (Annex 1, reference *160*). Some additional data on absorption and metabolism have been submitted on one compound evaluated previously (perillyl alcohol or *p*-mentha-1,8-dien-7-ol, No. 974) (O'Brien, 2004), and these are in line with the information described in the report of the fifty-ninth meeting.

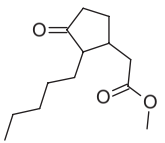
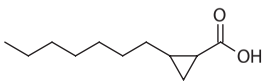
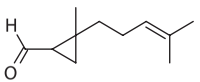
Table 2

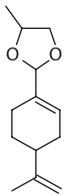
Summary of the results of the safety evaluations of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I						
cis-4-(2,2,3-Trimethylcyclopentyl)butanoic acid	1899	957136-80-0 	A3. Yes, SPET: 3000	A5. Yes. The NOEL of 12 mg/kg bw per day from a 90-day study in rats with the structurally related substance 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde (No. 967) is at least 240 times the estimated daily dietary exposure to No. 1899 when used as a flavouring agent.	Note 1	No safety concern

Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	1900	27939-60-2 	A3. No, SPET: 150	NR	Note 1	No safety concern
(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	1902	22451-50-9 (*cis*); 22451-49-6 (*trans*) 	A3. No, MSDI: Europe ND USA 0.7 Japan ND	NR	Note 1	No safety concern
d-Limonen-10-ol	1903	38142-45-9 	A3. No, SPET: 3	NR	Note 1	No safety concern

p-Menthan-7-ol	1904	5502-75-0 	A3. No, SPET: 150	NR	Note 1	No safety concern
p-Menth-1-en-9-ol	1905	18479-68-0 	A3. No, SPET: 30	NR	Note 1	No safety concern
1,3-*p*-Menthadien-7-al	1906	1197-15-5 	B3. No, SPET: 30	B4. Yes. The NOELs of 15, 33.9 and 33 mg/kg bw per day for, respectively, *trans*,*trans*-2,4-hexadienal (No. 1175), 2-*trans*-4-*trans*-decadienal (No. 1190) and 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (No. 1198) from 14-week studies in rats (Nos 1175 and 1190) and a 4-week study in rats (No. 1198) are at least 30 000–67 800 times the estimated daily dietary exposure to No. 1906 when used as a flavouring agent.	Note 1	No safety concern

Structural class II						
Methyl dihydrojasmonate	1898	24851-98-7 	A3. Yes, MSDI: Europe 676 USA 1875 Japan 23	A5. Yes. The NOEL of 80 mg/kg bw per day for maternal toxicity from a study of prenatal developmental toxicity in rats is at least 2580 times the estimated daily dietary exposure to No. 1898 when used as a flavouring agent.	Notes 1 and 2	No safety concern
cis- and *trans*-2-Heptylcyclopropanecarboxylic acid	1907	697290-76-9 (*cis*); 697290-77-0 (*trans*) 	A3. No, SPET: 1	NR	Note 1	No safety concern
(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl) cyclopropanecarbaldehyde	1908	130932-16-0 (*cis*); 97231-35-1 (*trans*) 	A3. No, SPET: 240	NR	Note 1	No safety concern

Structural class III						
Perillaldehyde propyleneglycol acetal	1901	121199-28-8 	A3. No, SPET: 3	NR	Note 3	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

[a] Twenty-six flavouring agents belonging to the same chemical group were previously evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*).

[b] *Step 1:* Seven of the flavouring agents (Nos 1899, 1900 and 1902–1906) in this group were assigned to structural class I, three of the flavouring agents (Nos 1898, 1907 and 1908) were assigned to structural class II and the remaining flavouring agent (No. 1901) was assigned to structural class III.

[c] *Step 2:* Ten of the flavouring agents in this group are expected to be metabolized to innocuous products. The remaining substance (No. 1906), which contains two endocyclic double bonds, cannot be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. Expected to be metabolized largely by oxidation of the side-chain to the corresponding carboxylic acid, which is excreted unchanged and as conjugates.
2. Expected to undergo hydrolysis to form the corresponding alcohol and carboxylic acid, followed by metabolism in the fatty acid pathway or tricarboxylic acid cycle.
3. Hydrolysis of the ketal to yield propylene glycol and perillaldehyde, which will mainly be oxidized to perillic acid. Propylene glycol is oxidized to pyruvic acid and completely oxidized in the citric acid cycle.

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 11 flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters, the Committee assigned 7 flavouring agents (Nos 1899, 1900 and 1902–1906) to structural class I, 3 flavouring agents (Nos 1898, 1907 and 1908) to structural class II and 1 flavouring agent (No. 1901) to structural class III.

Step 2. Ten flavouring agents in this group (Nos 1898–1905, 1907 and 1908) are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. The remaining substance, 1,3-*p*-menthadien-7-al (No. 1906), which contains two endocyclic double bonds and is an α,β-unsaturated aldehyde, cannot be predicted to be metabolized to innocuous products and therefore was assessed via the B-side of the procedure.

Step A3. The highest estimated daily per capita intakes of five of the six flavouring agents in structural class I (Nos 1900 and 1902–1905) are below the threshold of concern (i.e. 1800 μg/person per day for class I). The safety of these five flavouring agents raises no concern at current estimated dietary exposures. The highest estimated daily intake of the remaining flavouring agent in structural class I (*cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid, No. 1899; 3000 μg using the SPET) is above the threshold of concern for class I. Accordingly, the evaluation of this flavouring agent proceeded to step A4.

The highest estimated daily per capita intakes of two of the three flavouring agents in structural class II (Nos 1907 and 1908) are below the threshold of concern (i.e. 540 μg/person per day for class II). The safety of these two flavouring agents raises no concern at current estimated dietary exposures. The highest estimated daily per capita intake of the remaining agent in structural class II (methyl dihydrojasmonate, No. 1898; 1875 μg as the MSDI) is above the threshold of concern for class II. Accordingly, the evaluation of this flavouring agent proceeded to step A4.

The highest estimated daily per capita intake of the flavouring agent in structural class III (No. 1901) is below the threshold of concern (i.e. 90 μg/person per day for class III). The safety of this flavouring agent raises no concern at current estimated dietary exposures.

Step A4. Neither the flavouring agents methyl dihydrojasmonate (No. 1898) and *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899) nor their metabolites are endogenous substances. Accordingly, the evaluation of these two flavouring agents proceeded to step A5.

Step A5. For methyl dihydrojasmonate (No. 1898), the no-observed-effect level (NOEL) of 80 mg/kg bw per day for maternal toxicity from a study of prenatal developmental toxicity in rats is 2580 times the estimated dietary exposures from its use as a flavouring agent (1875 µg/day as the MSDI).

For *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899), the NOEL of 12 mg/kg bw per day for the structurally related substance 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde (No. 967) from a 90-day study of toxicity in rats is 240 times the estimated dietary exposures to No. 1899 from its use as a flavouring agent (3000 µg/day using the SPET).

The Committee therefore concluded that methyl dihydrojasmonate (No. 1898) and *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899) would not pose a safety concern at current estimated dietary exposures.

Step B3. The highest estimated daily per capita intake of 1,3-*p*-menthadien-7-al (No. 1906) is below the threshold of concern (i.e. 1800 µg/person per day for class I). Accordingly, its evaluation proceeded to step B4.

Step B4. The NOELs of 15, 33.9 and 33 mg/kg bw per day for, respectively, the structurally related substances *trans*,*trans*-2,4-hexadienal (No. 1175), 2-*trans*-4-*trans*-decadienal (No. 1190) and 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (No. 1198) from 14-week studies in rats (Nos 1175 and 1190) and a 4-week study in rats (No. 1998) are 30 000–67 800 times higher than the highest estimated intake of 1,3-*p*-menthadien-7-al (No. 1906) from its use as a flavouring agent (30 µg/day using the SPET). Although these three structurally related compounds are linear compounds, they contain the same toxicologically relevant groups as No. 1906 (i.e. an α,β-unsaturated aldehyde with two or more double bonds) and are therefore considered suitable for the evaluation of No. 1906. The Committee therefore concluded that 1,3-*p*-menthadien-7-al (No. 1906) would not pose a safety concern at current estimated dietary exposures.

Table 2 summarizes the evaluations of the 11 alicyclic primary alcohols, aldehydes, acids and related esters (Nos 1898–1908) in this group.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*). No common metabolites or homologous series could be identified for the additional flavouring agents in this group. When also considering the flavouring agents in this group evaluated at the fifty-ninth meeting (Annex 1, reference *160*), the different flavouring agents were

not members of homologous series, despite having some common structural characteristics. However, two common metabolites were identified: *p*-menth-1-en-9-ol (No. 1905) and perillic alcohol (No. 974), both of which are in structural class I. In the unlikely event that the flavouring agents with the common metabolite *p*-menth-1-en-9-ol (i.e. Nos 971 and 972) and *p*-menth-1-en-9-ol itself were to be consumed concurrently on a daily basis, the estimated combined intakes for Europe, the USA and Japan would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I). In the unlikely event that the flavouring agents with the common metabolite perillic alcohol (i.e. Nos 973 and 975), perillic alcohol itself and No. 1901, which would be metabolized to a structural isomer of perillic acid, were to be consumed concurrently on a daily basis, the estimated combined intakes for Europe, the USA and Japan would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Consideration of secondary components

Five members of this group of flavouring agents (Nos 1898, 1901, 1902, 1906 and 1908) have assay values of less than 95%. The secondary component of methyl dihydrojasmonate (No. 1898), methyl epi-dihydrojasmonate, is expected to share the same metabolic fate as the primary substance and was considered not to present a safety concern at current estimated dietary exposures. The secondary components of perillaldehyde propyleneglycol acetal (No. 1901), perillaldehyde (No. 973) and propylene glycol, are metabolites of the primary substance and were considered not to present a safety concern at current estimated dietary exposures. The secondary components of (±)-*cis*- and *trans*-dihydroperillaldehyde (No. 1902), *trans*-4-isopropyl-cyclohexane-1-carboxaldehyde, *cis*-4-isopropyl-cyclohexane-1-carboxaldehyde and 4-isopropenyl-cyclohex-1-enecarboxaldehyde, are expected to share the same metabolic fate as the primary substance and were considered not to present a safety concern at current estimated dietary exposures. The secondary component of 1,3-*p*-menthadien-7-al (No. 1906), cumin aldehyde (No. 868), was evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and was considered not to present a safety concern at estimated dietary exposures. The secondary component of (±)-*cis*- and *trans*-2-methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarbaldehyde (No. 1908), [2-methyl-2-(4-methylpent-3-en-1-yl)cyclopropyl]methanol, is a metabolite of the primary substance and is expected to share the same metabolic fate. It was considered not to present a safety concern at current estimated dietary exposures.

Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the previous evaluation of substances in the group of alicyclic primary alcohols, aldehydes, acids and related esters, studies of acute toxicity, short-term and long-term toxicity and genotoxicity were available (Annex 1, reference *161*). None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation.

The Committee concluded that these 11 flavouring agents, which are additions to the group of 26 alicyclic primary alcohols, aldehydes, acids and related esters previously evaluated, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.3 *Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones: additional compounds*

The Committee evaluated eight additional flavouring agents belonging to the group of aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones, which was evaluated previously. The additional flavouring agents included two aliphatic α-diketones, two aliphatic α-hydroxyketones, one aliphatic β-diketone, one alicyclic α,β-unsaturated α-hydroxyketone and two α-hydroxyketals. The group of substances was selected on the basis of the structural criteria of possessing an aliphatic acyclic and alicyclic α-diketone and related α-hydroxyketone. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee previously evaluated 22 other members of this group of flavouring agents at its fifty-first meeting (Annex 1, reference *138*). The Committee concluded that all 22 flavouring agents in the group were of no safety concern based on estimated dietary exposures.

Five of the eight additional flavouring agents (Nos 2032 and 2035–2038) in this group have been reported to occur naturally and have been found in black tea, green tea, sherry, beef fat, mutton, lamb, fish, turkey, chicken, guinea hen, coffee, roasted peanuts, soya bean, mushroom, prickly pear, lovage leaf, cocoa, black currants, peppermint oil and buchu oil. Quantitative intake data from natural occurrence were available for two substances, 3-methyl-2,4-nonedione (No. 2032) and octan-2,3-dione (No. 2036). The consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were calculated to be 177 and 125, respectively.

Assessment of dietary exposure

The total annual volumes of production of the eight aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones are 2 kg in Europe, 6 kg in the USA and 39 kg in Japan. In Europe, 65% of the annual volume of production is accounted for by 3-methyl-2,4-nonedione (No. 2032) and octan-2,3-dione (No. 2036), and in the USA, 83% of the annual volume of production is accounted for by octan-2,3-dione (No. 2036). Over 84% of the annual volume of production in Japan is accounted for by acetoin propyleneglycol acetal (No. 2033).

The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 3. The highest estimates are for acetoin propyleneglycol acetal (No. 2033) and the mixture of 3-hydroxy-5-methyl-2-hexanone and 2-hydroxy-5-methyl-3-hexanone (No. 2034) (450 µg for both, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in the group, the daily dietary exposures range from 0.01 to 400 µg, with the SPET yielding the highest estimates for all, except for 4,5-octanedione (No. 2037).

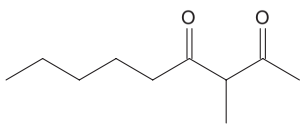
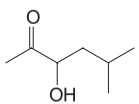
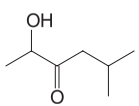
Absorption, distribution, metabolism and elimination

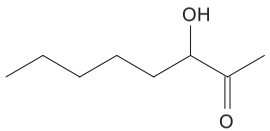
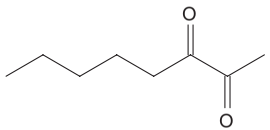
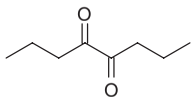
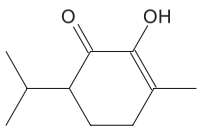
In the report of the fifty-first meeting, biodisposition of flavouring agents in this group was extensively discussed. In rats and mice, orally administered aliphatic α-diketones are rapidly absorbed from the gastrointestinal tract. It is anticipated that at low levels of exposure, humans will metabolize aliphatic acyclic α-diketones principally by α-hydroxylation and subsequent oxidation of the terminal methyl group to yield the corresponding ketocarboxylic acid. The acid may undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which could be completely metabolized in the fatty acid pathway and citric acid cycle. At higher concentrations, another detoxication pathway is used, which involves reduction to the diol and subsequent conjugation with glucuronic acid. Aliphatic α-diketones and alicyclic α-hydroxyketones, diketones and hydroxyketones are mainly metabolized by reduction to the corresponding diol, followed by glucuronic acid conjugation and excretion. Ketals (dioxolanes) are predicted to undergo hydrolysis to yield the corresponding alcohol and ketone (Nos 405, 408 and 2033).

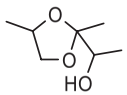
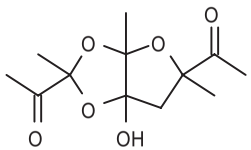
Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned six flavouring agents (Nos 2032 and 2034–2038) to structural class II and the remaining two flavouring agents (Nos 2033 and 2039) to structural class III (*7*).

Table 3
Summary of the results of the safety evaluations of aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II							
3-Methyl-2,4-nonedione	2032	113486-29-6 	No, SPET: 20	NR	NR	Note 1	No safety concern
Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	2034	63038-04-8  246511-74-0 	No, SPET: 450	NR	NR	Note 1	No safety concern

3-Hydroxy-2-octanone	2035	37160-77-3 	No, SPET: 400	NR	NR	Note 1	No safety concern
2,3-Octanedione	2036	585-25-1 	No, SPET: 3.6	NR	NR	Note 1	No safety concern
4,5-Octanedione	2037	5455-24-3 	No, MSDI: Europe 0.01 USA ND Japan 0.9	NR	NR	Note 1	No safety concern
(±)-2-Hydroxypiperitone	2038	490-03-9 	No, SPET: 400	NR	NR	Note 2	No safety concern

Structural class III							
Acetoin propyleneglycol ketal	2033	94089-23-3 	Yes, SPET: 450	No	Yes. The NOAEL of 330 mg/kg bw per day for the metabolite acetoin (No. 405) in a 90-day study in rats is at least 41 200 times the estimated daily dietary exposure to No. 2033 when used as a flavouring agent.	Note 3	No safety concern
1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	2039	18114-49-3 	Yes, SPET: 400	No	Yes. The NOAEL of 90 mg/kg bw per day for the metabolite 2,3-butanedione (No. 408) in a 90-day study in rats is at least 12 800 times the estimated daily dietary exposure to No. 2039 when used as a flavouring agent.	Note 3	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure.

[a] Twenty-two flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *138*).

[b] *Step 1:* Six flavouring agents in this group (Nos 2032 and 2034–2038) are in structural class II. Two flavouring agents in this group (Nos 2033 and 2039) are in structural class III.

[c] *Step 2:* All of the flavouring agents in this group can be expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

Notes:

1. Metabolized by α-hydroxylation, followed by oxidation of the terminal methyl group to the corresponding ketocarboxylic acid. The acid may undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolized in the fatty acid pathway and citric acid cycle.
2. Reduction of the hydroxyketone to yield the corresponding diol, which is conjugated with glucuronic acid and excreted primarily in the urine.
3. Hydrolysis to form the α-hydroxyketone or diketone, followed by oxidation of the terminal methyl group, or reduction to the corresponding diol, followed by conjugation with glucuronic acid and excretion in the urine.

Step 2. All eight of the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily intakes for the six flavouring agents in structural class II are below the threshold of concern (i.e. 540 μg/person per day for class II). Therefore, the safety of these six flavouring agents raises no concern at their current estimated dietary exposures. The estimated daily intakes for the two flavouring agents in structural class III are above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. Neither the flavouring agents—acetoin propyleneglycol ketal (No. 2033) and 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (No. 2039)—nor their metabolites are endogenous substances. Accordingly, the evaluation of these flavouring agents proceeded to step A5.

Step A5. For acetoin propyleneglycol ketal (No. 2033), the NOAEL of 330 mg/kg bw per day for the metabolite acetoin (No. 405) in a 90-day study in rats provides a margin of safety of over 40 000 in relation to the highest estimated intake of acetoin propyleneglycol ketal (SPET = 450 μg/person per day) when used as a flavouring agent.

For 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (No. 2039), the NOAEL of 90 mg/kg bw per day for the metabolite 2,3-butanedione (No. 408) in a 90-day study in rats provides a margin of safety of approximately 13 000 in relation to the highest estimated intake of 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (SPET = 400 μg/person per day) when used as a flavouring agent.

The Committee concluded that the margins of safety indicate that these flavouring agents would not pose safety concerns at current estimated dietary exposures.

Table 3 summarizes the evaluations of the eight aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents (Nos 2032–2039) in this group.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was undertaken based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting;

Annex 1, reference *190*). In addition, at this meeting, the Committee also considered combined intakes for structurally closely related series of flavouring agents.

Flavouring agents in this series that are members of a structurally closely related series of aliphatic acyclic α-diketones or related α-hydroxyketones, which are in structural class II, or predicted to be metabolized to such compounds are Nos 2033–2037 and 2039. The five related flavouring agents with the highest intakes in Europe are Nos 405, 408, 410, 412 and 413 and in the USA are Nos 405, 406, 408, 410 and 412. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be approximately 6000 μg/person per day in Europe and approximately 10 000 μg/person per day in the USA. These would exceed the threshold of concern (i.e. 540 μg/person per day for class II). However, all of these flavouring agents are expected to be efficiently metabolized and would not saturate available detoxication pathways. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of the substances in this group would not raise concern about safety.

The flavouring agent No. 2038 is a member of a structurally closely related series of alicyclic α-diketones or related α-hydroxyketones, which are in structural class II, or predicted to be metabolized to such compounds. The five related flavouring agents with the highest intakes in Europe and in the USA are Nos 418–421 and 425; the flavouring agent with the highest intake in Japan is No. 2033. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be approximately 1000 μg/person per day in Europe, 6 μg/person per day in Japan and 800 μg/person per day in the USA. These would exceed the threshold of concern (i.e. 540 μg/person per day for class II). However, all of these flavouring agents are expected to be efficiently metabolized and would not saturate available detoxication pathways. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of these substances would not raise concern about safety.

The remaining flavouring agent (No. 2032) does not share close structural characteristics with others in the group, and consideration of combined intake is not indicated.

The Committee concluded that under the conditions of use as flavouring agents, the combined intakes of flavouring agents in this group would not pose a safety concern.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of less than 95%.

Conclusion

In the previous evaluation of this group of flavouring agents, studies of biological properties, acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None raised safety concerns. The additional biochemical and toxicological data available for this evaluation supported those from the previous evaluation (Annex 1, reference *138*).

The Committee concluded that these eight flavouring agents, which are additions to the group of aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.4 *Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances: additional compounds*

The Committee evaluated seven additional flavouring agents belonging to the group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances, which was evaluated previously. The additional flavouring agents included one aliphatic terpene tertiary alcohol (No. 2031), four alicyclic tertiary alcohols (Nos 2027–2030) and two esters of phenyl-substituted aliphatic tertiary alcohols (Nos 2025 and 2026). The group of flavouring agents was selected on the basis of the structural criteria of possessing a tertiary alcohol or an ester derived from a tertiary alcohol. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has been evaluated previously by the Committee.

The Committee previously evaluated 23 other members of this group of flavouring agents at its fifty-first meeting (Annex 1, reference *137*). The Committee concluded that 22 of the 23 flavouring agents in that group were of no safety concern based on estimated dietary exposures. For one flavouring agent, methyl 1-acetoxycyclohexylketone (No. 442), the available metabolic data were inadequate to allow the Committee to predict whether it would be metabolized to innocuous products, a relevant NOEL was lacking and the intake exceeded 1.5 μg/day. The Committee concluded that additional data were required for the evaluation of methyl 1-acetoxycyclohexylketone.

The Committee subsequently evaluated 15 other members of this group of flavouring agents at the sixty-eighth meeting (Annex 1, reference *187*). The Committee concluded that all 15 flavouring agents in that group were of no safety concern based on estimated dietary exposures.

Five of the seven additional flavouring agents (Nos 2027–2031) in this group have been reported to occur naturally and have been found in camomile, figs, lemon juice, black and green teas, calamus, soya bean, pepper and strawberry guava.

Assessment of dietary exposure

The total annual volumes of production of the seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances are approximately 18 kg in Europe and 5 kg in Japan. More than 94% of the total annual volume of production in Europe is accounted for by (+)-cedrol (No. 2030).

The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 4. The highest estimates are for (–)-sclareol (No. 2029) and (+)-cedrol (No. 2030) (1500 μg for both, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in this group, the daily dietary exposures range from 0.01 to 900 μg, with the SPET yielding the highest estimates for all.

Absorption, distribution, metabolism and elimination

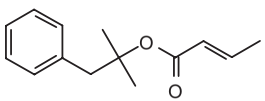
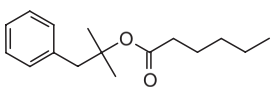
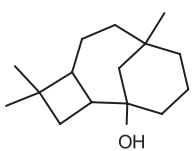
In the report of the fifty-first meeting, biodisposition of substances in this group was extensively discussed. The esters in this group (Nos 2025 and 2026) can be readily hydrolysed to their component tertiary alcohols and carboxylic acids. The hydrolysis products would be readily detoxified primarily by conjugation with glucuronic acid and then excreted primarily in the urine. The alicyclic tertiary alcohols and alcohols with unsaturation (Nos 2027–2031) undergo ω-oxidation at the allylic position to yield polar metabolites, which can be conjugated and excreted. Metabolites of acyclic alcohols can be further oxidized to eventually yield carbon dioxide.

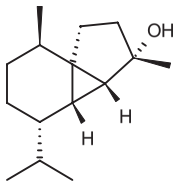
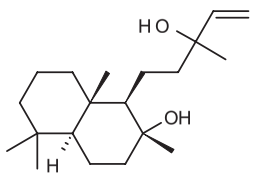
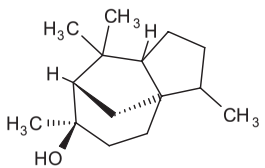
Application of the Procedure for the Safety Evaluation of Flavouring Agents

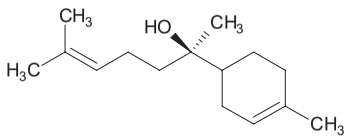
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned all seven of the flavouring agents (Nos 2025–2031) to structural class I.

Table 4

Summary of the results of the safety evaluations of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I					
Dimethylbenzyl carbinyl crotonate	2025	93762-34-6 	No, SPET: 400	Note 1	No safety concern
Dimethylbenzyl carbinyl hexanoate	2026	891781-90-1 	No, SPET: 900	Note 1	No safety concern
Caryophyllene alcohol	2027	472-97-9 	No, SPET: 50	Note 2	No safety concern

Cubebol	2028	23445-02-5 	No, SPET: 3	Note 2	No safety concern
(−)-Sclareol	2029	515-03-7 	No, SPET: 1500	Notes 2 and 3	No safety concern
(+)-Cedrol	2030	77-53-2 	No, SPET: 1500	Note 2	No safety concern

α-Bisabolol	2031	23089-26-1 	No, SPET: 150	Note 3	No safety concern

CAS, Chemical Abstracts Service

[a] Thirty-eight flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *137* and *187*).

[b] *Step 1:* All seven flavouring agents in this group (Nos 2025–2031) are in structural class I.

[c] *Step 2:* All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

Notes:

1. Esters are rapidly hydrolysed, and the corresponding tertiary alcohols are metabolized primarily by conjugation with glucuronic acid and excretion in the urine.
2. Alicyclic tertiary alcohols are metabolized primarily by conjugation with glucuronic acid and excretion in the urine.
3. Tertiary unsaturated alcohols are metabolized primarily by conjugation with glucuronic acid and excretion in the urine. Oxidation of the allylic methyl group may occur after repeated exposure.

Step 2. All seven of the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily intakes of all seven flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I).

The Committee concluded that exposures to these seven flavouring agents would not pose a safety concern at current estimated dietary exposures.

Table 4 summarizes the evaluations of the seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances (Nos 2025–2031) in this group.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Flavouring agents in this series with the common metabolite α,α-dimethylphenethyl alcohol (No. 1653), which is in structural class I, are Nos 2025 and 2026. The highest intakes of flavouring agents that are part of a homologous series with No. 1653 or have this as a common metabolite are Nos 1649, 1650, 1653, 1655 and 1656 in Europe, Nos 1649, 1650, 1653, 1655 and 1656 in Japan and Nos 1650 and 1653–1656 in the USA. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 120 μg/person per day in Europe, 124 μg/person per day in Japan and 1155 μg/person per day in the USA, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Flavouring agents in this group that are bicyclic tertiary alcohols or related esters are Nos 2027–2030. The highest intakes in this series are, in Europe, Nos 2029 and 2030 in structural class I and Nos 1647 and 1648 in structural class II; in Japan, Nos 2027, 2028 and 2030 in structural class I and Nos 1647 and 1648 in structural class II; and in the USA, No. 1648 in structural class II. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 2.1 μg/person per day in Europe, 1.5 μg/person per day in Japan and 0.05 μg/person per day in the USA, which would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

The Committee concluded that under the conditions of use as flavouring agents, the combined intakes at currently estimated dietary exposures would not pose a safety concern.

Consideration of secondary components

Two flavouring agents in this group (Nos 2027 and 2031) have minimum assay values of less than 95%. The secondary component of caryophyllene alcohol (No. 2027), dihydrocloven-9-ol, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as caryophyllene alcohol, and is considered not to present a safety concern at current estimated dietary exposures. The secondary component of α-bisabolol (No. 2031), β-bisabolol, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as caryophyllene alcohol, and is considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the two previous evaluations of this group of flavouring agents, studies of biological properties, acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None raised safety concerns. Additional biochemical and toxicological data that were available for this evaluation supported those from the previous evaluations (Annex 1, references *137* and *187*).

The Committee concluded that these seven flavouring agents, which are additions to the group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.5 *Aliphatic and aromatic amines and amides: additional compounds*

The Committee evaluated an additional group of nine flavouring agents belonging to the group of aliphatic and aromatic amines and amides. The additional flavouring agents included one quaternary ammonium salt, one primary amine, three branched-chain aliphatic amides and four amides with alicyclic or aromatic alkyl side-chains, one of which contains a benzeneacetonitrile group. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee evaluated 49 other members of this group of flavouring agents at its sixty-fifth and sixty-eighth meetings (Annex 1, references *178* and *187*). For 36 of the 37 flavouring agents evaluated at the sixty-fifth meeting, the Committee concluded that they would not give rise to safety concerns based on estimated dietary exposures. For 1 of the 37 flavouring agents—namely, acetamide (No. 1592)—the Committee considered it inappropriate for use as a flavouring agent or for food additive purposes, based on the available data indicating carcinogenicity in mice and rats. For 27 flavouring agents, the dietary exposure estimates were based on anticipated annual volumes of production, and these evaluations were conditional pending submission of use levels or poundage data, which were provided at the sixty-ninth meeting (Annex 1, reference *190*).

For the evaluation of 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595), additional data available at the sixty-ninth meeting raised safety concerns, and the Committee concluded that the Procedure could not be applied to this flavouring agent until additional safety data became available.

For all 12 flavouring agents evaluated at the sixty-eighth meeting (Annex 1, reference *187*), the Committee concluded that they would not give rise to safety concerns at estimated dietary exposures. The Committee noted, while making this conclusion, that 4-aminobutyric acid (No. 1771) is an endogenous neurotransmitter; however, the tissue levels arising from consumption of food containing this flavouring agent would be biologically insignificant.

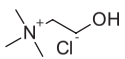
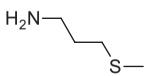
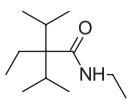
One of the nine flavouring agents considered at the current meeting—namely, choline chloride (No. 2003)—is a natural component of food and has been detected in beef liver, chicken liver, eggs, wheat germ, bacon, dried soya beans and pork.

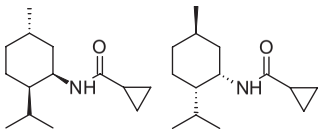
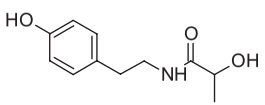
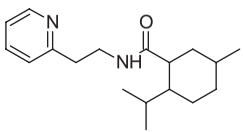
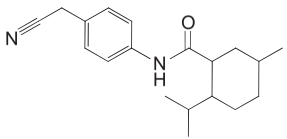
Assessment of dietary exposure

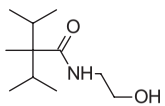
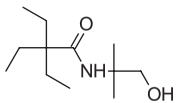
The total annual volumes of production of the nine additional flavouring agents in this group are 21 kg in Europe, 1001 kg in the USA and 3 kg in Japan. In Europe and the USA, greater than 99% of the annual volume of production is accounted for by *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) and *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), respectively. In Japan, 100% of the annual volume of production is accounted for by 3-(methylthio)propylamine (No. 2004).

The estimated dietary exposures for each of the flavouring agents, calculated as the MSDI or using the SPET, are reported in Table 5. The highest estimate is for choline chloride (No. 2003) (200 000 μg, the SPET value obtained from bread and ordinary bakery ware). For the other flavouring agents in the group, the daily dietary exposures range from 0.02 to 48 000 μg, with the SPET yielding the highest estimate for all.

Table 5
Summary of the results of the safety evaluations of aliphatic and aromatic amines and amides used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Are additional data available for substances with an estimated intake exceeding the threshold of concern? (follow-on from step B3)[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I							
Choline chloride	2003	67-48-1 	A3: Yes, SPET: 200 000	Choline is endogenous		Note 1	No safety concern
3-(Methylthio)propylamine	2004	4104-45-4 	A3: No, SPET: 200	NR		Note 2	No safety concern
Structural class III							
N-Ethyl-2,2-diisopropylbutanamide	2005	51115-70-9 	B3: Yes, SPET: 27 000		Additional data are not available	Note 3	Additional data required to complete evaluation

Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	2006	958660-02-1; 958660-04-3 	B3: Yes, SPET: 200	The NOAEL of 8 mg/kg bw per day in a 28-day study in rats for the structurally related *N*-ethyl 2-isopropyl-5-methylcyclo-hexanecarboxamide (No. 1601) is at least 2400 times the estimated daily dietary exposure to No. 2006 when used as a flavouring agent.	Note 3	No safety concern
(±)-*N*-Lactoyl tyramine	2007	781674-18-8 	B3: Yes, SPET: 20 000	Additional data are available, but inadequate margins of safety are provided from the NOELs for structurally related substances.	Notes 3 and 4	Additional data required to complete evaluation
N-(2-(Pyridin-2-yl)-ethyl)-3-*p*-menthanecarboxamide	2008	847565-09-7 	B3: Yes, SPET: 2400	The NOAEL of 10 mg/kg bw per day in a 28-day study in rats is at least 250 times the estimated daily dietary exposure to No. 2008 when used as a flavouring agent.	Note 3	No safety concern
N-*p*-Benzeneacetonitrile menthanecarboxamide	2009	852379-28-3 	B3: Yes, SPET: 3000	The NOEL of 300 mg/kg bw per day in a 90-day study in rats is at least 6000 times the estimated daily dietary exposure to No. 2009 when used as a flavouring agent.	Note 3	No safety concern

N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	883215-02-9 	B3: Yes, SPET: 48 000		Additional data are not available.	Notes 3 and 4	Additional data required to complete evaluation
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	51115-77-6 	B3: Yes, SPET: 27 000	NR	Additional data are not available.	Notes 3 and 4	Additional data required to complete evaluation

CAS, Chemical Abstracts Service; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

[a] Forty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *178* and *187*).

[b] *Step 1:* Two flavouring agents (Nos 2003 and 2004) are in structural class I, and seven flavouring agents (Nos 2005–2011) are in structural class III.

[c] *Step 2:* Flavouring agents Nos 2003 and 2004 are predicted to be metabolized to innocuous products. The remaining seven amides (Nos 2005–2011) cannot be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. Choline is endogenous and excreted as such in human urine.
2. Aliphatic primary amines readily undergo oxidative deamination, with the resulting aldehydes and ketones entering existing pathways of metabolism and excretion.
3. Amides are expected to undergo oxidation and enter known pathways of metabolism.
4. It is anticipated that the free hydroxyl group will form conjugates with sulfate or glucuronic acid, followed by excretion in the urine.

Absorption, distribution, metabolism and elimination

The metabolism of aliphatic and aromatic amines and amides was described previously in the report of the sixty-fifth meeting of the Committee (Annex 1, reference *178*) and further considered in the report of the sixty-eighth meeting (Annex 1, reference *187*).

In general, aliphatic and aromatic amines and amides are rapidly absorbed from the gastrointestinal tract and metabolized by deamination, hydrolysis or oxidation to polar metabolites that are readily eliminated in the urine. Many amines are endogenous and have been identified as normal constituents of urine in humans. Aliphatic amides have been reported to undergo hydrolysis in mammals; the rate of hydrolysis is dependent on the chain length and the extent of steric hindrance and may involve a number of different enzymes.

Additional studies were provided on *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768), which was previously considered at the sixty-eighth meeting (Annex 1, reference *187*). Rapid absorption and rapid blood clearance were noted in rats and dogs following gavage or intraperitoneal dosing and in humans following oral administration, after which blood levels returned to baseline by 24 h.

In relation to these additional flavouring agents, only limited information regarding metabolic pathways is available for specific substances. The available data suggest that the likely metabolic pathway for the amides in this group, which would be resistant to amide hydrolysis, is cytochrome P450–induced C-hydroxylation, followed by sulfation or glucuronidation and excretion.

Unpublished studies on (±)-*N*-lactoyl tyramine (No. 2007) indicate no significant hydrolysis of this amide, whereas a published study identified a glucuronic acid conjugate formed in an in vitro study with rat hepatocytes.

Published studies on choline chloride (No. 2009) show that it is absorbed readily, metabolized to betaine in the liver and kidney and used in the synthesis of endogenous substances, such as acetylcholine.

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 additional flavouring agents, the Committee assigned two flavouring agents (Nos 2003 and 2004) to structural class I. The remaining seven flavouring agents (Nos 2005–2011) were assigned to structural class III (*7*).

Step 2. The two flavouring agents in structural class I (Nos 2003 and 2004) are predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure. The remaining seven flavouring agents (Nos 2005–2011) could not be predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily intake (calculated either as the MSDI or by the SPET) of 3-(methylthio)propylamine (No. 2004) is below the threshold of concern (i.e. 1800 μg/person per day for class I). This substance would not be expected to be of safety concern at current estimated dietary exposures. The highest estimated daily intake (calculated by the SPET) of choline chloride (No. 2003) is above the threshold of concern (i.e. 1800 μg/person per day for class I). Accordingly, the evaluation of this substance proceeded to step A4.

Step A4. Choline derived from choline chloride (No. 2003) is endogenous. This substance would not be expected to be of safety concern.

Step B3. The highest estimated daily intake (calculated by the SPET) for the seven flavouring agents in structural class III are above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, for all of these substances, data are required on the substance or a closely related substance in order to perform a safety evaluation.

Consideration of flavouring agents with high exposure evaluated on the B-side of the decision-tree:

For cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (No. 2006), available data on the structurally related *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) give a NOAEL of 8 mg/kg bw per day from a 28-day study in rats. This provides a margin of safety of about 2400 in relation to the highest estimated dietary exposure to No. 2006 (SPET = 200 μg/day) when used as a flavouring agent.

For *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008), available data give a NOAEL of 10 mg/kg bw per day from a 28-day study in rats. This provides a margin of safety of 250 in relation to the highest estimated dietary exposure to No. 2008 (SPET = 2400 μg/day) when used as a flavouring agent. The Committee noted that the margin of safety of No. 2008 based on the MSDI of 0.01 μg/day exceeds 60 million and concluded that the values of 250 (based on the SPET) and greater than 60 million (based on the MSDI) provide an adequate margin of safety.

For *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009), available data give a NOEL of 300 mg/kg bw per day from a 90-day study in rats. This

provides an adequate margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2009 (SPET = 3000 μg/day) when used as a flavouring agent.

The Committee therefore concluded that these three flavouring agents, cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (No. 2006), *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008) and *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009), would not pose a safety concern at current estimated dietary exposures.

For (±)-*N*-lactoyl tyramine (No. 2007), available data on the structurally related nonanoyl 4-hydroxy-3-methoxybenzylamide (No. 1599) give a NOEL of 8.4 mg/kg bw per day from a 90-day study in rats. This provides a margin of safety of 25 in relation to the highest estimated dietary exposure to No. 2007 (SPET = 20 000 μg/day) when used as a flavouring agent. The NOELs for other structurally related flavouring agents, such as *N*-[2-(3,4-dimethoxy-phenyl)ethyl]-3,4-dimethoxycinnamic acid (No. 1777) or *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide (No. 1776), give similarly low margins of safety. The Committee therefore concluded that additional data on (±)-*N*-lactoyl tyramine (No. 2007) would be necessary to complete the safety evaluation.

For *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), *N*-(2-hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide (No. 2010) and *N*-(1,1-dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide (No. 2011), NOELs for these substances or structurally related substances were not available. Therefore, for these three substances, the Committee concluded that additional data would be necessary to complete the safety evaluation. For these three substances, the previously considered substance, 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595), is structurally related; however, at the sixty-ninth meeting (Annex 1, reference *190*), the Committee concluded that additional data would be necessary to complete the evaluation for this substance, and therefore this substance was not suitable to support the evaluation of these three flavouring agents.

Table 5 summarizes the evaluations of the nine aliphatic and aromatic amines and amides used as flavouring agents in this group (Nos 2003–2011).

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series as proposed at the sixty-eighth meeting (Annex 1, reference *187*) and using the MSDI exposure assessment as proposed at the sixty-ninth meeting (Annex 1, reference *190*).

This group of flavouring agents contains members of several homologous or closely related series—namely, aliphatic primary amines, aliphatic tertiary amines, amines with an alkyl aromatic side-chain and aliphatic unsaturated amides. In the unlikely event that the flavouring agents in this group in any of these homologous, closely related series were to be consumed concurrently on a daily basis, the estimated combined intakes would be as shown in Table 6.

Table 6
Combined dietary exposure for the homologous or closely related series within this group of aliphatic and aromatic amines and amides

Homologous or closely related series	Substances with highest per capita dietary exposure (Nos)	Structural class	Estimated combined dietary exposure in Europe, USA and Japan (μg/person per day)	Dietary exposure relative to the threshold of concern for that structural class
Aliphatic primary amines	1582, 1584, 1587, 1591, 2004	I	160 (Europe), 21 (USA) and 1 (Japan)	Not exceeded
Aliphatic tertiary amines	1610–1612, 1614	I	195 (Japan) and 90 (Europe and USA)	Not exceeded
Amines with an alkyl aromatic side-chain	1589, 1590, 1613	III	0.1 (Europe and USA)	Not exceeded
Aliphatic unsaturated amides	1596–1600, 1779	III	102 (Japan) and 259 (Europe and USA)	Exceeded

For the homologous or closely related series of aliphatic unsaturated amides, the combined intakes would exceed the threshold of concern (i.e. 90 μg/person per day for class III) in Europe, the USA and Japan. However, in this case, all of the flavouring agents are expected to be efficiently metabolized and would not saturate available detoxication pathways. Therefore, the combined intake of these substances is not expected to raise any safety concerns.

Consideration of secondary components

Two flavouring agents in this group (Nos 2007 and 2009) have minimum assay values of less than 95%. The secondary components of (±)-*N*-lactoyl tyramine (No. 2007) are lactic acid and ethyl lactate. Lactic acid (No. 930) is endogenous, and ethyl lactate (No. 931) is expected to be hydrolysed to lactic acid. These substances were evaluated at the fifty-seventh meeting of the

Committee (Annex 1, reference *154*) and concluded to be of no safety concern at estimated dietary exposures as flavouring agents. The secondary component of *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) is *N*-*p*-benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*). This substance is a stereoisomer of No. 2009, is expected to share the same metabolic fate as the primary substance and is not considered to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the previous evaluations of members of this group (Annex 1, references *178*, *187* and *190*), studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. The toxicity data available for the evaluation of these additional substances supported those from the previous evaluations.

The Committee concluded that five of the nine additional flavouring agents evaluated at the present meeting do not raise any safety concerns at current estimated dietary exposures. For one of the remaining four flavouring agents (No. 2007), the available additional data did not provide an adequate margin of safety, and for the other three flavouring agents (Nos 2005, 2010 and 2011), no additional data were available. The Committee concluded that for these four flavouring agents, further data would be required to complete the safety evaluation.

An addendum to the toxicological monograph was prepared.

4.1.6 *Aliphatic lactones: additional compounds*

The Committee evaluated 14 additional flavouring agents belonging to the group of aliphatic lactones. The additional flavouring agents included three saturated γ-lactones (Nos 1992, 1995 and 1998), four unsaturated γ-lactones (Nos 1989 and 2000–2002), six saturated δ-lactones (Nos 1990, 1993, 1994, 1996, 1997 and 1999) and one unsaturated ω-lactone (No. 1991). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee previously evaluated 35 other members of this group of flavouring agents at its forty-ninth meeting (Annex 1, reference *132*). At that meeting, the Committee concluded that 31 flavouring agents in that group were of no safety concern based on estimated dietary exposures. The evaluations of four flavouring agents that are α,β-unsaturated were deferred, pending consideration of other α,β-unsaturated carbonyl flavouring agents. The Committee reconsidered these flavouring agents at the fifty-fifth meeting

(Annex 1, reference *159*) and concluded that there were no safety concerns associated with α,β-unsaturated flavouring agents at the dietary exposures that would arise from their use as flavouring agents. An additional 26 non-lactone α,β-unsaturated flavouring agents were considered at the sixty-first meeting (Annex 1, reference *166*); at this meeting, the Committee concluded that there were no safety concerns associated with these flavouring agents.

Seven of the additional 14 flavouring agents are natural components of food (Nos 1989, 1990, 1992, 1998–2000 and 2002) and have been detected in roasted hazelnuts, peanuts, soya beans, onion, asparagus, tomato, coffee, green teas, mate, beef, fatty fish, shrimp, chicken fat, butter, saffron, wheat and rye breads, wheaten bread, beer and traditional rice (*8*).

Assessment of dietary exposure

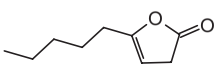
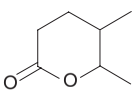
The total annual volumes of production of these additional 14 aliphatic lactones are approximately 109 kg in Europe, 6 kg in Japan and 13 kg in the USA (*9–12*). In Europe, approximately 99% of the total annual volume of production is accounted for by isoambrettolide (No. 1991). In the USA, approximately 62% of the total annual volume of production is accounted for by 5-pentyl-3H-furan-2-one (No. 1989). In Japan, 66% of the total annual volume of production is accounted for by 8-decen-5-olide (No. 1994) and 4-hydroxy-2-butenoic acid γ-lactone (No. 2000).

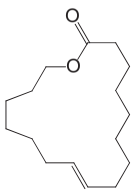
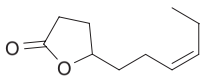
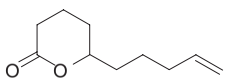
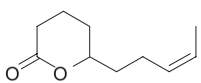
The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 7. The highest estimates are for four substances: 9-decen-5-olide (No. 1993), 9-dodecen-5-olide (No. 1996), 9-tetradecen-5-olide (No. 1997) and γ-octadecalactone (No. 1998) (1000 μg, all using the SPET value obtained from milk [dairy] and fermented milk products). For the other flavouring agents in the group, the daily dietary exposures range from 0.03 to 800 μg, with the SPET yielding the highest estimate for all except isoambrettolide (No. 1991). Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 8.

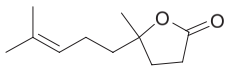
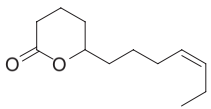
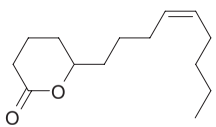
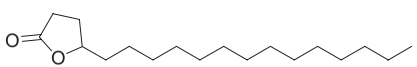
Absorption, distribution, metabolism and elimination

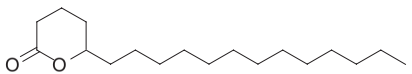
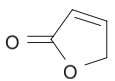
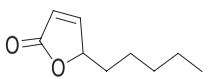
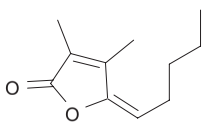
The metabolism of aliphatic lactones has been previously described in detail in the report of the forty-ninth meeting (Annex 1, reference *132*). The metabolism of these additional aliphatic lactones was considered in three subgroups—namely, (i) lactones from saturated linear and branched-chain hydroxycarboxylic acids, (ii) lactones from unsaturated linear and branched-chain hydroxycarboxylic acids and (iii) lactones containing α,β-unsaturation—and is briefly described below.

Table 7
Summary of the results of the safety evaluations of aliphatic lactones used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A4/A5/B4*[e] *A4.* Is the substance or are its metabolites endogenous? *A5/B4.* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II						
5-Pentyl-3H-furan-2-one	1989	51352-68-2 	No, SPET: 0.04	NR	Notes 1 and 2	No safety concern
5-Hydroxy-4-methylhexanoic acid δ-lactone	1990	10413-18-0 	Yes, SPET: 800	A4. No. A5. Yes. The NOEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day study in rats (*13*) is at least 900 times the estimated daily intake of No. 1990 when used as a flavouring agent.	Note 1	No safety concern

Isoambrettolide	1991	28645-51-4 	No, MSDI: Europe 12 USA 0.05 Japan 0.1	NR	Note 1	No safety concern
7-Decen-4-olide	1992	67114-38-9 	No, SPET: 125	NR	Note 1	No safety concern
9-Decen-5-olide	1993	74585-00-5 	Yes, SPET: 1000	A4. No. A5. Yes. The NOEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day study in rats (*13*) is at least 700 times the estimated daily intake of No. 1993 when used as a flavouring agent.	Note 1	No safety concern
8-Decen-5-olide	1994	32764-98-0 	No, SPET: 200	NR	Note 1	No safety concern

Orin lactone	1995	134359-15-2 	No, SPET: 300	NR	Note 1	No safety concern
9-Dodecen-5-olide	1996	15456-68-5 	Yes, SPET: 1000	A4. No. A5. Yes. The NOEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day study in rats (*13*) is at least 700 times the estimated daily intake of No. 1996 when used as a flavouring agent.	Note 1	No safety concern
9-Tetradecen-5-olide	1997	15456-70-9 	Yes, SPET: 1000	A4. No. A5. Yes. The NOEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day study in rats (*13*) is at least 700 times the estimated daily intake of No. 1997 when used as a flavouring agent.	Note 1	No safety concern
γ-Octadecalactone	1998	502-26-1 	Yes, SPET: 1000	A4. No. A5. Yes. The NOEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a	Note 1	No safety concern

				90-day study in rats (*13*) is at least 700 times the estimated daily intake of No. 1998 when used as a flavouring agent.		
δ-Octadecalactone	1999	1227-51-6 	No, SPET: 30	NR	Note 1	No safety concern
Structural class III						
4-Hydroxy-2-butenoic acid γ-lactone	2000	497-23-4 	Yes, SPET: 500	A4. No. A5. Yes. The NOAEL of 17.4 mg/kg bw per day for the related substance 4-hydroxy-3-pentenoic acid (*14*) is at least 2000 times the estimated daily intake of No. 2000 when used as a flavouring agent.	Notes 1 and 2	No safety concern
2-Nonenoic acid γ-lactone	2001	21963-26-8 	No, SPET: 60	NR	Notes 1 and 2	No safety concern
4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	2002	774-64-1 	No, SPET: 62.5	B4. Yes. The NOAEL of 12.1 mg/kg bw per day for the related substance 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) (*13*) is at least 12 000 times the estimated daily intake of No.	Notes 1 and 2	No safety concern

2002 when used as a flavouring agent.

CAS, Chemical Abstracts Service; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

[a] Thirty-five flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *131*).

[b] *Step 1:* Eleven flavouring agents in this group (Nos 1989–1999) are in structural class II. Three flavouring agents in this group (Nos 2000–2002) are in structural class III.

[c] *Step 2:* The 11 flavouring agents in structural class II and 2 flavouring agents in structural class III (Nos 2000 and 2001) can be predicted to be metabolized to innocuous products, and their evaluation therefore proceeded via the A-side of the Procedure. The evaluation of the remaining flavouring agent in structural class III (No. 2002) proceeded via the B-side of the Procedure.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 μg/day, respectively. All intake values are expressed in μg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by SPET or as the MSDI.

Notes:

1. Aliphatic lactones are expected to undergo hydrolysis and oxidative metabolism in the fatty acid pathway.
2. α,β-Unsaturated lactones may directly form conjugates with glutathione, followed by excretion in the urine.

Table 8
Annual production volumes and dietary exposure of aliphatic lactones

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
5-Pentyl-3H-furan-2-one (1989)				0.04	0.001	+
Europe	ND	ND	ND			
USA	8	1.0	0.02			
Japan	ND	ND	ND			
5-Hydroxy-4-methylhexanoic acid δ-lactone (1990)				800	13	+
Europe	0.1	0.01	0.0002			
USA	5	0.6	0.01			
Japan	ND	ND	ND			
Isoambrettolide (1991)				0.1	0.002	–
Europe	108	12	0.2			
USA	0.4	0.05	0.0008			
Japan	0.3	0.1	0.002			
7-Decen-4-olide (1992)				90	2	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
9-Decen-5-olide (1993)				1000	17	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
8-Decen-5-olide (1994)				200	3	–
Europe	0.5	0.06	0.001			
USA	ND	ND	ND			
Japan	2	0.7	0.01			
Orin lactone (1995)				300	5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
9-Dodecen-5-olide (1996)				1000	17	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.3	0.08	0.0014			
9-Tetradecen-5-olide (1997)				1000	17	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
γ-Octadecalactone (1998)				1000	17	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.4	0.1	0.0019			

Table 8 (continued)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
δ-Octadecalactone (1999)				40	1	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
4-Hydroxy-2-butenoic acid γ-lactone (2000)				500	8	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	2	0.5	0.008			
2-Nonenoic acid γ-lactone (2001)				60	1	−
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone (2002)				80	1	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.3	0.1	0.002			
Total						
Europe	109					
USA	13					
Japan	6					

ND, no data reported; +, reported to occur naturally in foods (*8*), but no quantitative data; −, not reported to occur naturally in foods

[a] From references *9–12*. Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1×10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*9–12*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (*12*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Qualitative data only are available (*8*).

Subgroup i: Lactones from saturated linear and branched-chain hydroxycarboxylic acids

The aliphatic lactones considered in this subgroup that are formed from saturated hydroxycarboxylic acids include one δ-lactone with a branched chain (No. 1990) and one γ-lactone and one δ-lactone with linear chains (Nos 1998 and 1999). These lactones would be predicted to be readily hydrolysed to the corresponding hydroxycarboxylic acid, followed by β-oxidative cleavage to yield metabolites that are completely oxidized in the fatty acid pathway and citric acid cycle.

Subgroup ii: Lactones from unsaturated linear and branched-chain hydroxycarboxylic acids

The aliphatic lactones considered in this subgroup that are formed from unsaturated hydroxycarboxylic acids include two γ-lactones with linear chains (Nos 1989 and 1992) and one with a branched chain (No. 1995). The group also contains four linear unsaturated δ-lactones (Nos 1993, 1994, 1996 and 1997) and one ω-lactone (No. 1991), which contains 16 carbons. There are three other unsaturated γ-lactones, but these contain α,β-unsaturation (discussed below). These lactones would be predicted to be readily hydrolysed to the corresponding hydroxycarboxylic acid, followed by β-oxidative cleavage to yield metabolites that are completely metabolized in the fatty acid pathway and citric acid cycle.

Subgroup iii: Lactones containing α,β-unsaturation

Metabolic processes such as oxidation and conjugation effectively eliminate reactive aldehyde groups from such substances when they are consumed in the amounts that would arise from their use as flavouring agents. The aliphatic lactones considered in this subgroup that contain α,β-unsaturation are all γ-lactones. Two are linear γ-lactones (Nos 2000 and 2001), and one is a branched γ-lactone (No. 2002). These lactones would be predicted to be readily hydrolysed to the corresponding hydroxycarboxylic acid. Two of the flavouring agents (Nos 2000 and 2001) would undergo β-oxidative cleavage to yield metabolites that are completely metabolized in the fatty acid pathway and citric acid cycle. One of the flavouring agents (No. 2002) would be hydrolysed to a substituted 2,4-dienoic acid, which can undergo oxidation and/or excretion in the urine.

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 this group of flavouring agents, the Committee assigned 11 of the flavouring agents (Nos 1989–1999) to structural class II and 3 flavouring

agents (Nos 2000–2002) to structural class III (*7*). The Committee noted that the open-chain forms that are in equilibrium with the lactone forms would be in structural class I or II.

Step 2. The 11 flavouring agents that were assigned to structural class II (Nos 1989–1999) are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. Of the three flavouring agents that were assigned to structural class III (Nos 2000–2002), two (Nos 2000 and 2001) are expected to be metabolized via simple α,β-unsaturated acids to innocuous products, and therefore their evaluation proceeded via the A-side of the Procedure; one (No. 2002) may undergo more complex metabolism, and its evaluation therefore proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily intakes (calculated either as the MSDI or by the SPET) of six of the flavouring agents in structural class II (Nos 1989, 1991, 1992, 1994, 1995 and 1999) are below the threshold of concern (i.e. 540 μg/person per day for class II). The safety of these six flavouring agents raises no concern at current estimated dietary exposures. The highest estimated daily intakes (calculated by the SPET) of the other five flavouring agents in structural class II (Nos 1990, 1993 and 1996–1998) are above the threshold of concern (i.e. 540 μg/person per day for class II). Therefore, the evaluation of these five flavouring agents proceeded to step A4.

The highest estimated daily intakes (calculated either as the MSDI or by the SPET) of one of the flavouring agents in structural class III (No. 2001) is below the threshold of concern (90 μg/person per day for class III), and therefore this flavouring agent would not be expected to be of safety concern. For the other flavouring agent in structural class III (No. 2000), the highest estimated daily intake (calculated by the SPET) is above the threshold of concern (90 μg/person per day for class III), and therefore the evaluation proceeded to step A4.

Step A4. None of the five flavouring agents in structural class II or their metabolites are endogenous. Therefore, their evaluation proceeded to step A5.

Neither the structural class III flavouring agent, 4-hydroxy-2-butenoic acid γ-lactone (No. 2000), nor its metabolites are endogenous; therefore, the evaluation proceeded to step A5.

Step A5. For the five flavouring agents in structural class II—namely, 5-hydroxy-4-methylhexanoic acid δ-lactone (No. 1990), 9-decen-5-olide (No. 1993), 9-dodecen-5-olide (No. 1996), 9-tetradecen-5-olide (No. 1997) and γ-octadecalactone (No. 1998)—the NOEL of 12.1 mg/kg bw per day for the structurally related flavouring agent 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day dietary study in rats (*13*) is appropriate. The

NOEL of 12.1 mg/kg bw per day for 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) provides a margin of safety of at least 700 or at least 900 in relation to the estimated dietary exposure to each of these flavouring agents. Therefore, the Committee concluded that all of these five flavouring agents in structural class II would not pose a safety concern at current estimated dietary exposures.

For the structural class III flavouring agent, 4-hydroxy-2-butenoic acid γ-lactone (No. 2000), the NOAEL of 17.4 mg/kg bw per day for the structurally related 4-hydroxy-3-pentenoic acid (the open-chain form of 4-hydroxy-3-pentenoic acid lactone [No. 221]) in a 90-day study in rats (*14*) provides a margin of safety of approximately 2000 in relation to the highest estimated dietary exposure to No. 2000. Therefore, the Committee concluded that 4-hydroxy-2-butenoic acid γ-lactone (No. 2000) would not pose a safety concern at current estimated dietary exposures.

Step B3. For the flavouring agent in structural class III, 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone (No. 2002), the highest estimated daily intake (calculated either as the MSDI or by the SPET) is below the threshold of concern (90 μg/person per day for class II), and its evaluation therefore proceeded to step B4.

Step B4. For 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone (No. 2002), the NOEL of 12.1 mg/kg bw per day for the structurally related flavouring agent 5-hydroxy-2,4-decadienoic acid δ-lactone (No. 245) from a 90-day dietary study in rats (*13*) is appropriate. This NOEL provides a margin of safety of at least 12 000 in relation to the estimated dietary exposure to No. 2002. Therefore, the Committee concluded that 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone (No. 2002) would not pose a safety concern at current estimated dietary exposures.

Table 7 summarizes the evaluations of the 14 additional flavouring agents belonging to the group of aliphatic lactones used as flavouring agents (Nos 1989–2002).

Additional biochemical data and toxicological studies

Data from additional biochemical and toxicological studies on this group of flavouring agents have been submitted since the initial consideration by the Committee at the forty-ninth meeting (Annex 1, reference *132*). These data are summarized below.

Lactones have been reported to undergo hydrolysis with the human serum enzyme paraoxanase (PON1). This enzyme is synthesized in the liver and exported to the blood. It has a variety of substrates, including carboxylic acid esters and lactones. With a lactone substrate, it causes the lactone ring to open

hydrolytically, yielding a corresponding hydroxyl-substituted carboxylic acid. To better characterize the lactonase activity of PON1, the hydrolysis of a series of lactones was investigated. PON1 was able to readily hydrolyse a series of 30 lactones containing different structural features. Only the lactones that are pseudoaromatic (e.g. coumarin) did not undergo extensive hydrolysis by PON1 (*15*).

Oral median lethal dose (LD_{50}) values have been reported for one of the flavouring agents of this group. In male rats, an LD_{50} value of >5000 mg/kg bw was reported for 8-decen-5-olide (No. 1994) (*16*).

In vitro genotoxicity studies have been reported for three flavouring agents in this group (Nos 1990–1992). For 5-hydroxy-4-methylhexanoic acid δ-lactone (No. 1990), negative results were reported in reverse mutation assays with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 incubated with 100, 316, 1000 and 3160 μg/plate with and without metabolic activation (*17*). For isoambrettolide (No. 1991), negative results were reported in reverse mutation assays with *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 incubated with 33, 100, 333, 1000, 2500 and 5000 μg/plate with and without metabolic activation. Negative results for isoambrettolide (No. 1991) were also reported in a modified reverse mutation assay using the preincubation method with *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 incubated with 33, 100, 333, 1000, 2500 and 5000 μg/plate with and without metabolic activation (*18*). For 7-decen-4-olide (No. 1992), negative results were reported in Ames assays with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 incubated with 15, 50, 150, 500, 1500 and 5000 μg/plate with and without metabolic activation (*19*).

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series as proposed at the sixty-eighth meeting (Annex 1, reference *187*) and using the MSDI exposure assessment as proposed at the sixty-ninth meeting (Annex 1, reference *190*).

The consideration of combined intakes from the use of aliphatic lactones as flavouring agents was discussed in the report of the forty-ninth meeting (Annex 1, reference *132*). The additional aliphatic lactones considered at this meeting from each of the structural classes all have very low dietary exposures compared with the aliphatic lactones considered previously and would not contribute significantly to the combined intakes of this flavouring group. All of these additional aliphatic lactones would be expected to be efficiently metabolized to innocuous substances and would not saturate metabolic pathways.

Consideration of secondary components

One member of this group of flavouring agents (No. 2002) has a minimum assay value of less than 95%. The secondary component of 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone is 3,4-dimethyl-5-ketobutanoic acid γ-lactone. This substance is expected to share the same metabolic fate as the primary substance and is not considered to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary component of this flavouring agent is summarized in Annex 4.

Conclusion

In the previous evaluation of aliphatic lactones in this group at the forty-ninth meeting and in the subsequent evaluation of α,β-unsaturated flavouring agents at the fifty-fifth meeting, studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. The toxicity data available for the evaluation of these additional flavouring agents supported the data from previous evaluations.

The Committee concluded that these 14 additional members of the group of aliphatic lactones when used as flavouring agents would not present safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was not prepared.

4.1.7 *Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups: additional compounds*

The Committee evaluated 44 additional flavouring agents belonging to the group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups, which was evaluated previously. The additional flavouring agents included 23 esters, 11 diesters, 5 acids, 2 primary alcohols, 2 ketals and 1 acetal. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee previously evaluated 47 other members of this group of flavouring agents at its fifty-third meeting (Annex 1, reference *144*). The Committee concluded that all 47 flavouring agents in that group were of no safety concern based on estimated dietary exposures.

Eleven of the additional 44 flavouring agents are natural components of food (Nos 1945, 1949, 1951, 1955, 1956, 1959, 1962, 1964, 1967, 1976 and 1987). They have been detected in pineapple, coconut, cape gooseberry, melon, licorice, potato, raspberry, papaya, pear, honey, scallop, pork, beef, guinea hen, mushroom, tamarind, cheese, beer and apple and pear brandy (*8*).

Assessment of dietary exposure

The total annual volumes of production of this group of 44 additional flavouring agents are approximately 7 kg in Europe, 2 kg in the USA and 980 kg in Japan (*9–12*). In Europe, greater than 70% of the annual volume of production is accounted for by hydroxyacetone (No. 1945), and in the USA, 100% of the annual volume of production is accounted for by (±)-ethyl 3-hydroxy-2-methylbutyrate (No. 1949).

The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 9. The highest estimate is for dipropyl adipate (No. 1965) (2000 μg, the SPET value for fine bakery ware). For the other flavouring agents in the group, the daily dietary exposures range from 0.02 to 1600 μg, with the SPET yielding the higher estimate for all except the mixture of 6-(5-decenoyloxy)decanoic acid and 6-(6-decenoyloxy)decanoic acid (No. 1977). Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 10.

Absorption, distribution, metabolism and elimination

Studies on the metabolism of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups were considered at the fifty-third meeting (Annex 1, reference *144*).

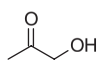
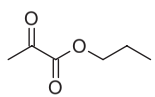
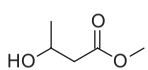
Many of the substances in this group are esters, diesters, acetals or ketals and are expected to undergo hydrolysis to their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols or branched-chain hydroxyl- or keto-alcohols). The presence of a second oxygenated functional group is expected to have little effect on ester hydrolysis. The β-keto acids and derivatives easily undergo decarboxylation and, with α-keto and α-hydroxyacids, yield breakdown products that are incorporated into normal biochemical pathways. The γ-keto acids and related substances may undergo complete or partial β-oxidation to yield metabolites that are eliminated in the urine. The ω-substituted derivatives are predicted to be readily oxidized and/or excreted in the urine. The simple aliphatic dicarboxylic and tricarboxylic acids either occur endogenously in humans or are structurally related to endogenous substances. These substances are metabolized through the fatty acid β-oxidation pathway or the tricarboxylic acid cycle (*21*).

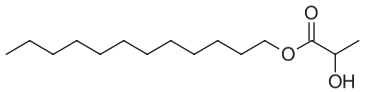
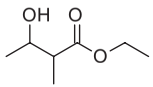
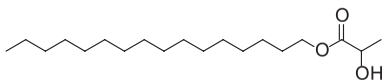
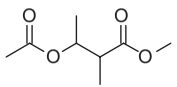
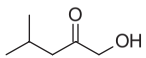
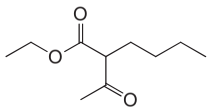
Application of the Procedure for the Safety Evaluation of Flavouring Agents

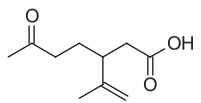
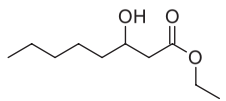
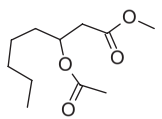
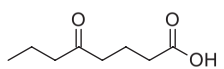
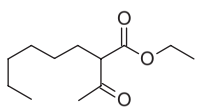
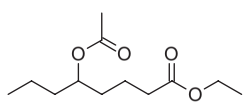
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to this group of flavouring agents, the Committee assigned 40 flavouring agents (Nos 1945–1968, 1970–1972, 1974 and 1976–1987) to structural class I and 4 flavouring agents (Nos 1969, 1973, 1975 and 1988) to structural class III (*7*).

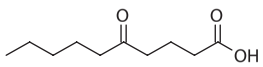
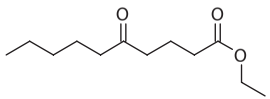
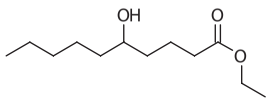
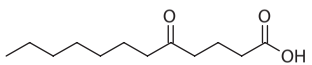
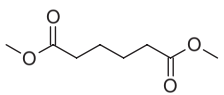
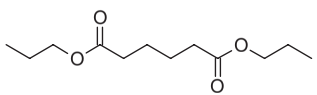
Table 9

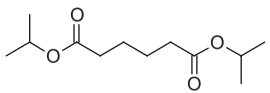
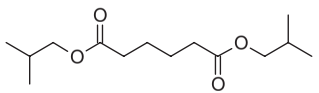
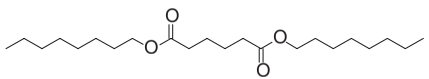
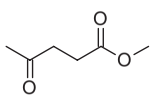
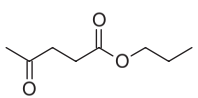
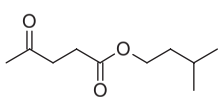
Summary of the results of the safety evaluations of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups used as flavouring agents[a,b,c]

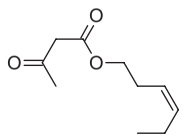
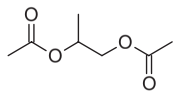
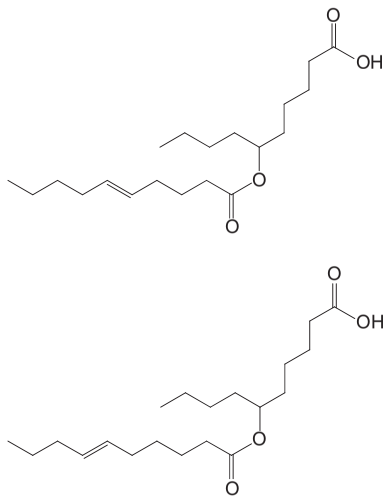
Flavouring agent	No.	CAS No. and structure	*Step A3*[d,e] Does intake exceed the threshold for human intake?	*Step A4/A5* *A4.* Is the substance or are its metabolites endogenous? *A5.* Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I						
Hydroxyacetone	1945	116-09-6 	No, SPET: 1500	NR	Note 1	No safety concern
Propyl pyruvate	1946	20279-43-0 	No, SPET: 200	NR	Notes 2 and 3	No safety concern
Methyl 3-hydroxybutyrate	1947	1487-49-6 	No, SPET: 6	NR	Notes 2 and 3	No safety concern

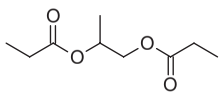
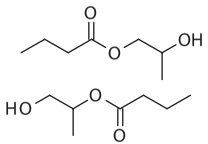
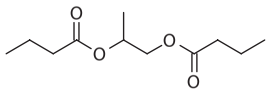
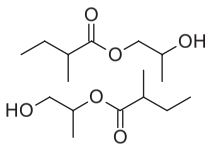
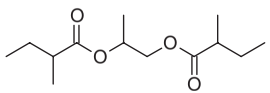
Dodecyl lactate	1948	6283-92-7		No, SPET: 600	NR	Notes 2 and 3	No safety concern
(±)-Ethyl 3-hydroxy-2-methylbutyrate	1949	27372-03-8		No, SPET: 210	NR	Notes 2 and 3	No safety concern
Hexadecyl lactate	1950	35274-05-6		No, SPET: 100	NR	Notes 2 and 3	No safety concern
Methyl 3-acetoxy-2-methylbutyrate	1951	139564-42-4		No, SPET: 300	NR	Notes 2 and 3	No safety concern
1-Hydroxy-4-methyl-2-pentanone	1952	68113-55-3		No, SPET: 80	NR	Notes 3 and 4	No safety concern
Ethyl 2-acetylhexanoate	1953	1540-29-0		No, SPET: 400	NR	Notes 2, 4 and 5	No safety concern

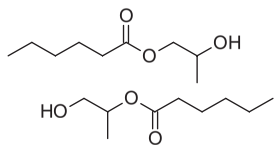
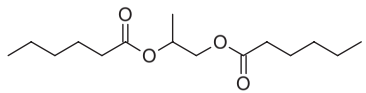
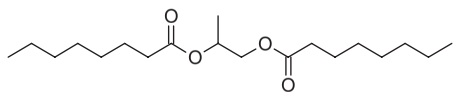
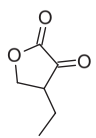
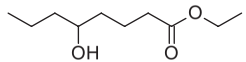
3-Isopropenyl-6-oxoheptanoic acid	1954	4436-82-2		No, SPET: 3	NR	Notes 5 and 6	No safety concern
Ethyl 3-hydroxyoctanoate	1955	7367-90-0		No, SPET: 15	NR	Notes 2 and 3	No safety concern
Methyl 3-acetoxyoctanoate	1956	35234-21-0		No, SPET: 300	NR	Notes 2 and 3	No safety concern
5-Oxooctanoic acid	1957	3637-14-7		No, SPET: 2	NR	Notes 3, 4 and 6	No safety concern
Ethyl 2-acetyloctanoate	1958	29214-60-6		No, SPET: 1200	NR	Notes 2, 4 and 6	No safety concern
Ethyl 5-acetoxyoctanoate	1959	35234-25-4		No, SPET: 1200	NR	Notes 2 and 3	No safety concern

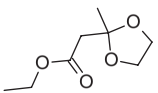
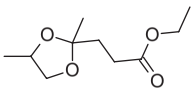
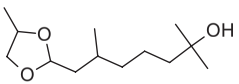
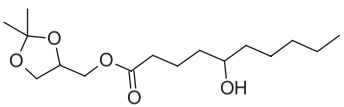
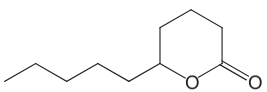
5-Oxodecanoic acid	1960	624-01-1		No, SPET: 2	NR	Notes 3, 4 and 6	No safety concern
Ethyl 5-oxodecanoate	1961	93919-00-7		No, SPET: 1000	NR	Notes 2, 3, 4 and 6	No safety concern
Ethyl 5-hydroxydecanoate	1962	75587-06-3		No, SPET: 300	NR	Notes 2 and 3	No safety concern
5-Oxododecanoic acid	1963	3637-16-9		No, SPET: 2	NR	Notes 3, 4 and 6	No safety concern
Dimethyl adipate	1964	627-93-0		No, SPET: 1000	NR	Notes 2 and 3	No safety concern
Dipropyl adipate	1965	106-19-4		No, SPET: 2000	NR	Notes 2 and 3	No safety concern

Diisopropyl adipate	1966	6938-94-9 	No, SPET: 1200	NR	Notes 2 and 3	No safety concern
Diisobutyl adipate	1967	141-04-8 	No, SPET: 1000	NR	Notes 2 and 3	No safety concern
Dioctyl adipate	1968	123-79-5 	No, SPET: 1600	NR	Notes 2 and 3	No safety concern
Methyl levulinate	1970	624-45-3 	No, SPET: 600	NR	Notes 2, 3, 4 and 6	No safety concern
Propyl levulinate	1971	645-67-0 	No, SPET: 625	NR	Notes 2, 3, 4 and 6	No safety concern
Isoamyl levulinate	1972	71172-75-3 	No, SPET: 300	NR	Notes 2, 3, 5 and 6	No safety concern

cis-3-Hexenyl acetoacetate	1974	84434-20-8 	No, SPET: 1200	NR	Notes 2, 3 and 4	No safety concern
Propyleneglycol diacetate	1976	623-84-7 	No, SPET: 320	NR	Notes 2 and 7	No safety concern
Mixture of 6-(5-Decenoyloxy)decanoic acid and 6-(6-Decenoyloxy)decanoic acid	1977	85392-05-8; 85392-06-9 	No, MSDI: Europe: ND USA: ND Japan: 61	NR	Notes 2, 3, 5 and 6	No safety concern

Propyleneglycol dipropionate	1978	10108-80-2 	No, SPET: 1250	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol monobutyrate (mixture of isomers)	1979	29592-95-8 	No, SPET: 1600	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol dibutyrate	1980	50980-84-2 	No, SPET: 400	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol mono-2-methylbutyrate (mixture of isomers)	1981	923593-56-0; 923593-57-1 	No, SPET: 1600	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol di-2-methylbutyrate	1982	15514-30-0 	No, SPET: 400	NR	Notes 2, 3 and 7	No safety concern

Propyleneglycol monohexanoate (mixture of isomers)	1983	39556-41-7; 170678-49-6 	No, SPET: 1600	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol dihexanoate	1984	50343-36-7 	No, SPET: 1600	NR	Notes 2, 3 and 7	No safety concern
Propyleneglycol dioctanoate	1985	7384-98-7 	No, SPET: 300	NR	Notes 2, 3 and 7	No safety concern
2-Oxo-3-ethyl-4-butanolide	1986	923291-29-6 	No, SPET: 150	NR	Notes 3 and 8	No safety concern
Ethyl 5-hydroxyoctanoate	1987	75587-05-2 	No, SPET: 900	NR	Notes 2, 5 and 6	No safety concern

Structural class III						
Ethyl acetoacetate ethyleneglycol ketal	1969	6413-10-1 	No, SPET: 80	NR	Notes 2, 9 and 10	No safety concern
Ethyl levulinate propyleneglycol ketal	1973	5413-49-0 	Yes, SPET: 800	A4. Not endogenous A5. Additional data required	Notes 2, 3, 7 and 9	Additional data required to complete evaluation
Hydroxycitronellal propyleneglycol acetal	1975	93804-64-9 	No, SPET: 30	NR	Notes 7, 9 and 11	No safety concern
Mixture of Isopropylideneglyceryl 5-hydroxyoctanoate and δ-Decalactone (No. 232)	1988	172201-58-0; 705-86-2  	Yes, SPET: 1600	A4. Not endogenous A5. Additional data required	Notes 2, 6, 9 and 11	Additional data required to complete evaluation

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

[a] Forty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *144*).

[b] *Step 1:* Forty flavouring agents in this group (Nos 1945–1968, 1970–1972, 1974 and 1976–1987) are in structural class I. Four flavouring agents in this group (Nos 1969, 1973, 1975 and 1988) are in structural class III.

[c] *Step 2:* All of the agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. Hydroxyacetone is readily biotransformed into metabolites that eventually enter the citric acid cycle.
2. The ester is expected to be hydrolysed to the corresponding alcohol and carboxylic acid.
3. Biotransformed by endogenous metabolism to carbon dioxide and water.
4. Biotransformed by reduction to the ketone and subsequent conjugation and excretion and/or oxidative metabolism.
5. It is anticipated that the ketone group will be reduced to the secondary alcohol and excreted in the urine as the glucuronic acid conjugate.
6. Acid metabolites will be excreted in the urine.
7. Propylene glycol is readily oxidized to lactic acid.
8. Butanolides readily undergo lactone hydrolysis, followed by decarboxylation.
9. The acetal or ketal is expected to be hydrolysed, liberating the aldehyde or ketone.
10. Acetoacetate is readily converted to acetyl coenzyme A and completely metabolized.
11. The alcohol is anticipated to be excreted in the urine as the glucuronic acid conjugate.

Table 10

Annual volumes of production and dietary exposure for aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Hydroxyacetone (1945)				1500	25	72
Europe	5.0	0.5	0.01			
USA	ND	ND	ND			
Japan	37	11	0.2			
Propyl pyruvate (1946)				200	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.0	0.3	0.005			
Methyl 3-hydroxybutyrate (1947)				6	0.1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
Dodecyl lactate (1948)				800	13	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.5	0.1	0.002			
(±)-Ethyl 3-hydroxy-2-methylbutyrate (1949)				210	4	+
Europe	ND	ND	ND			
USA	2	0.2	0.004			
Japan	ND	ND	ND			
Hexadecyl lactate (1950)				160	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	33	9	0.2			
Methyl 3-acetoxy-2-methylbutyrate (1951)				300	5	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	2	1	0.01			
1-Hydroxy-4-methyl-2-pentanone (1952)				80	1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.1	0.001			
Ethyl 2-acetylhexanoate (1953)				400	7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.04	0.001			

Table 10 (continued)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
3-Isopropenyl-6-oxoheptanoic acid (1954)				3	0.1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.02	0.0003			
Ethyl 3-hydroxyoctanoate (1955)				15	0.3	+
Europe	2.0	0.2	0.004			
USA	ND	ND	ND			
Japan	0.3	0.1	0.002			
Methyl 3-acetoxyoctanoate (1956)				300	5	32
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.04	0.001			
5-Oxooctanoic acid (1957)				2	0.03	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.3	0.1	0.001			
Ethyl 2-acetyloctanoate (1958)				1200	20	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	19	5	0.1			
Ethyl 5-acetoxyoctanoate (1959)				1200	20	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.3	0.01			
5-Oxodecanoic acid (1960)				2	0.03	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.3	0.005			
Ethyl 5-oxodecanoate (1961)				1000	17	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	27	8	0.1			

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Ethyl 5-hydroxydecanoate (1962)				300	5	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	121	35	0.6			
5-Oxododecanoic acid (1963)				2	0.03	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.0	0.3	0.00			
Dimethyl adipate (1964)				1000	17	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
Dipropyl adipate (1965)				2000	33	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	145	41	0.7			
Diisopropyl adipate (1966)				1200	20	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	53	15	0.3			
Diisobutyl adipate (1967)				1000	17	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.5	0.1	0.002			
Dioctyl adipate (1968)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	15	4	0.07			
Ethyl acetoacetate ethyleneglycol ketal (1969)				80	1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	16	5	0.1			
Methyl levulinate (1970)				600	10	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	7	2	0.03			
Propyl levulinate (1971)				625	10	–
Europe	ND	ND	ND			

Table 10 (continued)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
USA	ND	ND	ND			
Japan	2	0.4	0.01			
Isoamyl levulinate (1972)				300	5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	20	5.7	0.1			
Ethyl levulinate propyleneglycol ketal (1973)				800	13	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	112	32	0.5			
cis-3-Hexenyl acetoacetate (1974)				1200	20	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	7	2	0.03			
Hydroxycitronellal propyleneglycol acetal (1975)				30	0.5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.7	0.2	0.003			
Propyleneglycol diacetate (1976)				320	5	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	36	10	0.2			
Mixture of 6-(5-Decenoyloxy)decanoic acid and 6-(6-Decenoyloxy)decanoic acid (1977)				15	0.3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	215	61	1.0			
Propyleneglycol dipropionate (1978)				1250	21	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.02	0.0003			

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Propyleneglycol monobutyrate (mixture of isomers) (1979)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	47	13	0.2			
Propyleneglycol dibutyrate (1980)				400	7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.3	0.01			
Propyleneglycol mono-2-methylbutyrate (mixture of isomers) (1981)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	5	1	0.02			
Propyleneglycol di-2-methylbutyrate (1982)				400	7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
Propyleneglycol monohexanoate (mixture of isomers) (1983)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	3	0.9	0.02			
Propyleneglycol dihexanoate (1984)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.7	0.2	0.003			
Propyleneglycol dioctanoate (1985)				300	5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	5	1	0.02			
2-Oxo-3-ethyl-4-butanolide (1986)				150	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			

Table 10 (continued)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Japan	0.1	0.03	0.001			
Ethyl 5-hydroxyoctanoate (1987)				900	15	1014
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.5	0.1	0.002			
Mixture of Isopropylideneglyceryl 5-hydroxydecanoate and δ-Decalactone (1988)				1600	27	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	43	12	0.2			
Total						
Europe	7					
USA	2					
Japan	980					

ND, no data reported; +, reported to occur naturally in foods (*8*), but no quantitative data; –, not reported to occur naturally in foods

[a] From references *9–12*. Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1×10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*9–12*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (*12*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Qualitative data reported by Nijssen, van Ingen-Visscher & Donders (*8*).

[e] Quantitative data for the USA reported by Stofberg & Grundschober (*20*). The consumption ratio (annual consumption via food, kg)/(most recent reported production volume as a flavouring substance, kg) was not determined, as consumption data from the USA only were available.

Step 2. All of the flavouring agents in structural class I or III are expected to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure.

Step A3. The highest estimated daily intakes (calculated either as the MSDI or by the SPET) of the 40 flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). The highest estimated daily intakes (calculated either as the MSDI or by the SPET) of two flavouring agents (Nos 1969 and 1975) in structural class III are below the threshold of concern (i.e. 90 μg/person per day for class III). The safety of these 42 flavouring agents at their current estimated dietary exposures raises no concern. The highest estimated daily intakes (calculated by the SPET) of the other two flavouring agents (Nos 1973 and 1988) in structural class III are above the threshold of concern (i.e. 90 μg/person per day for class III). For these two flavouring agents, the evaluation proceeded to step A4.

Step A4. Neither of the two flavouring agents (Nos 1973 and 1988) is endogenous, and therefore the evaluation proceeded to step A5.

Step A5. For ethyl levulinate propyleneglycol ketal (No. 1973) and the mixture of isopropylideneglyceryl 5-hydroxydecanoate and δ-decalactone (No. 1988), adequate data on the rate and extent of hydrolysis were not available. NOELs were not available for these substances or for structurally related substances. Therefore, for these two substances, the Committee concluded that additional data would be necessary to complete the evaluation.

Table 9 summarizes the evaluations of the 44 additional members of the group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups used as flavouring agents (Nos 1945–1988).

Additional toxicological studies

Toxicity data on these additional flavouring agents have been submitted.

Oral LD_{50} values have been reported for 2 of the 44 additional flavouring agents in this group. For diisobutyl adipate (No. 1967) and ethyl acetoacetate ethyleneglycol ketal (No. 1969), LD_{50} values in rats were reported as greater than 5000 mg/kg bw (*22*, *23*).

Genotoxicity studies have been reported for acetoacetate ethyleneglycol ketal (No. 1969). No genotoxic potential was observed when *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 or TA1537 were incubated with 0, 33, 100, 333, 1000, 2500 or 5000 μg of ethyl acetoacetate ethyleneglycol ketal per plate in the absence and presence of S9 metabolic activation (*24*).

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series as proposed at the sixty-eighth meeting (Annex 1, reference *187*) and using the MSDI exposure assessment as proposed at the sixty-ninth meeting (Annex 1, reference *190*).

This group of flavouring agents contains several homologous series that have common metabolites—namely, pyruvate, 3-hydroxybutyrate, levulinic acid, propylene glycol, adipate and lactate. In the unlikely event that any of these flavouring agents with a common metabolite or that are members of a homologous series were to be consumed concurrently on a daily basis, the estimated combined intakes would be as shown in Table 11.

Table 11
Combined dietary exposure for the homologous series with a common metabolite within this group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups used as flavouring agents

Common metabolite	Substances with highest dietary exposure (Nos)	Estimated combined dietary exposure in Europe, USA and Japan (μg/person per day)	Dietary exposure relative to the threshold of concern (1800 μg/person per day)
Pyruvate	936, 937, 938, 1946	183 (Europe), 88 (USA), 0.2 (Japan)	Not exceeded
3-Hydroxybutyrate	600, 601, 604, 1947, 1949, 1955, 1956	90 (Europe), 3.2 (USA), 0.1 (Japan)	Not exceeded
Levulinic acid	606, 607, 608, 1970, 1971, 1972, 1973	896 (Europe), 310 (USA), 24.3 (Japan)	Not exceeded
Propylene glycol	Propylene glycol, 926, 1973, 1976, 1979, 1981, 1985	2 414 660 (Europe), 24.7 (USA and Japan)	Exceeded (USA)
Adipate	623, 1964, 1965, 1966, 1967, 1968	12 (Europe), 18 000 (USA), 38.1 (Japan)	Exceeded (USA)
Lactate	930, 931, 932, 934, 935, 1948, 1950	1820 (Europe), 48 811 (USA), 3 (Japan)	Exceeded (Europe and USA)

For flavouring agents with common metabolites of propylene glycol, adipate or lactate, the combined intakes would exceed the threshold of concern (i.e. 1800 μg/person per day for class I) in the USA, and also in Europe in the case of lactate. For compounds metabolized to propylene glycol, the vast majority of the intake in the USA was due to propylene glycol itself (2 400 000 μg/person per day), which has an ADI of 0–25 mg/kg bw. For compounds

metabolized to adipate and lactate, the flavouring agents are expected to be efficiently metabolized and would not saturate available detoxication pathways.

Consideration of secondary components

Seven flavouring agents in this group (Nos 1948, 1950, 1962, 1974, 1979, 1987 and 1988) have minimum assay values of less than 95%. The secondary components of these flavouring agents are shown in Table 12.

Table 12
Secondary components of flavouring agents in the group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups used as flavouring agents

No.	Flavouring agent	Secondary components
1948	Dodecyl lactate	Dodecanol
1950	Hexadecyl lactate	Hexadecanol (No. 114)
1962	Ethyl 5-hydroxydecanoate	δ-Decalactone (No. 232)
1974	*cis*-3-Hexenyl acetoacetate	*cis*-3-Hexenol
1979	Propyleneglycol monobutyrate	Propyleneglycol dibutyrate (No. 1980)
1987	Ethyl 5-hydroxyoctanoate	Ethanol (No. 41); 1,5-octanolide; 5-hydroxydecanoic acid; ethyl-5-hydroxyoctanoate ester
1988	Mixture of Isopropylideneglyceryl 5-hydroxydecanoate and δ-Decalactone	2,2-Dimethyl-1,3-dioxolane-4-methanol; 2-propyl 5-hydroxydecanoate

The secondary components of each of these flavouring agents are expected to undergo rapid absorption, distribution, metabolism and excretion and are considered not to present a safety concern at current dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the previous evaluation of flavouring agents in this group at the fifty-third meeting, studies of acute toxicity, short-term toxicity and genotoxicity were available. The toxicity data available for the additional flavouring agents support those from the previous evaluation (Annex 1, reference *144*).

The Committee concluded that 42 of 44 additional flavouring agents evaluated at the present meeting do not raise any safety concerns at current estimated dietary exposures.

For ethyl levulinate propyleneglycol ketal (No. 1973) and the mixture of isopropylideneglyceryl 5-hydroxydecanoate and δ-decalactone (No. 1988), the Committee concluded that additional data would be necessary to complete the evaluation.

An addendum to the toxicological monograph was not prepared.

4.1.8 *Aliphatic secondary alcohols, ketones and related esters and acetals: additional compounds*

Seven flavouring agents were proposed to be evaluated as additions to the previously evaluated group of saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters. These seven agents included one secondary unsaturated alcohol (No. 2071), one ketone (No. 2074), three esters (Nos 2070, 2072 and 2073) and two cyclic acetals (Nos 2075 and 2076). The Committee decided that these seven agents fit better in the previously evaluated group of aliphatic secondary alcohols, ketones and related esters. The Committee therefore evaluated these compounds as additions to this group and extended the group name to "aliphatic secondary alcohols, ketones and related esters and acetals" to include the acetals. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these agents has previously been evaluated by the Committee.

The Committee previously evaluated 39 members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*) and an additional 17 members at its sixty-ninth meeting (Annex 1, reference *190*). All 56 flavouring agents were concluded to be of no safety concern at estimated dietary exposures.

Two of the seven flavouring agents evaluated at the present meeting are natural components of foods (Nos 2071 and 2074). No. 2071 (*R*-(–)-1-octen-3-ol) can be found in mushrooms. No. 2074 (2-decanone) can be found in a wide range of food products, including meat (beef, poultry, pork, lamb), milk and milk products, cheeses, eggs, fish, shellfish, brandy, tea, coffee, fruits (banana, mountain papaya, berries), vegetables (potato, mushroom, endive, soya bean, chayote, kumazase), grains (maize, rice, oats), nuts, honey, ginger, garlic, vanilla, hop oil and mate. The highest levels have been reported in milk and milk products and hop oil (*8*).

Assessment of dietary exposure

The total annual volumes of production of the seven flavouring agents in this group are approximately 12 kg in Europe, 0.3 kg in the USA and 22 kg

in Japan (*9–12*). In the USA, 100% of the total annual volume of production is accounted for by *R*-(–)-1-octen-3-ol (No. 2071). In Europe and Japan, 2-decanone (No. 2074) makes the biggest contribution to the total annual volume of production (99% and 95%, respectively).

The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 13. The highest estimate is for (±)-octan-3-yl formate (No. 2070) (900 µg, the SPET value obtained from non-alcoholic beverages). For the other flavouring agents in the group, the daily dietary exposures range from 0.01 to 400 µg, with the SPET yielding the highest estimate for all. Reported annual volumes of production for this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 14.

Absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic secondary alcohols, ketones and related esters and acetals has previously been described in the reports of the fifty-ninth and sixty-ninth meetings (Annex 1, references *160* and *190*). The two acetals are predicted to be metabolized to propylene glycol and the corresponding ketones; this has been previously described in the report of the fifty-seventh meeting (Annex 1, reference *154*).

No additional relevant data have been reported since the fifty-ninth, sixty-ninth and fifty-seventh meetings.

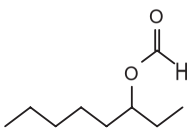
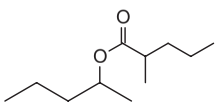
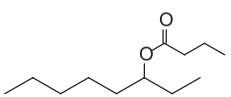
Application of the Procedure for the Safety Evaluation of Flavouring Agents

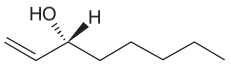
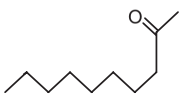
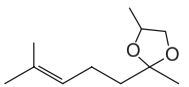
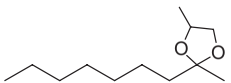
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the seven flavouring agents in this group of aliphatic secondary alcohols, ketones and related esters and acetals, the Committee assigned three flavouring agents (Nos 2070, 2072 and 2073) to structural class I, two flavouring agents (Nos 2071 and 2074) to structural class II and two flavouring agents (Nos 2075 and 2076) to structural class III (*7*).

Step 2. All flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all three flavouring agents in structural class I (Nos 2070, 2072 and 2073) are below the threshold of concern (i.e. 1800 µg/person per day for class I). The safety of these three flavouring agents raises no concern at current estimated dietary exposures.

Table 13
Summary of the results of the safety evaluations of aliphatic secondary alcohols, ketones and related esters and acetals used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I					
(±)-Octan-3-yl formate	2070	84434-65-1 	No, SPET: 900	Note 1	No safety concern
2-Pentyl 2-methylpentanoate	2072	90397-36-7 	No, SPET: 75	Note 1	No safety concern
3-Octyl butyrate	2073	20286-45-7 	No, SPET: 300	Note 1	No safety concern

Structural class II					
(*R*)-(−)-1-Octen-3-ol	2071	3687-48-7 	No, SPET: 400	Note 2	No safety concern
2-Decanone	2074	693-54-9 	No, SPET: 400	Note 3	No safety concern
Structural class III					
6-Methyl-5-hepten-2-one propyleneglycol acetal	2075	68258-95-7 	No, SPET: 30	Note 4	No safety concern
2-Nonanone propyleneglycol acetal	2076	165191-91-3 	No, SPET: 16	Note 4	No safety concern

CAS, Chemical Abstracts Service

[a] Thirty-nine flavouring agents belonging to the renamed group of aliphatic secondary alcohols, ketones and related esters and acetals were previously evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*), and 17 additional members were evaluated at its sixty-ninth meeting (Annex 1, reference *190*).

[b] *Step 1:* Three of the flavouring agents (Nos 2070, 2072 and 2073) in this group were assigned to structural class I, two of the flavouring agents (Nos 2071 and 2074) were assigned to structural class II and the remaining two flavouring agents (Nos 2075 and 2076) were assigned to structural class III.

[c] *Step 2:* All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

Notes:

1. Hydrolysed to the corresponding alcohol and carboxylic acid. The carboxylic acids can be metabolized via the β-oxidation pathway, yielding shorter-chain carboxylic acids that are subsequently metabolized to carbon dioxide via the tricarboxylic acid pathway. The alcohols participate in the pathway cited in note 2.
2. Conjugated with glucuronic acid and excreted primarily in the urine.
3. Reduced to the corresponding alcohol, followed by glucuronic acid conjugation.
4. Hydrolysis of the acetal to yield propylene glycol and the corresponding ketone, which is reduced to the corresponding alcohol and excreted as the glucuronic acid conjugate. Propylene glycol is oxidized to pyruvic acid and completely oxidized in the citric acid cycle.

Table 14

Annual volumes of production and daily dietary exposures for aliphatic secondary alcohols, ketones and related esters and acetals used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
(±)-Octan-3-yl formate (2070)				900	15	–
Europe	0.1	0.01	0.00018			
USA	ND	ND	ND			
Japan	ND	ND	ND			
R-(−)-1-Octen-3-ol (2071)				400	7	+
Europe	ND	ND	ND			
USA	0.3	0.04	0.001			
Japan	ND	ND	ND			
2-Pentyl 2-methylpentanoate (2072)				75	1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
3-Octyl butyrate (2073)				300	5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.5	0.1	0.002			
2-Decanone (2074)				400	7	+
Europe	11	1	0.02			
USA	ND	ND	ND			
Japan	21	6	0.09			
6-Methyl-5-hepten-2-one propyleneglycol acetal (2075)				30	1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.3	0.1	0.001			
2-Nonanone propyleneglycol acetal (2076)				16	0.3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			

Table 14 (continued)

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Total						
Europe	12					
USA	0.3					
Japan	22					

ND, no data reported; +, reported to occur naturally in foods (*8*), but no quantitative data; –, not reported to occur naturally in foods

[a] From references *9–12*. Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 12 × 10^6 for Japan; and where correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*9–12*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (*12*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

The estimated daily per capita intakes of the two flavouring agents in structural class II (Nos 2071 and 2074) are below the threshold of concern (i.e. 540 µg/person per day for class II). The safety of these two flavouring agents raises no concern at current estimated dietary exposures.

The estimated daily per capita intakes of the two flavouring agents in structural class III (Nos 2075 and 2076) are below the threshold of concern (i.e. 90 µg/person per day for class III). The safety of these two flavouring agents raises no concern at current estimated dietary exposures.

Table 13 summarizes the evaluations of the seven additional flavouring agents (Nos 2070–2076) in this group of aliphatic secondary alcohols, ketones and related esters and acetals.

Additional toxicological studies

Studies of acute oral toxicity report an LD_{50} value of 550 mg/kg bw for *R*-(–)-1-octen-3-ol (No. 2071) in female rats (*25*) and an LD_{50} value of 175 mg/kg bw for the previously evaluated racemic mixture of 1-octen-3-ol (No. 1152) in female rats (*26*). These results support the findings in the

previous evaluations (Annex 1, references *161* and *190*) that the oral acute toxicity of aliphatic secondary alcohols, ketones and related esters and acetals is low to moderate.

Additional studies of genotoxicity in vitro have also been reported for 1-octen-3-ol (No. 1152). There was no evidence of mutagenicity in a standard and modified (preincubation method) reverse mutation assay when various strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 *uvrA* were incubated with up to 5000 μg of 1-octen-3-ol per plate, with or without metabolic activation (*27*).

In an alkaline single cell gel electrophoresis (comet) assay using human lung carcinoma epithelial A549 cells, human peripheral blood cells and Chinese hamster V79 cells, 1-octen-3-ol (No. 1152; 0.6 and 6.4 mmol/l) produced varying results. The test was negative in A549 cells. In V79 cells, a significant increase in tail moment was observed at the highest concentration tested. At this concentration, cytotoxic effects were observed in peripheral blood cells (*28*).

In a micronucleus assay using Chinese hamster V79 cells, 1-octen-3-ol tested negative in the absence and presence of metabolic activation at concentrations up to 6.4 and 3.2 mmol/l, respectively (*28*). In a hypoxanthine–guanine–phosphoribosyl transferase (HPRT) gene mutation assay, 1-octen-3-ol tested negative at concentrations up to 5 mmol/l in the absence and presence of S9 preparation (*28*).

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

No homologous series could be identified for the flavouring agents currently under evaluation, but 3-octanol (No. 291) and propylene glycol were identified as common metabolites. When also considering the flavouring agents in this group evaluated at the fifty-ninth and sixty-ninth meetings (Annex 1, references *160* and *190*) and the flavouring agents in the related group of saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters evaluated at the fifty-first meeting (Annex 1, reference *137*), the following additional common metabolites were identified: 1-octen-3-ol (No. 1153), formic acid (No. 79), 2-pentanol (No. 280), butyric acid (No. 87), 6-methyl-5-hepten-2-one (No. 1120) and 2-nonanol (No. 293), which are all in structural class I, with the exception of 1-octen-3-ol, which

is in structural class II. In addition, two flavouring agents currently under evaluation, (*R*)-(–)-1-octen-3-ol (No. 2071) and 2-decanone (No. 2074), belong to a homologous series of 1-alken-3-ols and 2-alkanones, respectively.

When calculating, for each common metabolite, the combined intakes in Europe, the USA and Japan for up to five flavouring agents with the highest intakes (for the compounds evaluated during the aforementioned meetings) (i.e. Nos 290, 291, 313, 448 and 2073 for 3-octanol; Nos 79, 304 and 2070 for formic acid; Nos 279, 280, 1146 and 2072 for 2-pentanol; Nos 87, 307, 1142, 1144 and 2073 for butyric acid; Nos 1148, 1152, 1836, 1837 and 2071 for 1-octen-3-ol; and propylene glycol itself and Nos 2075 and 2076 for propylene glycol), they were all below their respective thresholds of concern (i.e. 1800 μg/person per day for structural class I and 540 μg/person per day for structural class II), except for butyric acid and propylene glycol.

For butyric acid, the estimated combined intakes if the three flavouring agents that lead to the formation of butyric acid (Nos 87, 307 and 2073) were to be consumed concurrently on a daily basis would be 10 000 μg/person per day in Europe, 5900 μg/person per day in the USA and 0.04 μg/person per day in Japan. Almost 100% of the total intake in Europe and the USA was accounted for by butyric acid. Butyric acid was evaluated at the forty-ninth meeting (Annex 1, reference *131*), at which the Committee concluded that butyric acid can be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways and that the endogenous levels of metabolites resulting from butyric acid would not give rise to perturbations outside the physiological range.

For propylene glycol, the estimated combined intakes if the three substances that lead to the exposure to propylene glycol (propylene glycol itself and Nos 2075 and 2076) were to be consumed concurrently on a daily basis would be 0 μg/person per day in Europe, 2 400 000 μg/person per day in the USA and 0.05 μg/person per day in Japan. The total intake in the USA for propylene glycol exceeds the threshold of concern; however, 100% of the intake is accounted for by propylene glycol (i.e. Nos 2075 and 2076 do not contribute to the intake of propylene glycol). The Committee established an ADI of 0–25 mg/kg bw for propylene glycol at its seventeenth meeting (Annex I, reference *32*).

(*R*)-(–)-1-Octen-3-ol (No. 2071) is a member of a homologous series of 1-alken-3-ols. The members of this homologous series belong to structural class II. In the unlikely event that the five flavouring agents of this homologous series with the highest intake (Nos 1150–1153 and 2071) were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold of concern for class II (i.e. 540 μg/person per day).

2-Decanone (No. 2074) is a member of a homologous series of long-chain 2-ketones, belonging to structural class II. The estimated combined intakes for the five flavouring agents of this homologous series with the highest intakes (Nos 283, 288, 292, 296 and 298) would be 1100 μg/person per day in Europe, 200 μg/person per day in the USA and 0 μg/person per day in Japan; the estimated combined intake for Europe would exceed the threshold of concern for class II (i.e. 540 μg/person per day). However, the estimated intakes of 2-decanone are 1 and 6 μg/day in Europe and Japan, respectively, and therefore 2-decanone does not contribute significantly to the intake of this homologous series of long-chain 2-ketones.

The Committee at its current meeting therefore concluded that under the conditions of use as flavouring agents, the combined intakes of the substances leading to a common metabolite or substances of a homologous series would not raise safety concerns.

Consideration of secondary components

One member of this group of flavouring agents, 6-methyl-5-hepten-2-one propyleneglycol acetal (No. 2075), has an assay value of less than 95%. The secondary component of 6-methyl-5-hepten-2-one propyleneglycol acetal, 6-methyl-6-hepten-2-one propyleneglycol acetal, is expected to share the same metabolic fate as the primary substance and is considered not to present a safety concern at current estimated dietary exposures.

Conclusion

In the previous evaluations of flavouring agents in this group of aliphatic secondary alcohols, ketones and related esters and acetals, studies of acute toxicity, short-term toxicity and genotoxicity were available (Annex 1, references *161* and *190*). None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluations.

The Committee concluded that these seven flavouring agents, which are additions to the renamed group of aliphatic secondary alcohols, ketones and related esters and acetals evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

No addendum to the toxicological monograph was prepared.

4.1.9 *Aromatic substituted secondary alcohols, ketones and related esters: additional compounds*

The Committee was requested to evaluate nine additional flavouring agents that belong to the group of aromatic substituted secondary alcohols, ketones and related esters. This group of nine compounds includes eight ketones

(Nos 2040–2045 and 2047–2048) and one diester (No. 2046). The safety of one submitted substance, 2-aminoacetophenone (No. 2043), was not assessed, because the Committee decided that this compound should be evaluated in the future in a group of aliphatic and aromatic amines and amides. The evaluations of the remaining eight were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these agents has previously been evaluated.

The Committee previously evaluated 38 other members of this group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *154*). The Committee concluded that all 38 flavouring agents in that group were of no safety concern based on estimated dietary exposures.

Six of the eight flavouring agents (Nos 2040–2042 and 2044–2046) have been reported to occur naturally in various foods and have been detected in honey, milk, tomato, mango, coffee, cloudberry, starfruit, peas, whiskey, papaya, chicken, sherry, beer and white wine. For No. 2041, the consumption from natural sources is estimated to be 7 times the volume used as a flavouring agent.

Assessment of dietary exposure

The total annual volumes of production of the eight aromatic substituted secondary alcohols, ketones and related esters are approximately 5 kg in Europe, 52 kg in the USA and 2 kg in Japan. Approximately 80% and 96% of the total annual volumes of production in Europe and the USA, respectively, are accounted for by 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048). In Japan, approximately 50% of the total annual volume of production is accounted for by 4-hydroxyacetophenone (No. 2040).

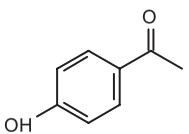
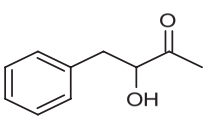
The estimated dietary exposures for each flavouring agent, calculated either as the MSDI or using the SPET, are reported in Table 15. The estimated daily intake is greatest for dihydrogalangal acetate (No. 2046) (10 000 μg, calculated using the SPET obtained from six different food categories). For the other flavouring agents, the estimated daily intakes ranged from 0.01 to 1600 μg, with the SPET yielding the highest estimates for all.

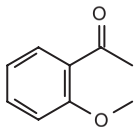
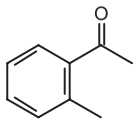
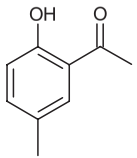
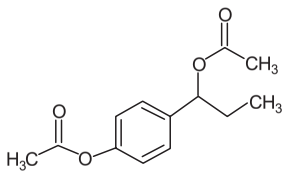
Absorption, distribution, metabolism and elimination

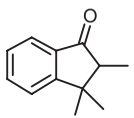
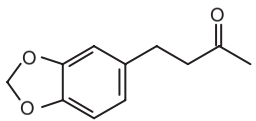
Aromatic substituted secondary alcohols, ketones and related esters are rapidly absorbed from the gut. Hydrolysis of the esters occurs in the intestine and liver. The aromatic substituted secondary alcohols (and aromatic ketones after reduction to the corresponding secondary alcohols) are then either conjugated with glucuronic acid and excreted primarily in the urine or further oxidized to carboxylic acids, which are excreted mainly as glycine conjugates.

Table 15

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

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/High exposure B-side*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I						
4-Hydroxyacetophenone	2040	99-93-4 	No, SPET: 300	NR	Notes 1 and 2	No safety concern
3-Hydroxy-4-phenylbutan-2-one	2041	5355-63-5 	No, SPET: 1600	NR	Notes 1 and 2	No safety concern

2-Methoxyacetophenone	2042	579-74-8 	No, SPET: 1500	NR	Notes 1, 2, 3 and 4	No safety concern
2-Methylacetophenone	2044	577-16-2 	No, SPET: 80	NR	Notes 1 and 4	No safety concern
2-Hydroxy-5-methylacetophenone	2045	1450-72-2 	No, SPET: 10	NR	Notes 1 and 2	No safety concern
Dihydrogalangal acetate	2046	129319-15-9 	Yes, SPET: 10 000	A5. No. The NOEL of 15 mg/kg bw per day for the structurally related substance α-methylbenzyl acetate from an oral toxicity study in rats is at least 86 times greater than the estimated daily dietary exposure to No. 2046 when used as a flavouring agent.	Notes 1 and 5	Additional data required to complete evaluation

2,3,3-Trimethylindan-1-one	2047	54440-17-4 	No, SPET: 25	NR		Notes 1 and 4	No safety concern
Structural class III							
4-(3,4-Methylenedioxyphenyl)-2-butanone	2048	55418-52-5 	Yes, SPET: 640	Yes. The NOEL of 57 mg/kg bw per day for No. 2048 in a 90-day study in rats is at least 5000 times its estimated dietary exposure when used as a flavouring agent.		Notes 1, 2 and 3	No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

[a] Thirty-eight flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *131*).

[b] *Step 1:* Seven flavouring agents in this group (Nos 2040–2042 and 2044–2047) are in structural class I. One flavouring agent in this group (No. 2048) is in structural class III.

[c] *Step 2:* All flavouring agents in this group except 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) can be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. Acetophenone derivatives (or analogues) are expected to undergo reduction at the ketone function and form α-methylbenzyl alcohol derivatives, which will be conjugated with glucuronic acid and excreted primarily in the urine. The ketone may also undergo α-methyl oxidation.
2. Detoxication of the phenol derivative primarily involves conjugation of the hydroxyl group with sulfate or glucuronic acid.
3. May undergo demethylation, generating a phenol derivative, which is expected to undergo conjugation with sulfate or glucuronic acid.
4. Aromatic rings may undergo cytochrome P450–mediated oxidation to a phenolic metabolite, which can be conjugated with glucuronic acid or sulfate prior to excretion in the urine or bile.
5. Ester groups will undergo hydrolysis to form the corresponding alcohol or phenol and acid.

Studies on absorption, distribution, metabolism and elimination were considered at the fifty-seventh meeting of the Committee (Annex 1, reference *154*).

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 seven flavouring agents (Nos 2040–2042 and 2044–2047) to structural class I. One flavouring agent (No. 2048) was assigned to structural class III.

Step 2. Seven flavouring agents in this group (Nos 2040–2042 and 2044–2047) are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. One flavouring agent (No. 2048) cannot be predicted to be metabolized to innocuous products, and its evaluation therefore proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily intakes of six flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). The safety of these flavouring agents raises no concern at current estimated dietary exposures. The highest estimated daily intake of one of the flavouring agents (No. 2046) in structural class I is above the threshold of concern (i.e. 1800 μg/person per day for class I). Accordingly, the evaluation of this flavouring agent proceeded to step A4.

Step A4. Neither the flavouring agent dihydrogalangal acetate (No. 2046) nor its metabolites are endogenous. Accordingly, the evaluation of this flavouring agent proceeded to step A5.

Step A5. The NOEL of 15 mg/kg bw per day for the structurally related substance, α-methylbenzyl acetate, from an oral study of toxicity in rats provided a margin of safety of less than 100 in relation to the highest estimated dietary exposure to dihydrogalangal acetate (No. 2046) (SPET = 10 000 μg/day) when used as a flavouring agent. The Committee expressed concern that the reported NOEL was insufficient to accommodate any potential differences in toxicity between No. 2046 and the related substance. The Committee therefore concluded that additional data are required to complete the evaluation of this flavouring agent.

Step B3. The highest daily intake of the flavouring agent in structural class III (No. 2048) is above the threshold of concern (i.e. 90 μg/person per day for class III). Therefore, additional data are necessary for the evaluation of this flavouring agent (see below).

Consideration of flavouring agents with high exposure evaluated on the B-side of the decision-tree:

In accordance with the Procedure, additional data were evaluated for 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048), as the estimated intake exceeded the threshold for structural class III (90 μg/person per day).

A NOEL for 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) of approximately 57 mg/kg bw per day in a 90-day study in rats was identified. Groups of 10–16 male and female rats per group were fed a diet formulated to provide intake in excess of 100 times the maximum estimated daily human dietary exposure. The animals were monitored for food intake and body weight. End-points evaluated included haematology, clinical chemistry, organ weights and organ pathology. No adverse effects on any of these parameters were observed. The NOEL provides a margin of safety of more than 5000 in relation to the highest estimated dietary exposure to 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) (SPET = 640 μg/day) when used as a flavouring agent. The Committee therefore concluded that 4-(3,4-methylenedioxyphenyl)-2-butanone would not pose a safety concern at current estimated dietary exposures.

Table 15 summarizes the evaluations of the eight aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents (Nos 2040–2042 and 2044–2048) in this group.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Flavouring agents in this group with the highest intakes and with the common metabolite α-methylbenzyl alcohol (No. 799), which is in structural class I, are Nos 799, 801, 804, 807 and 810. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 395, 753 and 76 μg/person per day, respectively, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Other members of this group with intakes greater than 20 μg/person per day do not share common metabolites or represent members of a homologous series.

Consideration of secondary components

One member of this group of flavouring agents, 3-hydroxy-4-phenylbutan-2-one (No. 2041), has a minimum assay value of less than 95%. The secondary component of No. 2041, 4-hydroxy-4-phenylbutan-2-one, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as the primary substance, and is considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary component of this flavouring agent is summarized in Annex 4.

Conclusion

In the previous evaluation of the flavouring agents in this group, studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, and genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *154*).

The Committee concluded that seven of these eight flavouring agents, which are additions to the group of aromatic substituted secondary alcohols, ketones and related esters evaluated previously, would not give rise to safety concerns at current estimated dietary exposures. For dihydrogalangal acetate (No. 2046), the Committee concluded that additional data would be necessary to complete the evaluation of this flavouring agent.

An addendum to the toxicological monograph was prepared.

4.1.10 *Benzyl derivatives: additional compounds*

The Committee evaluated eight additional flavouring agents belonging to the group of benzyl derivatives, which was previously evaluated. The structural feature common to all members of the group is a primary oxygenated functional group bonded directly to a benzene ring or a functional group metabolized to a benzyl alcohol or benzoic acid derivative. The ring may also have alkyl substituents. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee previously evaluated 37 other members of this group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *155*). The Committee concluded that all 37 flavouring agents in this group were of no safety concern based on estimated dietary exposures.

Three of the additional eight flavouring agents (Nos 2061, 2062 and 2068) have been reported to occur naturally and can be found in passion fruit juice, cinnamon bark, cassia leaf, Tahitian vanilla and raw cabbage.

Assessment of dietary exposure

The total annual volumes of production of the eight benzyl derivatives are approximately 27 kg in Europe, 3 kg in the USA and 17 in Japan. Approximately 70% and 100% of the total annual volumes of production in Europe and in the USA, respectively, are accounted for by *o*-anisaldehyde (No. 2062). In Japan, approximately 50% of the total annual volume of production is accounted for by benzyl levulinate (No. 2064).

The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 16. The highest estimate is for benzyl hexanoate (No. 2061) (300 µg, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in the group, the daily dietary exposures range from 0.004 to 240 µg, with the SPET yielding the highest estimates for all.

Absorption, distribution, metabolism and elimination

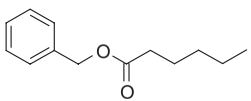
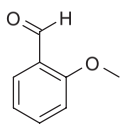
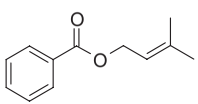
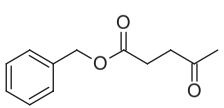
Metabolic information on this group was considered at the fifty-seventh meeting of the Committee (Annex 1, reference *155*). In general, aromatic esters and acetals are hydrolysed in vivo through the catalytic activity of A-type carboxylesterases that predominate in hepatocytes. Benzyl esters and acetals are hydrolysed to benzyl alcohol and benzaldehyde, respectively, followed by oxidation to yield benzoic acid. Benzoate esters are hydrolysed to benzoic acid.

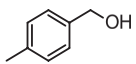
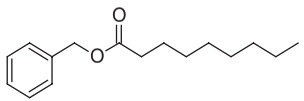
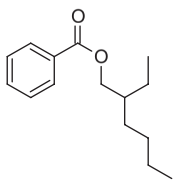
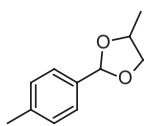
Benzyl derivatives have been shown to be rapidly absorbed through the gut, metabolized primarily in the liver and excreted in the urine as glycine conjugates of benzoic acid derivatives. At high dose levels, formation of the glycine conjugate is glycine limited. When glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted unchanged or as the glucuronic acid conjugate. Alkyl substituents on the aromatic ring have little influence on the principal pathways of metabolism.

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 of the flavouring agents (Nos 2061–2066) to structural class I, one of the flavouring agents (No. 2068) to structural class II and one (No. 2067) to structural class III.

Table 16
Summary of the results of the safety evaluations of benzyl derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I					
Benzyl hexanoate	2061	6938-45-0 	No, SPET: 300	Note 1	No safety concern
o-Anisaldehyde	2062	135-02-4 	No, SPET: 40	Note 2	No safety concern
Prenyl benzoate	2063	5205-11-8 	No, SPET: 180	Note 3	No safety concern
Benzyl levulinate	2064	6939-75-9 	No, SPET: 240	Note 1	No safety concern

4-Methylbenzyl alcohol	2065	589-18-4 	No, SPET: 3	Note 4	No safety concern
Benzyl nonanoate	2066	6471-66-5 	No, SPET: 125	Note 1	No safety concern
Structural class II					
2-Ethylhexyl benzoate	2068	5444-75-7 	No, SPET: 3	Note 3	No safety concern
Structural class III					
4-Methylbenzaldehyde propyleneglycol acetal	2067	58244-29-4 	No, SPET: 80	Notes 4 and 5	No safety concern

CAS, Chemical Abstracts Service

[a] Thirty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *155*).

[b] *Step 1:* Six of the flavouring agents in this group (Nos 2061–2066) are in structural class I; one (No. 2068) is in structural class II; and one (No. 2067) is in structural class III.

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

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

Notes:

1. It is anticipated that the ester will hydrolyse to form benzyl alcohol and an alkanoic acid. The benzyl alcohol is anticipated to undergo oxidation to benzoic acid, which forms conjugates with glycine that are excreted in the urine. The alkanoic acid will undergo fatty acid degradation.
2. Benzaldehydes are anticipated to undergo oxidation to the corresponding benzoic acid derivative and form conjugates with glycine that are eliminated in the urine.
3. It is anticipated that the ester will readily hydrolyse, forming benzoic acid and prenyl alcohol. Benzoic acid readily forms conjugates with glycine, which are eliminated in the urine. Prenyl alcohol will undergo oxidative metabolism.
4. Oxidized to a benzoic acid analogue and excreted in the urine as a glycine or glucuronic acid conjugate.
5. Hydrolysis of the acetal to a benzaldehyde derivative.

Step 2. All the flavouring agents in this group (Nos 2061–2068) are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The highest estimated daily intakes of all six of the flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). The highest estimated daily intake for the one flavouring agent in structural class II is below the threshold of concern (i.e. 540 μg/person per day for class II). The highest estimated daily intake for the one flavouring agent in structural class III is below the threshold of concern (i.e. 90 μg/person per day for class III). The safety of these eight flavouring agents raises no concern at current estimated dietary exposures.

Table 16 summarizes the evaluations of the eight benzyl derivatives (Nos 2061–2068) in this group when used as flavouring agents.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Flavouring agents with the highest intakes in this group that have the common metabolite benzyl alcohol, which is in structural class I, are Nos 23–25, 842 and 843. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe and the USA would be 18 000 and 4700 μg/person per day, respectively, which would exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The majority of this combined intake would be from benzyl alcohol itself (No. 25). All of these agents are expected to be efficiently metabolized and would not saturate metabolic pathways. The Committee concluded that combined intake would not raise concern about safety.

Flavouring agents with the highest intakes in this group that have the common metabolite benzaldehyde, which is in structural class I, are Nos 22, 837–839 and 867. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe and the USA would be 9300 and 36 200 μg/person per day, respectively, which would exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The majority of this combined intake would be from benzaldehyde itself (No. 22). All of these agents are expected to be efficiently metabolized and would not saturate metabolic pathways. The Committee concluded that combined intake would not raise concern about safety.

Flavouring agents with the highest intakes in this group that have the common metabolite benzoic acid, which is in structural class I, are Nos 850–852, 854, 857 and 861. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe and the USA would be 800 and 1800 μg/person per day, respectively, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The Committee concluded that combined intake would not raise concern about safety.

Consideration of secondary components

No members of this group of flavouring agents have a minimum assay value of less than 95%.

Conclusion

In the previous evaluation of flavouring agents in this group, studies of acute toxicity, short-term toxicity and genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *155*).

The Committee concluded that these eight flavouring agents, which are additions to the group of benzyl derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.11 *Phenol and phenol derivatives: additional compounds*

The Committee evaluated 13 additional flavouring agents belonging to the group of phenol and phenol derivatives used as flavouring agents, which was evaluated previously. The additional substances included an ester of phenol (No. 2019), two polyphenols (Nos 2022 and 2024), a phenol glucoside (No. 2018), alkyl-, alkenyl- or aryl-substituted phenols or their esters (Nos 2012, 2013 and 2023), alkoxyphenols or their esters (Nos 2014–2017) and phenol derivatives with alkyl side-chains containing a ketone function (Nos 2020 and 2021). The group of substances was selected on the basis of the structural criteria that all members either possess an aromatic ring containing one or more free hydroxyl groups or are the esters of phenol derivatives. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these substances has been evaluated previously by the Committee.

The Committee previously evaluated 48 other members of this group of flavouring agents at its fifty-fifth meeting (Annex 1, reference *149*). The Committee concluded that all 48 flavouring agents in that group were of no safety concern based on estimated dietary exposures.

Four of the 13 additional flavouring agents (Nos 2012, 2013, 2019 and 2021) in this group have been reported to occur naturally and have been found in dried bonito, apple cider, various cheeses and ginger.

Assessment of dietary exposure

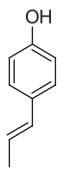
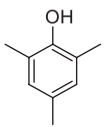
The total annual volumes of production of the 13 flavouring agents belonging to the group of phenol and phenol derivatives are approximately 241 kg in Europe, 0.05 kg in Japan and 2602 kg in the USA. Approximately 99% of the total annual volume of production in Europe is accounted for by 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024), and approximately 99% of the total annual volume of production in the USA is accounted for by magnolol (No. 2023) and 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024). Approximately 100% of the total annual volume of production in Japan is accounted for by phenyl butyrate (No. 2019).

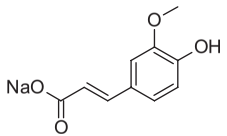
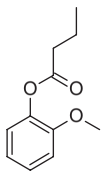
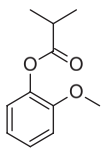
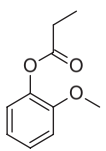
The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 17. The highest estimates are for 4-(2-propenyl)phenyl-β-D-glucopyranoside (No. 2018) and magnolol (No. 2023) (6000 μg for both, the SPET value from non-alcoholic beverages for No. 2018 and from chewing gum or other confections for No. 2023). For the other flavouring agents in this group, the daily dietary exposures range from 0.01 to 3000 μg, with the SPET yielding the highest estimates for all except 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024).

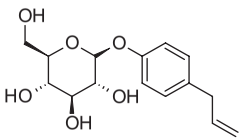
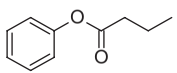
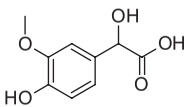
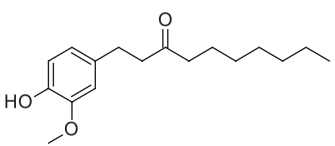
Absorption, distribution, metabolism and elimination

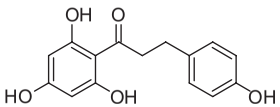
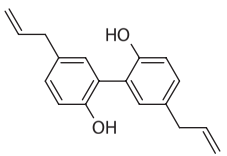
In the report of the fifty-fifth meeting, biodisposition of flavouring agents in this group was extensively discussed. When ingested as natural or added components of food, phenol and its derivatives are rapidly absorbed from the gastrointestinal tract and participate in common pathways of metabolic detoxication. Phenol and phenol derivatives are conjugated with sulfate and glucuronic acid and excreted primarily in the urine. Other metabolic pathways, observed mainly at high dose levels, include ring hydroxylation and side-chain oxidation. Phenols containing alkoxy groups and those that contain a ketone function on an alkyl side-chain are also detoxified mainly via conjugation. Alternative detoxication pathways include dealkylation of alkoxyphenols, reduction of side-chain ketones, side-chain oxidation and ring hydroxylation. At very high dose levels, a bioactivation pathway has been characterized; high dose levels of *p*-cresol (i.e. 4-methylphenol; No. 693), *p*-ethylphenol (No. 694), 2-methoxy-4-methylphenol (No. 715), 2-methoxy-4-propylphenol (No. 717), 2-methoxy-4-vinylphenol (No. 725) and 4-allyl-2,6-dimethoxyphenol (No. 726) are oxidized to reactive quinone methide intermediates.

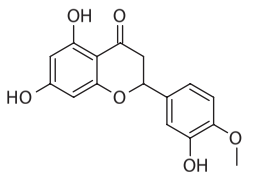
Table 17
Summary of the results of the safety evaluations of phenol and phenol derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I							
4-Propenylphenol	2012	539-12-8 	No, SPET: 400	NR	NR	Note 1	No safety concern
2,4,6-Trimethylphenol	2013	527-60-6 	No, SPET: 300	NR	NR	Note 1	No safety concern

Sodium 3-methoxy-4-hydroxycinnamate	2014	24276-84-4 	No, SPET: 1500	NR	NR	Notes 1 and 2	No safety concern
Guaicol butyrate	2015	4112-92-9 	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern
Guaicol isobutyrate	2016	723759-62-4 	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern
Guaicol propionate	2017	7598-60-9 	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern

4-(2-Propenyl)phenyl-β-D-glucopyranoside	2018	64703-98-6 	Yes, SPET: 6000	No	Yes. The NOAEL of 600 mg/kg bw per day for the structurally related eugenol (No. 1529) in a 90-day study in rats is at least 6000 times the estimated daily dietary exposure to No. 2018 when used as a flavouring agent.	Note 1	No safety concern
Phenyl butyrate	2019	4346-18-3 	No, SPET: 30	NR	NR	Notes 1 and 3	No safety concern
Hydroxy(4-hydroxy-3-methoxyphenyl)acetic acid	2020	55-10-7 	No, SPET: 1500	NR	NR	Note 1	No safety concern
Structural class II							
1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	2021	27113-22-0 	Yes, SPET: 3000	No	Yes. The NOAEL of 70 mg/kg bw per day for the structurally related 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in a 90-day study in rats is at least 1400 times the estimated daily dietary exposure to No. 2021	Note 1	No safety concern

					when used as a flavouring agent.		
Structural class III							
3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	2022	60-82-2 	Yes, SPET: 480	No	Yes. The NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats is at least 93 000 times the estimated daily dietary exposure to No. 2022 when used as a flavouring agent.	Note 1	No safety concern
Magnolol	2023	528-43-8 	Yes, SPET: 6000	No	Yes. The NOAEL of 240 mg/kg bw per day in a 90-day study in rats is at least 2400 times the estimated daily dietary exposure to magnolol when used as a flavouring agent.	Note 1	No safety concern

5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	2024	69097-99-0 	Yes, MSDI: Europe 26 USA 153 Japan ND	No	Yes. The NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats is at least 290 000 times the estimated daily dietary exposure to No. 2024 when used as a flavouring agent.	Note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

[a] Forty-eight flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *149*).

[b] *Step 1:* Nine flavouring agents in this group (Nos 2012–2020) are in structural class I. One flavouring agent in this group (No. 2021) is in structural class II. The remaining three flavouring agents (Nos 2022–2024) are in structural class III.

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

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated as the MSDI or by the SPET.

Notes:

1. Detoxication of phenol primarily involves conjugation of the hydroxyl group with sulfate and glucuronic acid and subsequent elimination in the urine.
2. Cinnamic acid derivatives are expected to undergo β-oxidation and are excreted as hippuric acid.
3. The phenolic ester will hydrolyse to phenol and the corresponding carboxylic 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 to the above-mentioned flavouring agents, the Committee assigned nine flavouring agents (Nos 2012–2020) to structural class I. One flavouring agent (No. 2021) was assigned to structural class II, and three flavouring agents (Nos 2022–2024) were assigned to structural class III.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. For all compounds in this group (except No. 2024; see below), the SPET resulted in the highest estimated daily intakes. Of eight of the nine flavouring agents (Nos 2012–2017, 2019 and 2020) in structural class I, all were below the threshold of concern (i.e. 1800 μg/person per day for class I). The safety of these eight flavouring agents raises no concern at current estimated dietary exposures. The estimated daily intake for one flavouring agent (No. 2018) in structural class I is above the threshold of concern (i.e. 1800 μg/person per day for class I). The estimated daily intake for the one flavouring agent (No. 2021) in structural class II is above the threshold of concern (i.e. 540 μg/person per day for class II). The estimated daily intake for all three flavouring agents (Nos 2022–2024) in structural class III are above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of these five substances proceeded to step A4.

Step A4. None of the flavouring agents—4-(2-propenyl)phenyl-β-D-glucopyranoside (No. 2018), 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one (No. 2021), 3-(4-hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (No. 2022), magnolol (No. 2023) and 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024)—or their metabolites are endogenous substances. Accordingly, the evaluation of these substances proceeded to step A5.

Step A5. For 4-(2-propenyl)phenyl-β-D-glucopyranoside (No. 2018), the NOAEL of 600 mg/kg bw per day for the structurally related eugenol (No. 1529) in a 90-day study in rats provides a margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2018 (SPET = 6000 μg/person per day) when used as a flavouring agent.

For 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one (No. 2021), the NOAEL of 70 mg/kg bw per day for the structurally related 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in a 90-day study in rats provides a margin of safety of 1400 in relation to the highest estimated dietary exposure to No. 2021 (SPET = 3000 μg/person per day) when used as a flavouring agent.

For 3-(4-hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (No. 2022), the NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats provides a margin of safety of greater than 93 000 in relation to the highest estimated dietary exposure to No. 2022 (SPET = 480 µg/person per day) when used as a flavouring agent.

The NOAEL of 240 mg/kg bw per day for magnolol (No. 2023) in a 90-day study in rats provides a margin of safety of 2400 in relation to the highest estimated dietary exposure to No. 2023 (SPET = 6000 µg/person per day) when used as a flavouring agent.

For 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024), the NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats provides a margin of safety of greater than 290 000 in relation to the highest estimated dietary exposure to No. 2024 (MSDI = 153 µg/person per day) when used as a flavouring agent.

The Committee concluded that the calculated margins of safety indicate that these flavouring agents would not pose safety concerns at current estimated dietary exposures.

Table 17 summarizes the evaluations of the 13 phenol and phenol derivatives (Nos 2012–2024) in this group.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined exposures to flavouring agents was undertaken based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*). In addition, at this meeting, the Committee also considered combined intakes for structurally closely related series of flavouring agents.

Flavouring agents in this series that are members of a structurally closely related series of simple phenols or alkylphenols or predicted to be metabolized to such compounds, in structural class I, are Nos 2012, 2013, 2018 and 2019. The five related flavouring agents with the highest intakes in Europe are Nos 690, 691, 694, 697 and 705 and in the USA are Nos 693, 695, 698, 699 and 703. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 316 µg/person in Europe and 81 µg/person in the USA, which would not exceed the threshold of concern (i.e. 1800 µg/person per day for class I).

The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

Flavouring agents in this series that are members of a structurally closely related series of methoxyphenols or predicted to be metabolized to such compounds, in structural class I, are Nos 2015, 2016 and 2017. The five related compounds with the highest intakes in Europe are Nos 713, 715, 717, 721 and 725 and in the USA are Nos 711, 713, 715, 721 and 726. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 307 μg/person in Europe and 43 μg/person in the USA, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

Flavouring agents in this series that are members of a structurally closely related series of phenols or methoxyphenols containing an additional oxygenated functional group or predicted to be metabolized to such compounds, in structural class I, are Nos 2014 and 2020. The related compounds with the highest intakes in Europe are Nos 727, 728, 736 and 731 and in the USA are Nos 727, 728, 736, 730 and 731. In the unlikely event that these substances were to be consumed concurrently on a daily basis, the estimated combined intakes would be approximately 3000 μg/person in Europe and approximately 4000 μg/person in the USA, which would exceed the threshold of concern (i.e. 1800 μg/person per day for class I). However, all five flavouring agents in this group are expected to be efficiently metabolized and would not saturate metabolic pathways. The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

The remaining flavouring agents (Nos 2022–2024) do not share close structural characteristics with others in the group, and consideration of combined intake is not indicated.

The Committee concluded that the combined intakes of these substances, when used as flavouring agents, would not raise safety concerns.

Consideration of secondary components

Two members of this group of flavouring agents, sodium 3-methoxy-4-hydroxycinnamate (No. 2014) and magnolol (No. 2023), have minimum assay values of less than 95%. The secondary component in No. 2014, vanillin (No. 889), was previously evaluated and found to be of no concern. The secondary components of magnolol (No. 2023), honokiol and eudesmol, are expected to share the same metabolic fate as the flavouring agent and are

considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 4.

Conclusion

In the previous evaluations of substances in this group of flavouring agents, studies of biological properties, acute toxicity, short-term toxicity and genotoxicity were available. None raised safety concerns. The additional biochemical and toxicological data available for this evaluation supported those from the previous evaluation (Annex 1, reference *149*).

The Committee concluded that these 13 flavouring agents, which are additions to the group of phenol and phenol derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.12 *Simple aliphatic and aromatic sulfides and thiols: additional compounds*

The Committee evaluated 36 additional flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols, which was evaluated previously. This group included 4 simple sulfides (Nos 1909–1911 and 1939), 13 acyclic sulfides with oxidized side-chains (Nos 1912, 1913, 1915–1922 and 1940–1942), 3 cyclic sulfides (Nos 1923, 1943 and 1944), 1 simple thiol (No. 1924), 8 thiols with oxidized side-chains (Nos 1914, 1925–1929, 1936 and 1938), 5 simple disulfides (Nos 1930–1933 and 1935), 1 trisulfide (No. 1934) and 1 thioester (No. 1937). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1; Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 137 other members of this group of flavouring agents at its fifty-third meeting (Annex 1, reference *143*). The group was divided into 12 subgroups on the basis of the position of the sulfur atom, in order to facilitate the assessment of the relevant data on metabolism and toxicity. The Committee concluded that all 137 flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee also evaluated 12 additional members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The Committee concluded that all 12 additional flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee evaluated another 51 additional members of this group of flavouring agents at its sixty-eighth meeting (Annex 1, reference *187*). The Committee concluded that all 51 additional flavouring agents in that group were of no safety concern at estimated dietary exposures.

Ten of the 36 flavouring agents evaluated at the current meeting are natural components of foods (Nos 1909, 1910, 1913, 1915, 1916, 1918, 1923, 1932, 1933 and 1937) and have been detected in beef, fish oil, onion, shallot, potato chips, cabbage, peanut, apple, pineapple, melon, yellow passion fruit, coffee and beer.

Assessment of dietary exposure

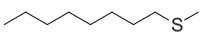
The total annual volumes of production of the 36 flavouring agents in this group are approximately 0.3 kg in Europe, 2 kg in the USA and 19 kg in Japan. In Europe, only methyl 1-propenyl sulfide (No. 1910), 2-(methylthio)-ethyl acetate (No. 1913) and 3-mercaptohexanal (No. 1929) are produced (each accounts for one third of the total annual volume of production). Only four are produced in the USA, with (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928) and 3-(methylthio)propyl hexanoate (No. 1941) accounting for the largest part of the total annual volume of production (42% each). All but five of these flavouring agents are produced in Japan, with methyl octyl sulfide (No. 1909) and 2-ethylhexyl 3-mercaptopropionate (No. 1938) making the largest contribution to the total annual volume of production (32% each).

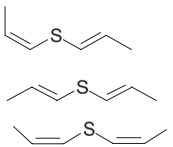
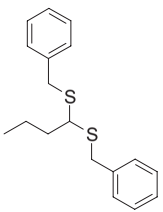
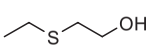
The estimated dietary exposures for each of the flavouring agents, calculated either as the MSDI or using the SPET, are reported in Table 18. The estimated daily dietary exposure is the highest for 3-(methylthio)propyl hexanoate (No. 1941) (1500 μg, the SPET value obtained for composite foods). For the other flavouring agents, the estimated daily per capita dietary exposures varied from 0.1 from 400 μg. For all of these flavouring agents except (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928) and 3-mercaptopropionic acid (No. 1936), the SPET gave the highest estimate.

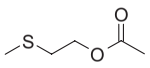
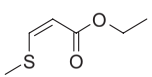
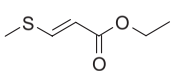
Absorption, distribution, metabolism and elimination

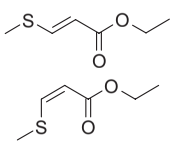
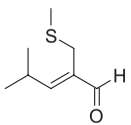
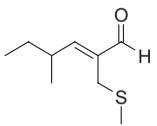
Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols has previously been described in the monographs of the fifty-third, sixty-first and sixty-eighth meetings (Annex 1, references *144*, *167* and *188*). No additional relevant data have been reported since these meetings.

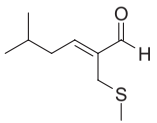
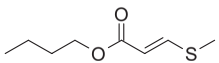
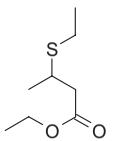
Table 18
Summary of the results of the safety evaluations of simple aliphatic and aromatic sulfides and thiols used as flavouring agents[a,b,c]

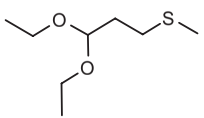
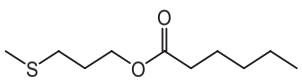
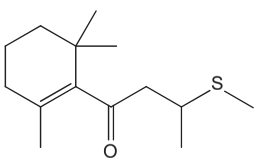
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Subgroup i: Simple sulfides							
Structural class I							
Methyl octyl sulfide	1909	3698-95-1 	No, SPET: 400	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is at least 37 500 times the estimated daily dietary exposure to No. 1909 when used as a flavouring agent.	NR	Note 1	No safety concern
Methyl 1-propenyl sulfide	1910	10152-77-9 	No, SPET: 2	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is at least 7 500 000 times the estimated daily dietary exposure to No.	NR	Note 1	No safety concern

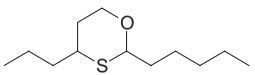
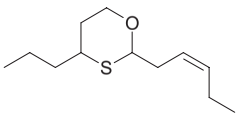
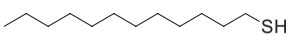
				1910 when used as a flavouring agent.			
Di-(1-propenyl)-sulfide (mixture of isomers)	1911	65819-74-1; 37981-37-6; 37981-36-5 	No, SPET: 80	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is at least 187 500 times the estimated daily dietary exposure to No. 1911 when used as a flavouring agent.	NR	Note 1	No safety concern
Structural class III							
Butanal dibenzyl thioacetal	1939	101780-73-8 	No, SPET: 40	B4. No.	Yes.	Note 1	Additional data required to complete evaluation
Subgroup ii: Acyclic sulfides with oxidized side-chains							
Structural class I							
Ethyl 2-hydroxyethyl sulfide	1912	110-77-0 	No, SPET: 3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is	NR	Notes 1 and 2	No safety concern

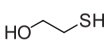
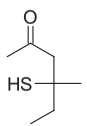
				at least 28 000 times the estimated daily dietary exposure to No. 1912 when used as a flavouring agent.			
2-(Methylthio)ethyl acetate	1913	5862-47-5 	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 280 times the estimated daily dietary exposure to No. 1913 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
Ethyl 3-(methylthio)-(2*Z*)-propenoate	1915	136115-66-7 	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 280 times the estimated daily dietary exposure to No. 1915 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
Ethyl 3-(methylthio)-(2*E*)-propenoate	1916	136115-65-6 	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 280 times the estimated daily dietary	NR	Notes 1 and 3	No safety concern

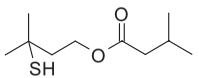
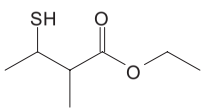
				exposure to No. 1916 when used as a flavouring agent.			
Ethyl 3-(methylthio)-2-propenoate (mixture of isomers)	1917	77105-51-2 	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 280 times the estimated daily dietary exposure to No. 1917 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
4-Methyl-2-(methylthiomethyl)-2-pentenal	1918	40878-73-7 	No, SPET: 0.125	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 672 000 times the estimated daily dietary exposure to No. 1918 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern
4-Methyl-2-(methylthiomethyl)-2-hexenal	1919	99910-84-6 	No, SPET: 1.5	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 56 000 times the estimated daily dietary exposure to No. 1919 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern

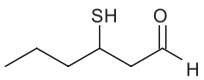
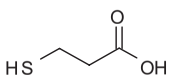
5-Methyl-2-(methylthiomethyl)-2-hexenal	1920	85407-25-6 	No, SPET: 3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 28 000 times the estimated daily dietary exposure to No. 1920 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern
Butyl β-(methylthio)-acrylate	1921	77105-53-4 	No, SPET: 0.3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 280 000 times the estimated daily dietary exposure to No. 1921 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
Ethyl 3-(ethylthio)-butyrate	1922	90201-28-8 	No, SPET: 24	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance ethyl 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 3500 times the estimated daily dietary exposure to No. 1922 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern

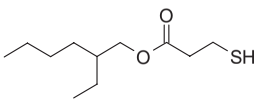
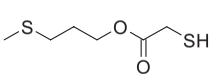
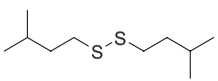
Methional diethyl acetal	1940	16630-61-8 	No, SPET: 6	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance ethyl 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 14 000 times the estimated daily dietary exposure to No. 1940 when used as a flavouring agent.	NR	Note 1	No safety concern
3-(Methylthio)propyl hexanoate	1941	906079-63-8 	No, SPET: 1500	B4. No.	Yes.	Notes 1 and 3	Additional data required to complete evaluation
Structural class III							
1-(3-(Methylthio)-butyryl)-2,6,6-trimethylcyclohexene	1942	68697-67-6 	No, SPET: 0.25	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 336 000 times the estimated daily dietary exposure to No. 1942 when used as a flavouring agent.	NR	Notes 1 and 5	No safety concern

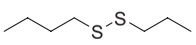
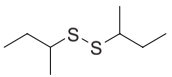
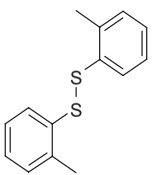
Subgroup iii: Cyclic sulfides							
Structural class II							
2-Oxothiolane	1923	1003-10-7 	No, SPET: 6	B4. Yes. The NOEL of 9.2 mg/kg bw per day for the related substance 4,5-dihydro-3(2H)-thiophenone (No. 498) is at least 92 000 times the estimated daily dietary exposure to No. 1923 when used as a flavouring agent.	NR	Note 1	No safety concern
Structural class III							
(±)-*cis*- and *trans*-2-Pentyl-4-propyl-1,3-oxathiane	1943	59323-81-8 	Yes, SPET: 300	Additional data: No.	NR	Note 1	Additional data required to complete evaluation
2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	1944	1094004-39-3 	Yes, SPET: 300	Additional data: No.	NR	Note 1	Additional data required to complete evaluation
Subgroup iv: Simple thiols							
Structural class I							
Dodecanethiol	1924	112-55-0 	No, SPET: 1.5	B4. Yes. The NOEL of 0.56 mg/kg bw per day for the related substance	NR	Notes 6 and 7	No safety concern

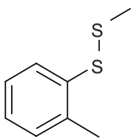
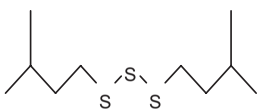
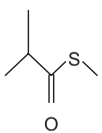
				cyclopentanethiol (No. 516) is at least 22 400 times the estimated daily dietary exposure to No. 1924 when used as a flavouring agent.			
Subgroup v: Thiols with oxidized side-chains							
Structural class I							
2-Hydroxyethanethiol	1925	60-24-2 	No, SPET: 600	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 190–280 times the estimated daily dietary exposure to No. 1925 when used as a flavouring agent.	NR	Notes 2, 6 and 7	No safety concern
4-Mercapto-4-methyl-2-hexanone	1926	851768-52-0 	No, SPET: 0.3	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from	NR	Notes 5, 6 and 7	No safety concern

				90-day studies in rats are at least 380 000–560 000 times the estimated daily dietary exposure to No. 1926 when used as a flavouring agent.			
3-Mercapto-3-methylbutyl isovalerate	1927	612071-27-9 	No, SPET: 20	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 5700–8400 times the estimated daily dietary exposure to No. 1927 when used as a flavouring agent.	NR	Notes 3, 6 and 7	No safety concern
(±)-Ethyl 3-mercapto-2-methylbutanoate	1928	888021-82-7 	No, MSDI: Europe ND USA 0.1 Japan ND	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 1 140 000–1 680 000 times the estimated daily dietary exposure to No. 1928	NR	Notes 3, 6 and 7	No safety concern

				when used as a flavouring agent.				
3-Mercaptohexanal	1929	51755-72-7 	No, SPET: 3	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 38 000–56 000 times the estimated daily dietary exposure to No. 1929 when used as a flavouring agent.	NR	Notes 4, 6 and 7	No safety concern
3-Mercaptopropionic acid	1936	107-96-0 	No, MSDI: Europe ND USA ND Japan 0.5	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 228 000–336 000 times the estimated daily dietary exposure to No. 1936 when used as a flavouring agent.	NR	Notes 6 and 7	No safety concern

2-Ethylhexyl 3-mercaptopropionate	1938	50448-95-8 	No, SPET: 30	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats are at least 3800–5600 times the estimated daily dietary exposure to No. 1938 when used as a flavouring agent.	NR	Notes 3, 6 and 7	No safety concern
Structural class III							
3-(Methylthio)propyl mercaptoacetate	1914	852997-30-9 	Yes, SPET: 300	Additional data: No.	NR	Notes 1, 3, 6 and 7	Additional data required to complete evaluation
Subgroup vii: Simple disulfides							
Structural class I							
Diisoamyl disulfide	1930	2051-04-9 	No, SPET: 10	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is at least 43 800 times the estimated daily dietary exposure to No. 1930 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern

Butyl propyl disulfide	1932	72437-64-0 	No, SPET: 0.2	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is at least 2 190 000 times the estimated daily dietary exposure to No. 1932 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Di-*sec*-butyl disulfide	1933	5943-30-6 	No, SPET: 50	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is at least 8760 times the estimated daily dietary exposure to No. 1933 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Structural class III							
Bis(2-methylphenyl) disulfide	1931	4032-80-8 	Yes, SPET: 350	Additional data: No.	NR	Notes 7, 8 and 9	Additional data required to complete evaluation

Methyl 2-methylphenyl disulfide	1935	35379-09-0 	No, SPET: 0.2	B4. Yes. The NOEL of 3.4 mg/kg bw per day for the related substance 2-naphthalenethiol (No. 531) is at least 1 020 000 times the estimated daily dietary exposure to No. 1935 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Subgroup ix: Trisulfides							
Structural class I							
Diisoamyl trisulfide	1934	955371-64-9 	No, SPET: 2	B4. Yes. The NOEL of 4.8 mg/kg bw per day for the related substance dipropyl trisulfide (No. 585) is at least 144 000 times the estimated daily dietary exposure to No. 1934 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Subgroup xi: Thioesters							
Structural class I							
Methyl isobutanethioate	1937	42075-42-3 	No, SPET: 60	B4. Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is at least 6500 times the estimated daily dietary exposure to No. 1937 when used as a flavouring agent.	NR	Note 10	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation

[a] One hundred and thirty-seven flavouring agents belonging to the chemical group of simple aliphatic and aromatic sulfides and thiols were previously evaluated by the Committee at its fifty-third meeting (Annex 1, reference *143*), 12 additional members at its sixty-first meeting (Annex 1, reference *166*) and 51 additional members at its sixty-eighth meeting (Annex 1, reference *187*).

[b] *Step 1:* Twenty-eight flavouring agents in this group are in structural class I (Nos 1909–1913, 1915–1922, 1924–1930, 1932–1934, 1936–1938, 1940 and 1941), 1 is in structural class II (No. 1923) and the remaining 7 are in structural class III (Nos 1914, 1931, 1935, 1939 and 1942–1944).

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

[d] The thresholds for human intake for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. Either the highest SPET estimate or the MSDI estimates, if at least one is higher than the highest SPET estimate, are given in the table.

[e] The margin of safety was calculated based on the highest daily dietary exposure calculated either by the SPET or as the MSDI.

Notes:

1. The sulfur is expected to be oxidized to the sulfoxide and sulfone.
2. The hydroxy group is expected to undergo oxidation to the carboxylic acid and/or conjugation with glucuronic acid, followed by excretion.
3. The ester is expected to undergo hydrolysis to the corresponding carboxylic acid and alcohol.
4. The aldehyde group is expected to be oxidized to the corresponding carboxylic acid, conjugated and subsequently excreted.
5. The ketone group is expected to be reduced to the alcohol, conjugated and subsequently excreted.
6. The sulfur is expected to be oxidized to sulfonic acid and/or undergo methylation, followed by excretion.
7. Free thiols may form mixed disulfides with glutathione or cysteine.
8. The disulfides or trisulfides are expected to be reduced to free thiols.
9. The geminal dithiols are expected to be hydrolysed to yield their parent aldehydes and hydrogen sulfide.
10. The thioester is expected to undergo hydrolysis to acetate and the corresponding thiol, which will be further oxidized.

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 36 flavouring agents in this group of simple aliphatic and aromatic sulfides and thiols, the Committee assigned 28 flavouring agents to structural class I (Nos 1909–1913, 1915–1922, 1924–1930, 1932–1934, 1936–1938, 1940 and 1941), 1 flavouring agent to structural class II (No. 1923) and 7 flavouring agents to structural class III (Nos 1914, 1931, 1935, 1939 and 1942–1944).

Step 2. None of the flavouring agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the B-side of the Procedure.

Step B3. The highest estimated daily per capita intakes of the 28 flavouring agents in structural class I and the 1 flavouring agent in structural class II are below the respective thresholds of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II). Accordingly, the evaluation of these 29 flavouring agents proceeded to step B4.

The highest estimated daily per capita intakes of three flavouring agents in structural class III (Nos 1935, 1939 and 1942) are below the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of these three flavouring agents proceeded to step B4. The highest estimated daily per capita intakes of the four remaining flavouring agents in structural class III (Nos 1914, 1931, 1943 and 1944) are 350 μg for No. 1931 and 300 μg for Nos 1914, 1943 and 1944 (calculated using the SPET) and are above the threshold of concern (i.e. 90 μg/person per day for class III). Therefore, additional data are necessary for the evaluation of these flavouring agents.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

In accordance with the Procedure, additional data were evaluated for 3-(methylthio)propyl mercaptoacetate (No. 1914), bis(2-methylphenyl) disulfide (No. 1931), (±)-*cis*- and *trans*-2-pentyl-4-propyl-1,3-oxathiane (No. 1943) and 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944), as the estimated intakes exceeded the threshold of concern for structural class III (90 μg/person per day).

No. 1914

No data are available for 3-(methylthio)propyl mercaptoacetate (No. 1914) or closely related substances to perform a safety evaluation. Therefore, the Committee determined that additional metabolic or toxicological data would

be necessary to complete the evaluation of No. 1914 at current estimated dietary exposures.

No. 1931

No data are available for bis(2-methylphenyl) disulfide (No. 1931) or closely related substances to perform a safety evaluation. Bis(2-methylphenyl) disulfide is expected to be reduced rapidly to a thiophenol analogue; however, the rate and extent of reduction are unknown. Therefore, the Committee determined that additional metabolic or toxicological data would be necessary to complete the evaluation of No. 1931 at current estimated dietary exposures.

No. 1943

No data are available for (±)-*cis*- and *trans*-2-pentyl-4-propyl-1,3-oxathiane (No. 1943). The NOEL of 0.44 mg/kg bw per day for the closely related substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) from a 90-day study in rats provides a margin of safety of 88 (SPET for No. 1943 = 300 µg/day). The Committee considered that this margin of safety is inadequate and that additional data would be necessary to complete the evaluation of No. 1943 at current estimated dietary exposures.

No. 1944

No data are available for 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944). The NOEL of 0.44 mg/kg bw per day for the closely related substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) from a 90-day study in rats provides a margin of safety of 88 (SPET for No. 1944 = 300 µg/day). The Committee considered that this margin of safety is inadequate and that additional data would be necessary to complete the evaluation of No. 1944 at current estimated dietary exposures.

Step B4. Subgroup i: Simple sulfides. The NOEL of 250 mg/kg bw per day for the structurally related substance methyl sulfide (No. 452) from a 14-week oral gavage study in rats provides adequate margins of safety (ranging from 37 500 to 7 500 000) for methyl octyl sulfide (No. 1909; SPET = 400 µg/day), methyl 1-propenyl sulfide (No. 1910; SPET = 2 µg/day) and di-(1-propenyl)-sulfide (mixture of isomers) (No. 1911; SPET = 80 µg/day) when used as flavouring agents. The Committee therefore concluded that these three flavouring agents are not of safety concern at current estimated dietary exposures.

No NOEL is available for butanal dibenzyl thioacetal (No. 1939). Although the thioacetal group in butanal dibenzyl thioacetal can be expected to be hydrolysed, the rate and extent of hydrolysis are unknown. A NOEL was not available for a structurally related substance. Accordingly, the evaluation of butanal dibenzyl thioacetal proceeded to step B5.

Subgroup ii: Acyclic sulfides with oxidized side-chains. The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from a 90-day oral study in rats provides adequate margins of safety, ranging from 3500 to 672 000, for ethyl 2-hydroxyethyl sulfide (No. 1912; SPET = 3 μg/day), 4-methyl-2-(methylthiomethyl)-2-pentenal (No. 1918; SPET = 0.125 μg/day), 4-methyl-2-(methylthiomethyl)-2-hexenal (No. 1919; SPET = 1.5 μg/day), 5-methyl-2-(methylthiomethyl)-2-hexenal (No. 1920; SPET = 3 μg/day), butyl β-(methylthio)acrylate (No. 1921; SPET = 0.3 μg/day), ethyl 3-(ethylthio)butyrate (No. 1922; SPET = 24 μg/day), methional diethyl acetal (No. 1940; SPET = 6 μg/day) and 1-(3-(methylthio)-butyryl)-2,6,6-trimethylcyclohexene (No. 1942; SPET = 0.25 μg/day) when used as flavouring agents. The Committee therefore concluded that these eight flavouring agents are not of safety concern at current estimated dietary exposures.

The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) provides a margin of safety of 280 for 2-(methylthio)ethyl acetate (No. 1913), ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915), ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) and ethyl 3-(methylthio)-2-propenoate (No. 1917) (SPET for Nos 1913 and 1915–1917 = 300 μg/day) when used as flavouring agents. This margin of safety is lower than the value of 1000 proposed at the forty-fourth meeting of the Committee as an adequate margin for flavouring agents on the B-side of the Procedure (Annex 1, reference *116*). However, No. 505 bears more structural alerts for toxicity compared with Nos 1913 and 1915–1917 because of its more complex molecular structure. Also, the value of 1000 was based on the comparison of the NOAEL with the MSDI. The Committee noted that the margin of safety for these compounds based on the MSDI (range 0.05–0.06 μg/day) is about 1 400 000. The Committee concluded that the values of 280 (based on the SPET) and about 1 400 000 (based on the MSDI) provide an adequate margin of safety and concluded that these four flavouring agents are not of safety concern at current estimated dietary exposures.

The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from a 90-day oral study in rats provides a margin of safety of 56 for 3-(methylthio)propyl hexanoate (No. 1941; SPET = 1500 μg/day). This margin of safety is approximately 20 times lower than the value of 1000 proposed at the forty-fourth meeting of the Committee (Annex 1, reference *116*) and is not considered adequate. Accordingly, the evaluation of 3-(methylthio)propyl hexanoate proceeded to step B5.

Subgroup iii: Cyclic sulfides. The NOEL of 9.2 mg/kg bw per day for the structurally related substance 4,5-dihydro-3(2H)-thiophenone (No. 498) from a 90-day study in rats provides an adequate margin of safety of 92 000 for 2-oxothiolane (No. 1923; SPET = 6 μg/day). The Committee concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup iv: Simple thiols. The NOEL of 0.56 mg/kg bw per day for the structurally related substance cyclopentanethiol (No. 516) from a 90-day study in rats provides an adequate margin of safety of 22 400 for dodecanethiol (No. 1924; SPET = 1.5 μg/day) when used as a flavouring agent. The Committee concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup v: Thiols with oxidized side-chains. For 2-hydroxyethanethiol (No. 1925), several studies of short-term toxicity were available, but it was not possible to derive an overall NOAEL for this compound. From the limitedly reported studies available, the NOAEL appears to be lower than 11 mg/kg bw per day. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, the structurally related substances 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats provide a margin of safety of at least 190 for No. 1925 (SPET = 600 μg/day). This margin of safety is lower than the value of 1000 proposed at the forty-fourth meeting of the Committee (Annex 1, reference *116*). However, the value of 1000 was based on the comparison of the NOAEL with the MSDI. The Committee noted that the margin of safety of No. 1925 based on the MSDI of 0.1 μg/person per day is at least 950 000. The Committee concluded that the values of at least 190 (based on the SPET) and at least 950 000 (based on the MSDI) provide an adequate margin of safety. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, Nos 546, 547 and 560 provide adequate margins of safety, ranging from 3800 to 1 680 000, for 4-mercapto-4-methyl-2-hexanone (No. 1926; SPET = 0.3 μg/day), 3-mercapto-3-methylbutyl isovalerate (No. 1927; SPET = 20 μg/day), (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928; MSDI = 0.1 μg/day), 3-mercaptohexanal (No. 1929; SPET = 3 μg/day), 3-mercaptopropionic acid (No. 1936; MSDI = 0.5 μg/day) and 2-ethylhexyl 3-mercaptopropionate (No. 1938; SPET = 30 μg/day) when used as flavouring agents. The Committee therefore concluded that these six flavouring agents are not of safety concern at current estimated dietary exposures.

Subgroup vii: Simple disulfides. The NOEL of 7.3 mg/kg bw per day for the structurally related substance propyl disulfide (No. 566) from a 90-day study in rats provides adequate margins of safety (range 8760–2 190 000) for diisoamyl disulfide (No. 1930; SPET = 10 µg/day), butyl propyl disulfide (No. 1932; SPET = 0.2 µg/day) and di-*sec*-butyl disulfide (No. 1933; SPET = 50 µg/day) when used as flavouring agents. The NOEL of 3.4 mg/kg bw per day for 2-naphthalenethiol (No. 531) from a 90-day study in rats provides an adequate margin of safety (1 020 000) for methyl 2-methylphenyl disulfide (No. 1935; SPET = 0.2 µg/day) when used as a flavouring agent. No. 1935 is predicted to be reduced rapidly to the corresponding thiophenol. The Committee therefore concluded that these four flavouring agents are not of safety concern at current estimated dietary exposures.

Subgroup ix: Trisulfides. The NOEL of 4.8 mg/kg bw per day for the structurally related substance dipropyl trisulfide (No. 585) from a 90-day study in rats provides an adequate margin of safety of 144 000 for diisoamyl trisulfide (No. 1934; SPET = 2 µg/day) when used as a flavouring agent. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup xi: Thioesters. The NOEL of 6.5 mg/kg bw per day for the structurally related substance ethyl thioacetate (No. 483) from a 90-day study in rats provides an adequate margin of safety of 6500 for methyl isobutanethioate (No. 1937; SPET = 60 µg/day) when used as a flavouring agent. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Step B5. The conditions of use for butanal dibenzyl thioacetal (No. 1939; SPET = 40) result in an intake greater than 1.5 µg/day. Therefore, the Committee determined that additional data would be necessary to complete the evaluation of this flavouring agent.

The conditions of use for 3-(methylthio)propyl hexanoate (No. 1941; SPET = 1500 µg/day) result in an intake greater than 1.5 µg/day. Therefore, the Committee determined that additional data would be necessary to complete the evaluation of this flavouring agent.

Table 18 summarizes the evaluations of the 36 additional members of the group of simple aliphatic and aromatic sulfides and thiols (Nos 1909–1944).

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the combined intakes of the five compounds with the highest estimated dietary exposure in each subgroup in which additional compounds were evaluated, using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Subgroup i: Simple sulfides

In the unlikely event that the flavouring agents belonging to the subgroup of simple sulfides, of which the highest estimated intakes are for Nos 452, 454, 455, 533 and 1909 (all structural class I) in Europe, the USA and Japan, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup ii: Acyclic sulfides with oxidized side-chains

In the unlikely event that the flavouring agents belonging to the subgroup of acyclic sulfides with oxidized side-chains, of which the highest estimated intakes are for Nos 466, 472, 476, 478 and 481 (all structural class I) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup iii: Cyclic sulfides

In the unlikely event that the flavouring agents belonging to the subgroup of cyclic sulfides, of which the highest estimated intakes correspond to Nos 464, 498, 499, 534 and 543 (all structural class II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

Subgroup iv: Simple thiols

In the unlikely event that the flavouring agents belonging to the subgroup of simple thiols, of which the highest estimated intakes correspond to Nos 508, 509, 520, 525 and 528 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup v: Thiols with oxidized side-chains

In the unlikely event that the flavouring agents in the subgroup of thiols with oxidized side-chains, of which the highest estimated intakes are for Nos 546, 551, 553, 558 and 561 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup vii: Simple disulfides

In the unlikely event that the flavouring agents in the subgroup of simple disulfides, of which the highest estimated intakes are for Nos 564, 565, 567, 570 and 572 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup ix: Trisulfides

In the unlikely event that the flavouring agents in the subgroup of trisulfides, of which the highest estimated intakes are for Nos 582, 585, 587, 588 and 1701 (all structural class I) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup xi: Thioesters

In the unlikely event that the flavouring agents in the subgroup of thioesters, of which the highest estimated intakes correspond to Nos 484, 492, 493, 1295 and 1676 in Europe, the USA and Japan (all structural class I), were to be consumed concurrently on a daily basis, the estimated combined intakes of 5 and 14 μg/person in Europe and the USA, respectively, would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Consideration of secondary components

Four flavouring agents in this group (Nos 1915, 1916, 1932 and 1944) have assay values of less than 95%. The secondary component of ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915) is ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916), and the secondary component of ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) is ethyl 3-(methylthio)-(*2Z*)-propenoate (No. 1915). These compounds are expected to share the same metabolic fate and are considered not to present a safety concern at current estimated dietary exposures. The secondary components of butyl propyl

disulfide (No. 1932) are dipropyl disulfide and dibutyl disulfide. They are both expected to share the same metabolic fate as the primary substance and are considered not to present a safety concern at current estimated dietary exposures. The secondary components of 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944) (2-[(2*E*)-pent-2-en-1-yl]-4-propyl-1,3-oxathiane and 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane) are expected to share the same metabolic fate as the primary substance and are considered not to present a safety concern at current estimated dietary exposures.

Conclusion

In the previous evaluations of flavouring agents in the group of simple aliphatic and aromatic sulfides and thiols, studies of biological properties, acute toxicity, short-term and long-term toxicity, genotoxicity and developmental toxicity as well as observations in humans were available (Annex 1, references *144*, *167* and *188*). The toxicity data available for this evaluation supported those from previous evaluations.

The Committee concluded that 30 flavouring agents (Nos 1909–1913, 1915–1930, 1932–1938, 1940 and 1942), which are additions to the group of simple aliphatic and aromatic sulfides and thiols, would not give rise to safety concerns at current estimated dietary exposures. For the other six flavouring agents (Nos 1914, 1931, 1939, 1941, 1943 and 1944), the Committee concluded that the evaluations could not be completed and that additional data would be necessary to complete these evaluations at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.2 Specifications of identity and purity of flavouring agents

4.2.1 *New specifications*

The Committee received information related to specifications for the 179 new flavouring agents on the agenda of the present meeting. In the case of two flavouring agents that were not assessed for safety at the current meeting, 2-aminoacetophenone (No. 2043) and (±)-2-phenyl-4-methyl-2-hexenal (No. 2069), no specifications were prepared. For the other 177 flavouring agents, the Committee prepared full specifications. The specifications prepared for 13 flavouring agents (Nos 1914, 1931, 1939, 1941, 1943, 1944, 1973, 1988, 2005, 2007, 2010, 2011 and 2046) include a statement that the safety evaluations for these flavouring agents had not been completed.

4.2.2 ***Revision of specifications***

4.2.2.1 *4-Carvomenthol (No. 439)*

The Committee revised the specifications for 4-carvomenthol (No. 439) in order to introduce new information on the physical form of the substance, its solubility as well as ranges of refractive index and specific gravity.

4.2.2.2 *5,6,7,8-Tetrahydroquinoxaline (No. 952)*

The Committee revised the specifications for 5,6,7,8-tetrahydroquinoxaline (No. 952) in order to introduce new information on the physical form of the substance, its solubility as well as ranges of refractive index and specific gravity.

5. Contaminants

5.1 Cadmium

Explanation

The presence of cadmium in food results from contamination of soil and water both from natural sources and from anthropogenic activities. Crops differ with respect to absorption of cadmium, and cadmium is known to accumulate in the tissues (particularly the liver and kidney) of terrestrial animals and in aquatic animals (particularly detritus feeders, such as molluscs).

Cadmium was evaluated by the Committee at its sixteenth, thirty-third, forty-first, fifty-fifth, sixty-first and sixty-fourth meetings (Annex 1, references *30*, *83*, *107*, *149*, *166* and *176*). At the thirty-third meeting, a provisional tolerable weekly intake (PTWI) of 400–500 μg or 7 μg/kg bw (assuming a body weight of 60 kg) was derived from a critical concentration of cadmium in the kidneys (200 mg/kg tissue), which caused an increase in β_2-microglobulin (β2MG) concentration in urine, and a toxicokinetic model that related cadmium bioaccumulation in the kidneys to dietary exposure. In 1992, Environmental Health Criteria 134 provided a detailed description of the model on which the PTWI was based and its various assumptions. At the forty-first meeting, the Committee concluded that the model estimates used to derive the PTWI were conservative, but it did not include a safety factor and reiterated that there was only a small margin of safety between exposure via the diet and the exposure that would result in deleterious effects.

At its fifty-fifth meeting, the Committee concluded that the prevalences of renal tubular dysfunction that correspond to various dietary exposures to cadmium were still appropriate for risk assessment and that the risk of renal tubular dysfunction in the general population would be negligible below a urinary cadmium excretion of 2.5 μg/g creatinine. The estimate of 2.5 μg/g creatinine was based on occupational data and involved a number of assumptions about creatinine excretion, cadmium absorption and bioavailability and the ratio of dietary exposure to cadmium to excreted cadmium.

At the sixty-first meeting, the Committee considered studies including epidemiological investigations of environmental exposure to cadmium, such as the CadmiBel studies from Belgium and a series of Japanese reports. The Committee reaffirmed that renal tubular dysfunction remained the critical health outcome with regard to the toxicity of cadmium and that an excess prevalence of renal tubular dysfunction would not be expected to occur if the urinary cadmium concentration did not exceed 2.5 μg/g creatinine. The Committee concluded that the new data did not provide a sufficient basis for revising the PTWI and therefore maintained the PTWI of 7 μg/kg bw.

At its sixty-fourth meeting, the Committee evaluated the impact of different maximum levels (MLs) for cadmium in commodities that contribute to dietary exposure. The dietary assessment took into account the potential impact of different MLs on the distribution of concentrations of cadmium in each commodity and the dietary exposures to cadmium from each individual commodity. The Committee concluded that a change in the proposed Codex Alimentarius Commission MLs would result in a change of only 1–6% in the dietary exposure to cadmium and therefore was of no significance in terms of risk to human health, considering that the total dietary exposure to cadmium was only 40–60% of the PTWI of 7 μg/kg bw.

At the request of the CCCF, the Committee considered new information that had become available since cadmium was last evaluated, together with the data it had previously reviewed. The Committee also considered new information on cadmium levels in food and dietary exposure. As it is now acknowledged that renal dysfunction is the most sensitive toxicological end-point arising from cadmium exposure, most of the new data involved the use of urinary biomarkers to estimate risk based on statistical modelling. The Committee considered whether these recent modelled risk estimates for cadmium would support the current PTWI.

Absorption, distribution, metabolism and excretion

In previously reviewed studies, the Committee noted that most ingested cadmium passes through the gastrointestinal tract largely without being absorbed. In mice, rats and monkeys, the absorption of cadmium from the gastrointestinal tract depends on the type of cadmium compound, dose and frequency, age and interaction with various dietary components. A recent study has shown that expression of divalent metal transporter 1 (*DMT1*) and metal transporter protein 1 (*MTP1*) genes is upregulated in response to iron-deficient diets. This upregulation may explain the observation that both the urinary cadmium excretion and kidney cadmium concentration were significantly higher in women with low iron stores (serum ferritin concentration below 30 μg/l).

The oral bioavailability of cadmium in laboratory animals ranges from 0.5% to 3.0%, on average. Following absorption, cadmium binds to metallothionein, but this binding can be overloaded at relatively moderate doses. Cadmium is distributed mainly to the liver, kidneys and placenta. The cadmium concentrations in liver and kidneys are comparable after short-term exposure, but the kidney concentration generally exceeds the liver concentration following prolonged exposure, except at very high exposures. Cadmium present in liver and kidney accounts for more than half of the body burden. The retention of cadmium in various tissues is variable, and its release appears to be multiphasic. The apparent half-life estimates range between 200 and 700 days in mice and rats and up to 2 years in the squirrel monkey.

In humans, about 50% of the cadmium body burden is found in kidneys. Other major bioaccumulating organs or tissues contributing to the body burden are liver (15%) and muscle (20%). The quantity of cadmium in bone is small. The slow excretion of cadmium results in a long biological half-life, which has been estimated to be between 10 and 33 years. A recent estimate, based on long-term dietary exposure data covering a period of 20 years from a Swedish cohort of 680 women aged between 56 and 70 years, indicated an apparent half-life of kidney cadmium of 11.6 years, with a standard deviation of 3.0 years (*29*). A one-compartment toxicokinetic model was applied to these dietary exposure data. The average daily dietary exposure was reported to be 14 μg (0.2 μg/kg bw), and the mean urinary cadmium level was 0.34 μg/g creatinine. Based on the model, the population distribution of the daily dietary cadmium exposure corresponding to a given level of urinary cadmium could be obtained (see section on Toxicokinetic modelling under Dose–response analyses).

Toxicological data

In previously reviewed studies, the Committee noted that long-term oral exposure to cadmium resulted in a variety of progressive histopathological changes in the kidney, including epithelial cell damage of proximal tubules, interstitial fibrosis and glomerular basal cell damage with limited tubular cell regeneration. Biochemical indications of renal damage were seen in the form of low molecular weight proteinuria, glucosuria and aminoaciduria. Tubular dysfunction also caused an increase in the urinary excretion of cadmium.

Observations in humans

A number of new epidemiological studies have assessed factors influencing cadmium concentrations in kidney and urine following environmental exposure, as well as the relationship between cadmium exposure and several health effects.

The kidney is the critical target organ for the long-term effects of cadmium, showing a variety of progressive histopathological changes, including epithelial cell damage in the proximal tubule, interstitial fibrosis and glomerular basal cell damage. The earliest manifestation of cadmium-induced nephrotoxicity is renal tubular dysfunction, which most often manifests as the urinary excretion of low molecular weight proteins and enzymes, such as β2MG, retinol-binding protein (RBP), α_1-microglobulin and *N*-acetyl-β-D-glucosaminidase. Urinary β2MG level has been the most widely used marker of renal tubular dysfunction.

Several studies monitoring populations following a reduction in cadmium exposure have attempted to address the question of the reversibility of early renal changes. A modest increase in urinary excretion of β2MG or RBP, in the range of 300–1000 μg/g creatinine, is unlikely to indicate compromised renal function and is usually reversible after cadmium exposure is reduced. With β2MG or RBP excretion above 1000 μg/g creatinine, proteinuria due to renal tubular dysfunction becomes irreversible, although glomerular filtration rate is normal or only slightly impaired; when the urinary excretion of these proteins is increased up to 10 000 μg/g creatinine, renal tubular dysfunction progresses to overt nephropathy, usually associated with a lower glomerular filtration rate. These values have been used as cut-off criteria to estimate cadmium nephrotoxicity (measured by urinary β2MG excretion) as a function of cadmium concentration in urine. Although there is good evidence demonstrating relationships between urinary excretion of cadmium and various renal biomarkers (e.g. urinary β2MG or RBP concentration), the health significance of these nonspecific biomarkers in relation to cadmium-induced renal damage remains somewhat uncertain. These biomarker changes in the lower range (i.e. 300–1000 μg/g creatinine) might reflect an early renal response to cadmium, which may be purely adaptive or reversible.

Previously reviewed studies have shown that effects on bone generally arise only after kidney damage has occurred and are likely to be secondary to resulting changes in calcium, phosphorus and vitamin D metabolism. Recent studies have evaluated the association between cadmium and bone mineral density or osteoporosis in populations with low-level cadmium exposure. Although these studies found a significant inverse association between the score of bone mineral density and urinary excretion of cadmium at low levels of exposure, they did not assess renal damage. In one of these studies, in Sweden, the incidence of forearm fractures was significantly increased (by 18%) per unit of urinary cadmium (1 μg/g creatinine). In a Belgian study, a significant relative risk of fractures of 1.73 was associated with a doubling of mean cadmium excretion in the urine (1.66 versus 0.83 μg/g creatinine) among women. There was no association between fractures and cadmium levels among men. Another study in Belgium that investigated the association

between urinary cadmium and bone mineral density also measured markers of bone resorption, renal tubular dysfunction and calcium metabolism. In this study, even in the absence of renal tubular dysfunction, urinary cadmium level was associated with reduced bone mineral density, increased calciuria and reduced levels of serum parathyroid hormone. However, four additional studies failed to show any association between urinary cadmium and bone mineral density or calcium metabolism, or the association was no longer significant after controlling for age, body weight and smoking, in the absence of renal tubular damage. The assessment of the association between urinary cadmium and bone mineral density is based upon different types of epidemiological designs, including prospective and cross-sectional studies, with variable power and different degrees of control of the relevant confounders. Although the overall evidence at present points to an association between urinary cadmium and a decrease in bone mineral density, it is unclear whether the effect is secondary to renal tubular dysfunction. Therefore, the data do not provide a basis for a dose–response analysis of the direct effects of cadmium on bone mineral density.

Cadmium has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (group 1), with sufficient evidence for lung cancer and limited evidence for kidney, liver and prostate cancer. Most of the evidence is derived from high cadmium exposure of exposed workers through inhalation. Some case–control studies have reported associations of bladder cancer with increased levels of blood cadmium, breast cancer with increased urinary excretion of cadmium and prostate cancer with increased levels of cadmium in toenails; the relationship between cadmium concentration in toenails and dietary exposure is unknown. A prospective study in Sweden reported a significantly increased risk of endometrial cancer in relation to dietary intake of cadmium in postmenopausal women.

In several cross-sectional studies, increased levels of cadmium measured in blood or urine have been found to be associated with various cardiovascular end-points, including myocardial infarction, stroke, heart failure, hypertension and changes in measures of arterial function (aortic pulse wave velocity and carotid, brachial and femoral pulse pressures). The epidemiological evidence for an association between cardiovascular diseases and cadmium is weak.

Prospective studies of the relationship between mortality and environmental exposure to cadmium were also available. In one study, based on a representative sample of the population of the USA with 9 years of follow-up, a doubling of the mean urinary cadmium level (0.64 versus 0.32 μg/g creatinine) was observed. This was associated with a 28% increased mortality by all causes, 55% increased mortality by cancer, 21% increased mortality

by cardiovascular diseases and 36% increased mortality by coronary heart disease, which were statistically significant among men. No significant effects were observed among women. In a study from Belgium of subjects from a cadmium-polluted area and a control area with a follow-up of 20 years, a doubling of the mean urinary cadmium concentration (1.36 versus 0.68 μg/g creatinine) was significantly associated with 20% increased risk of mortality by all causes, 43% increased mortality for cancer and 44% increased mortality for non-cardiovascular diseases. Two prospective studies assessed mortality, renal tubular dysfunction and environmental exposure to cadmium in cohorts of residents in highly polluted areas in Japan. One of them reported a significant increase of 41% in mortality for subjects with β2MG excretion greater than or equal to 1000 μg/g creatinine, compared with the regional reference death rate, after 20 years of follow-up. The other study, with a follow-up of 15 years, found a significant increase in overall mortality of 27% in men and 46% in women with β2MG urinary levels above 1000 μg/g creatinine; moreover, among subjects with β2MG urinary levels between 300 and 1000 μg/g creatinine, there was a significantly increased risk of death by cerebral infarction, digestive diseases (men) and heart failure (women).

Analytical methods

Analytical methods for the determination of cadmium in foods, water and biological materials are well established; the detection techniques include flame atomic absorption spectrometry (FAAS), electrothermal (graphite or Zeeman furnace) atomic absorption spectrometry (ETAAS), beam injection (thermospray) flame furnace atomic absorption spectrometry, hydride generation atomic fluorescence spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). The high-resolution continuum source electrothermal atomic absorption spectrometry allows direct analysis of solids with improved LODs. In recent years, the use of dynamic reaction cell technology combined with ICP-MS has allowed the removal of the interferences with a minimum loss of sensitivity. Although ETAAS has been extensively used, ICP-MS could be considered as the method of choice, as it offers lower LODs and wide dynamic range and allows simultaneous determination of several elements. Additionally, ICP-MS offers high specificity through spectral interpretation and isotopic information. Microwave-assisted acid digestion has been the preferred sample preparation technique, although other techniques, such as ashing and slurry preparation, have been used.

Most data submitted were obtained using the above methods, which were validated. Laboratories followed good quality assurance programmes; some had also participated in proficiency testing schemes and achieved good *z*-scores.

Sampling protocols

General guidance for sampling is described in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (*30*).

Prevention and control

There have been worldwide efforts to reduce cadmium exposure, including implementation of MLs for cadmium in foods, food additives and water. Other prevention and control measures include controlling cadmium levels in fertilizers and feeds and following good agricultural and manufacturing practices.

Levels and patterns of contamination in food

At its present meeting, the Committee reviewed new cadmium occurrence data submitted by EFSA, covering 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Estonia, France, Germany, Greece, Iceland, Ireland, Italy, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom), as well as data submitted by 11 other countries (Australia, Brazil, Canada, Chile, China, France, Ghana, Japan, Singapore, the USA and Viet Nam). The food industry also submitted data on cadmium levels in products that are distributed and used worldwide. The total number of analytical results (single or composite samples) was 155 496, with 84.4% coming from Europe, 5.2% from North America, 1.5% from Asia, 1.4% from Latin America, 0.3% from the Pacific region and 0.1% from Africa. The data submitted by industry accounted for 7.0% of the data.

A summary of the new occurrence data by food category is provided in Table 19. For all food categories, calculations of mean concentrations included results below the LOD or LOQ (i.e. non-detects or ND), although the values assigned to those results varied by country. National average concentrations of cadmium ranged between not detected and 0.04 mg/kg in most food categories. Higher national mean concentrations, ranging from 0.1 to 4.8 mg/kg, were reported for vegetables (including dried); meat and poultry offal; shellfish/molluscs; nuts and oilseeds; coffee, tea and cocoa; and spices.

Food consumption and dietary exposure assessment

New information on national estimates of dietary exposure to cadmium was submitted by Australia, China, Japan and the USA. EFSA submitted dietary exposure estimates for Europe. Additional information on national dietary exposure for Chile, Lebanon and the Republic of Korea was obtained from the scientific literature. National and regional exposure estimates were expressed on either a daily or weekly basis, as these estimates are based on

Table 19
Summary of cadmium occurrence data submitted for this meeting

Food category	Total no. of samples	Range of national or regional mean cadmium concentrations (mg/kg)
Wheat (including breads)	1 503	0.009–0.04
Rice	2 295	0.004–0.02
Oats	211	0.003–0.02
Baked goods	55	ND–0.02
Cereals/grains, other	12 637	ND–0.03
Roots and tubers	2 319	0.006–0.04
Pulses and legumes	169	0.003–0.03
Fruits	6 314	0.001–0.007
Fruit juices	3 932	ND–0.003
Dried fruit	79	0.003–0.009
Vegetables	18 183	0.006–0.1
Dried vegetables	348	0.09–1.0
Meat and poultry muscle, not further specified	20 154	0.008–0.04
Meat and poultry offal, not further specified	16 049	0.1
Meat muscle	1 715	0.001–0.003
Meat offal	1 406	0.03–0.5
Poultry muscle	2 500	0.0002–0.01
Poultry offal	1 224	0.006–0.5
Eggs	736	0.0001–0.007
Finfish	10 531	ND–0.008
Shellfish/molluscs	7 403	0.01–4.8
Dairy products	9 208	ND–0.004
Nuts and oilseeds	350	0.02–0.1
Animal and vegetable fats	1 610	ND–0.006
Coffee, tea and cocoa	3 505	0.0001–1.8
Sugar, honey and sweets	3 908	ND–0.03
Spices	2 237	0.006–0.2
Alcoholic beverages	3 443	ND–0.004
Drinking-water (bottled and tap)	21 472	ND–0.0004

1- to 7-day food consumption surveys. During the meeting, the Committee concluded that a provisional tolerable monthly intake (PTMI) was appropriate for cadmium (see Evaluation section). For contaminants such as cadmium that are widely distributed in foods at approximately constant levels, day-to-day variability in dietary exposure over the long term would be low, so extrapolating dietary exposure from a daily or weekly basis to a monthly basis would not have a substantial impact on exposure estimates. Therefore, the national and regional exposure estimates were extrapolated to a monthly basis by multiplying daily exposures by 30 or weekly exposures by 4.

Mean cadmium exposure for adults ranged from 2.2 to 12 µg/kg bw per month (Table 20). Estimates of high exposures reported for Europe, Lebanon and the USA ranged from 6.9 to 12.1 µg/kg bw per month. For Australia and the USA, dietary exposure for children 0.5–12 years of age ranged from 3.9 to 20.6 µg/kg bw per month. Dietary exposure for vegetarians, as reported by EFSA, was estimated to be 23.2 µg/kg bw per month.

Table 20
National and regional estimates of dietary exposure to cadmium for adults

Country or region	Treatment of ND occurrence data in exposure estimates	Mean exposure (µg/kg bw per month)	High exposure (µg/kg bw per month)
Australia	ND = 0 and LOD	2.2–6.9	—
Chile	Not specified	9	—
China	ND = LOD/2	9.9	—
Europe	ND = LOD/2	9.1[a]	12.1[b]
Japan	Not specified	12	—
Lebanon	ND = LOQ/2	5.2	6.9[c]
Republic of Korea	ND = LOD	7.7	—
USA	ND = 0	4.6	8.1[d]

[a] Median of mean exposure estimates for 16 European countries.
[b] Sum of 95th percentile exposure (consumers only) for the two food categories with highest exposure plus mean exposure (whole population) for the other food categories.
[c] Calculated from mean food consumption and highest cadmium concentrations in each food category.
[d] 90th percentile exposure calculated from distributions of both food consumption and cadmium occurrence data; high exposure equals 90th percentile of exposure.

The food categories that contributed most to cadmium exposure were reported by Chile, China, Europe, Lebanon and the Republic of Korea. For Chile, the major sources of cadmium in the diet were fish and shellfish, spices and cereals/grains. For China, the main contributions to dietary exposure to cadmium on a national basis were cereals/grains and vegetables; meat and seafood were found to be the main dietary sources of cadmium in several regions within China. Cereals/grains, vegetables/nuts/pulses and animal offal were the main dietary sources of cadmium in Europe. In the Republic of Korea, the main sources of cadmium in the diet were rice, vegetables/seaweed and seafood. The major sources of cadmium in the Lebanese diet were reported to be cereals/grains and vegetables.

The guidelines for conducting exposure assessments for contaminants in foods (*31*) recommend that regional dietary exposure estimates should be calculated using regional average contaminant values and the GEMS/Food consumption cluster diets. Such estimates were not calculated for the present meeting because occurrence data were submitted by countries that

represented only 2 of the 13 GEMS/Food clusters. Furthermore, national exposure estimates based on national food consumption data were submitted by the countries that also submitted the majority of new occurrence data. As the national estimates provided more refined estimates than could be calculated with the GEMS/Food consumption cluster diets, only the national estimates were considered in this assessment.

Dose–response analysis

The basis of the current PTWI is an estimate of a critical cadmium concentration in the kidney cortex at or below which there is no observed increase in β2MG concentrations in urine. A toxicokinetic model was used to estimate the dietary exposure required to reach this critical cadmium concentration in the kidney cortex. An alternative approach is to identify a threshold level of a urinary biomarker of renal tubular damage, such as β2MG, and then use a toxicokinetic model to calculate the dietary exposure corresponding to that threshold level.

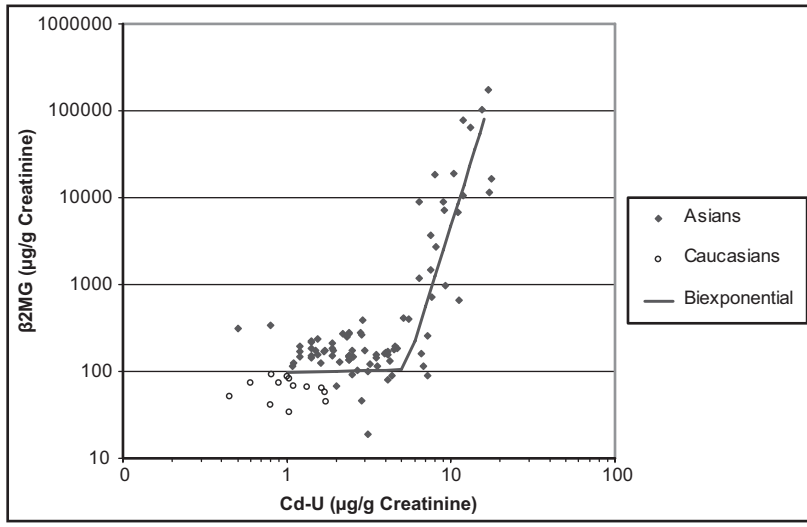
Biomarker meta-analysis

In order to determine a dose–response relationship between a suitable biomarker and urinary cadmium levels for the general population, the data available in published studies were compiled and used for a meta-analysis to characterize the relationship between urinary β2MG and urinary cadmium levels (*32*). Urinary β2MG level was chosen as the most suitable biomarker for the meta-analysis because it is widely recognized as a marker for renal pathology and consequently had the largest number of available data. The database covers approximately 30 000 non-occupationally exposed individuals reported in 35 studies, but the data are expressed only as group means with standard deviations. The majority of these non-occupationally exposed individuals were of Asian descent (93.5%) and female (75%). The age distribution was approximately equally divided above and below 50 years (i.e. ≥50 years: 51.5%; <50 years: 48.5%). As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved after 45–60 years of exposure. Therefore, data relating β2MG excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older should provide the most reliable basis to determine a critical concentration of cadmium in the urine. The data for the population aged 50 years and over in the 35 studies were categorized according to urinary cadmium concentration, resulting in 98 groups containing matched pairs of urinary cadmium and β2MG levels. The 98 groups ranged in size from 3 to 908 individuals, with a median of 56.

The Committee identified the biexponential model as being suitable to characterize the cadmium–β2MG dose–response relationship. In the model,

the first (low urinary cadmium concentration) slope is virtually flat, and only the second (high urinary cadmium concentration) slope was considered by the Committee to be indicative of renal pathology (Fig. 2). Therefore, the Committee chose the breakpoint for the second slope, which is the point at which the β2MG concentration begins to rapidly increase with increasing urinary cadmium level, as the basis of the evaluation. This breakpoint derived for the population aged 50 years and over corresponds to 5.24 (5th–95th percentiles 4.94–5.57) μg of cadmium per gram of creatinine (Fig. 2).

Figure 2
Dose–response relationship for cadmium and β2MG concentrations in urine



Toxicodynamic variability

Toxicodynamic variability in the dose–response relationship is not taken into account by the model, because the data represent only a population average rather than individual data points. The lack of empirical evidence of elevated β2MG levels below a urinary cadmium concentration of 5.24 (5th–95th percentiles 4.94–5.57) μg of cadmium per gram creatinine indicates that the variance is small.

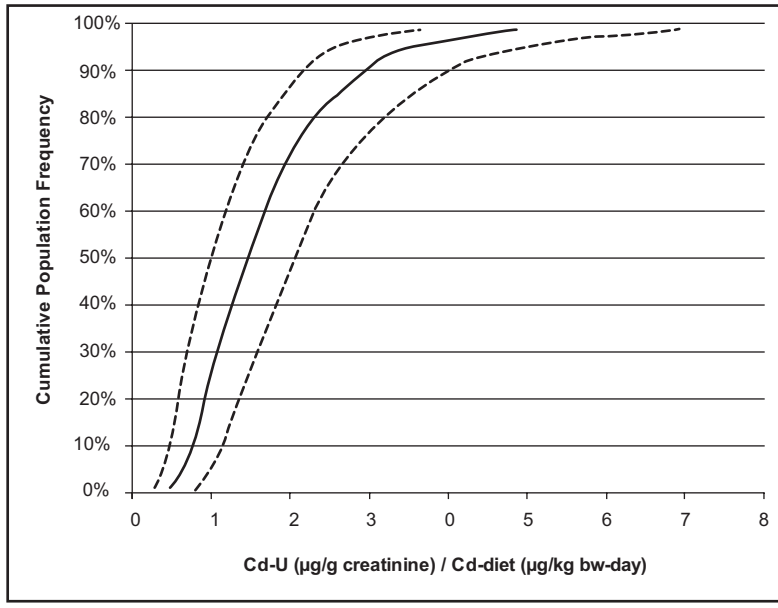
Toxicodynamic variability in the model was accounted for by incorporating a maximum variability that ranges from 1 to 3. The value of 3 approximately corresponds to the toxicodynamic component of the conventional 10-fold uncertainty factor for interindividual variability. Individual subjects were presumed to have a critical concentration (breakpoint) somewhere within the range defined by the mean multiplied or divided by the maximum value. As the same maximum value was used for both increased and reduced individual susceptibility, the adjustment resulted in broadened distributions of both

population variability and uncertainty without affecting the geometric central estimates.

Toxicokinetic modelling

A one-compartment model was used to characterize the relationship between urinary cadmium concentration and dietary cadmium exposure (see Absorption, distribution, metabolism and excretion). This model included a statistical parameter for variation in apparent half-life. The calculated relationship between dietary cadmium exposure and urinary cadmium concentration is linear; therefore, the outcome may be expressed as a population distribution of the ratio with confidence intervals (CIs) (Fig. 3).

Figure 3
Population distribution of urinary to dietary cadmium ratios

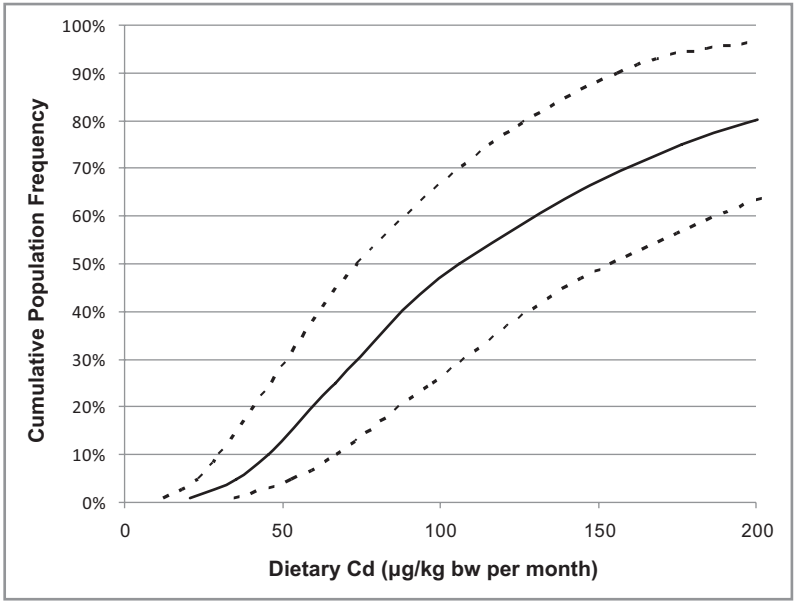


Estimation of the relationship between urinary cadmium excretion and dietary cadmium exposure

A two-dimensional Monte Carlo simulation was used to estimate the population percentiles with associated 5th to 95th percentile CIs from the variability and uncertainty in the breakpoint, the adjustment for toxicodynamic variability and the toxicokinetic model (Fig. 4). The dietary cadmium exposure (µg/kg bw per day) that equates to 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine in urine was estimated to be 1.2 (5th–95th percentiles 0.8–1.8) µg/kg bw per day at the 5th

population percentile. This is equivalent to 36 (5th–95th percentiles 24–54) μg/kg bw per month. The Committee decided to use the lower bound of the CI to account for particularly susceptible individuals so that they would remain below the dietary exposure at which renal pathology is indicated.

Figure 4
Cumulative population frequency of dietary cadmium exposure that would result in the urinary concentration at the breakpoint (5th–95th percentile CIs)



Evaluation

Since cadmium was last considered by the Committee, there have been a number of new epidemiological studies that have reported cadmium-related biomarkers in urine following environmental exposure. The Committee noted that a large meta-analysis of studies that measured the dose–response relationship between β2MG and cadmium excretion in urine was available. As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved after 45–60 years of exposure. Therefore, data relating β2MG excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older provided the most reliable basis on which to determine a critical concentration of cadmium in the urine. An analysis of the group mean data from individuals who were 50 years of age and older showed that the urinary excretion of less than 5.24 (5th–95th percentiles 4.94–5.57) μg of cadmium per gram creatinine was not associated with an increased excretion of β2MG. Higher urinary cadmium levels were associated with a steep increase in β2MG excretion.

To determine a corresponding dietary exposure that would result in a urinary cadmium concentration at the breakpoint of 5.24 (5th–95th percentiles 4.94–5.57) μg of cadmium per gram creatinine, a one-compartment toxicokinetic model was used. The lower bound of the 5th population percentile dietary cadmium exposure that equates to the breakpoint was estimated to be 0.8 μg/kg bw per day or about 25 μg/kg bw per month.

The Committee noted that the existing health-based guidance value for cadmium was expressed on a weekly basis (PTWI), but, owing to cadmium's exceptionally long half-life, considered that a monthly value was more appropriate. The PTWI of 7 μg/kg bw was therefore withdrawn.

In view of the long half-life of cadmium, daily ingestion in food has a small or even a negligible effect on overall exposure. In order to assess long- or short-term risks to health due to cadmium exposure, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a PTMI. The PTMI established was 25 μg/kg bw.

The estimates of exposure to cadmium through the diet for all age groups, including consumers with high exposure and subgroups with special dietary habits (e.g. vegetarians), examined by the Committee at this meeting are below the PTMI.

A detailed addendum to the monograph was prepared.

5.2 *Lead*

Explanation

Lead (Pb) occurs in Earth's crust primarily as the mineral galena (lead(II) sulfide) and, to a lesser extent, as anglesite (lead(II) sulfate) and cerussite (lead carbonate). It occurs in the environment both naturally and, to a greater extent, from anthropogenic activities such as mining and smelting, battery manufacturing and the use of leaded petrol (gasoline). Lead contamination of food arises mainly from the environment or from food processing, food handling and food packaging. Atmospheric lead can contaminate food through deposition on agricultural crops. Water is another source of lead contamination of food. Although lead exists in both organic and inorganic forms, only inorganic lead has been detected in food.

Lead was previously evaluated by the Committee at its sixteenth, twenty-second, thirtieth, forty-first and fifty-third meetings (Annex 1, references *30*, *47*, *73*, *107* and *143*). At the sixteenth meeting, the Committee established a PTWI of 3 mg of lead per person, equivalent to 50 μg/kg bw, stating that

this did not apply to infants and children (Annex 1, reference *30*). At its twenty-second meeting, the Committee retained the PTWI for adults, noting that establishing a PTWI for children was not yet possible owing to the lack of relevant scientific data (Annex 1, reference *47*). The health risks associated with exposure of infants and children to lead were evaluated at the thirtieth meeting, and a PTWI of 25 μg/kg bw was established for this population group, based on the information that a mean daily exposure to lead of 3–4 μg/kg bw for infants and children was not associated with an increase in blood lead levels (Annex 1, reference *73*). At the forty-first meeting, the Committee withdrew the previous PTWI of 50 μg/kg bw for adults and extended the PTWI of 25 μg/kg bw to all age groups (Annex 1, reference *107*). In these previous evaluations, it was emphasized that the PTWI applied to lead from all sources. At its fifty-third meeting, the Committee was asked to assess the risk of dietary exposure of infants and children to lead. It concluded that current concentrations of lead in food would have very little impact on the neurobehavioural development of infants and children but stressed that a full risk assessment of lead should take other sources of exposure into account (Annex 1, reference *143*).

At its present meeting, the Committee considered information on lead related to the toxicology, epidemiology, exposure assessment and analytical methodology, in particular for a dose–response analysis below blood lead levels of 10 μg/dl, at the request of the CCCF.

The literature relating to lead is extensive, and the present Committee used the recent (2010) review of EFSA as the starting point for its evaluation, together with newer studies that were considered to be informative. Only brief summaries of toxicological effects are given, but studies of the effects critical for the risk assessment are evaluated in more detail. The main emphasis is on studies in humans.

Absorption, distribution, metabolism and excretion

Absorption of lead from the gastrointestinal tract is influenced by physiological factors (e.g. age, fasting, calcium and iron status, pregnancy) and the physicochemical characteristics of the ingested material. Absorption is higher in children than in adults and is lower in the presence of food. Absorbed lead is transferred to soft tissues, including liver and kidney, and to bone tissue, where it accumulates with age. Under certain conditions, such as pregnancy and osteoporosis, bone resorption can result in increased concentrations of lead in blood. Lead readily crosses the placenta and is transferred into breast milk. In humans, the half-life of lead is approximately 30 days in blood and 10–30 years in bone. Urine and faeces are the major routes of excretion. Lead binds to thiol groups and other ligands in proteins.

Its toxicity has been attributed to inhibition of enzymes (e.g. those involved in haem synthesis) and to interference with calcium, magnesium and zinc homeostasis.

Toxicological data

The acute toxicity of lead is low. Chronic oral exposure of experimental animals to inorganic lead has effects on multiple organs, including kidney and liver, and systems, including the cardiovascular, haematological, immune, reproductive and nervous systems. IARC has concluded that there is sufficient evidence for the carcinogenicity of inorganic lead compounds in experimental animals, causing renal and brain tumours, and that the evidence for the carcinogenicity of organic lead compounds is inadequate. The results of genotoxicity studies and the inhibition of deoxyribonucleic acid (DNA) repair suggest a non-DNA-reactive mode of action for the carcinogenicity of lead.

Observations in humans

There is an extensive body of literature on epidemiological studies of lead. Blood is the tissue used most frequently to estimate exposure to lead, and blood lead levels generally reflect exposure in recent months. However, if the level of exposure is relatively stable, then blood lead level is a good indicator of exposure over the longer term. Longitudinal surveys in some countries have shown substantial reductions in population blood lead levels in recent decades. Programmes such as those that have eliminated the use of leaded petrol are considered to be an important factor, resulting in an average reduction of 39% in mean blood lead level over the 5-year period following implementation. Reductions in population blood lead levels in some countries have also been associated with the discontinued use of lead solder in food cans.

Exposure to lead has been shown to be associated with a wide range of effects, including various neurological and behavioural effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, delayed sexual maturation and impaired dental health. IARC concluded that there is *sufficient evidence* in animals but only *limited evidence* in humans for the carcinogenicity of inorganic lead and that inorganic lead compounds are *probably carcinogenic* to humans (group 2A). More recent studies do not indicate that any revision to the IARC conclusions is required.

For children, the weight of evidence is greatest, and evidence across studies is most consistent, for an association of blood lead levels with impaired neurodevelopment, specifically reduction of intelligence quotient (IQ).

Moreover, this effect has generally been associated with lower blood lead concentrations than those associated with the effects observed in other organ systems. Although the estimated IQ decrease per microgram of lead per decilitre of blood is small when viewed as the impact on an individual child (6.9 points over the range of 2.4–30 μg/dl), the decrement is considered to be important when interpreted as a reduction in population IQ. For example, if the mean IQ were reduced by 3 points, from 100 to 97, while the standard deviation and other characteristics of the distribution remained the same, there would be an 8% increase in the number of individuals with a score below 100. Moreover, there would be a 57% increase in the number of individuals with an IQ score below 70 (2 standard deviations below the expected population mean, commonly considered to be the cut-off for identifying individuals with an intellectual disability) and a 40% reduction in the number of individuals with an IQ score greater than 130 (considered to be the cut-off for identifying individuals with a "very superior" IQ). Furthermore, the Committee noted that a lead-associated reduction in IQ may be regarded as a marker for many other neurodevelopmental effects for which the evidence is not as robust but which have been observed in children at approximately the same blood lead levels (e.g. attention deficit hyperactivity disorder, reading deficit, executive dysfunction, fine motor deficit).

For adults, the adverse effect for which the weight of evidence is greatest and most consistent is a lead-associated increase in blood pressure. As with the lead-associated reduction in IQ, the increase is small when viewed as the effect on an individual's blood pressure, but important when viewed as a shift in the distribution of blood pressure within a population. Increased blood pressure is associated with increased risk of cardiovascular mortality. In a meta-analysis of 61 prospective studies involving more than 1 million adults, increased blood pressure was associated with age-specific increased mortality rates for ischaemic heart disease and stroke, and the proportional difference in risk associated with a given absolute difference in blood pressure was similar at all blood pressures above 115 mmHg (15 kPa) systolic or 75 mmHg (10 kPa) diastolic.

Analytical methods for the determination of lead in food and blood

The analytical methods for the determination of lead in food are well established. The techniques of choice are ETAAS and ICP-MS. To a minor extent, FAAS and ICP-OES are used. In the last decade, many technical improvements have been made to ETAAS, such as the design of the atomizer, background correction systems and improvement in the light source and detector. These have allowed the determination of lead in food at the low microgram per kilogram level. ICP-MS is increasingly used in food laboratories owing to its capability to perform multi-element measurements

in a wide variety of food matrices. In addition, the use of dynamic reaction cell technology combined with ICP-MS (DRC-ICP-MS) has allowed the removal of interferences with a minimum loss of sensitivity, while lowering the LOQs for lead, to allow the determination of lead in food at levels lower than 0.1 μg/kg.

The determination of lead in blood has been carried out using mainly ETAAS or ICP-MS. The methods are well established, and the LODs at the 0.1 ng/ml level are adequate to quantify lead in blood. Sample preparation is simple, but advances can be made in reducing the volume of sample required for analyses. One novel technique is the use of laser ablation coupled with ICP-MS, which requires a sample volume of less than 1 μl of whole blood for the quantification of lead.

The sample preparation procedure used most frequently for the determination of lead in food is acid digestion in the presence of strong oxidants in open or closed vessels. Microwave-assisted acid digestion has been extensively employed, which allows the use of large sample masses (1–2 g) under controlled temperature and pressure of the system, reducing contamination and avoiding losses of the element during mineralization.

Lead data for different food commodities submitted and evaluated at this meeting were almost all obtained by validated analytical methods or generated by accredited laboratories. The LODs and LOQs depend on the food matrix and the analytical technique employed. Analytical methods with poor LODs (>0.01 mg/kg) may erroneously lead to the conclusion that there is no lead present in the food.

As an example, Australia used a more sensitive analytical method for its 23rd TDS than previously used in its 19th and 20th TDSs. This resulted in a significant increase in the percentage of samples with detectable lead. However, more sensitive methods require greater resources, which may limit the number of samples that can be analysed. Therefore, an appropriate balance in number of samples that can be analysed and the sensitivity of the method will be required in the planning of surveillance programmes.

Sampling protocols

General guidance for sampling for foods is described in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (*30*).

Prevention and control

There have been widespread efforts to reduce lead exposure from food, focusing on implementing standards for lead levels in food, water and food additives; ending the use of lead-soldered cans; regulating the use of lead in

paint and petrol; controlling lead levels in water; reducing leaching from lead-containing vessels; and identifying and reacting to additional sources of lead contamination in foods or dietary supplements. Dust on foods should be removed before processing and/or consumption. For the prevention and control of lead in foods, good agricultural and manufacturing practices should be followed.

Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed data on lead occurrence in different food commodities received from seven countries—Australia, Brazil, China, France, Germany, Singapore and the USA. In addition, EFSA submitted data from Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Norway, Poland, Romania, Spain and Sweden and three commercial operators. The data from France and Germany were included in the assessment report of EFSA. In order to avoid duplicating the data in this analysis, the individual data submitted from both countries were not separately considered in the assessment of the current meeting.

The total number of analytical results (single or composite samples) was 110 899, with 84.9% coming from Europe, 7.6% from the USA, 1.9% from Latin America, 3.1% from Asia and 2.5% from the Pacific region. No data were received from Africa.

A summary of the occurrence data by food category is presented in Table 21. The weighted mean is provided for each food category and for the range of means across countries. All but one food category contained at least some foods with detectable lead levels. Maximum lead concentrations were determined for each category. However, two data sets, the Chinese TDS and 20th Australian TDS, provided only mean lead concentrations, and so it was not possible to determine maximum concentrations for these. Each category contains a number of foods with similar characteristics (e.g. baked goods, muscle). The miscellaneous category includes beverages, food supplements, infant formula, tap and bottled water and other foods for special dietary purposes as well as foods that did not fit in other categories. Within the miscellaneous category, generally the highest reported concentrations were for foods for special dietary uses and not for beverages. Infant formula essentially contained no detectable lead. EFSA reported that breast milk contained highly variable levels of lead. Sugar and sugar products and animal and vegetable fats rarely contained detectable levels of lead. Food categories with the highest frequency of detectable lead include meat, especially offal, organ meats and wild game, shellfish (particularly bivalves), cocoa, tea, cereal grains and products, and vegetables.

Table 21
Summary of lead occurrence data submitted for this meeting

Food category	*n*	Weighted mean lead concentration (mg/kg)[a]	Range of national mean concentrations (mg/kg)[b]	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	5 027	0.009	<LOD–0.029	7.12
Wheat (including breads)	506	0.005	<LOD–0.009	0.040
Rice	85	0.002	<LOD–0.004	0.021
Baked goods including "fancy breads"	203	0.047	0.001–0.23	16.5
Oats	63	0.001	<LOD–0.003	0.050
Roots and tubers	1 255	0.007	0.001–0.065	1.32
Pulses + legumes	326	0.004	<LOD–0.060	0.063
Fruits	7 480	0.030	<LOD–0.13	28.9
Dried fruit	282	0.086	0.006–0.34	1.34
Fruit juices	4 426	0.058	<LOD–0.35	74
Vegetables including juices	13 402	0.101	<LOD–0.40	27.6
Eggs	785	0.008	<LOD–0.039	0.21
All seafood (EFSA only)	11 453	0.054	—	4.06
Snails	11	0.069	0.065–0.074	0.19
Finfish	656	0.040	<LOD–0.22	0.45
Shellfish	765	0.070	0.010–0.19	11.80
Aquatic animals (China only)	12	0.015	—	—
Dairy foods	3 833	0.006	0.001–0.013	4.55
Nuts and oilseeds	184	0.005	<LOD–0.024	0.30
Animal fats	102	0.001	<LOD–0.002	0.029
Vegetable oils and fats	832	0.007	<LOD–0.039	7.30
Stimulants (coffee, tea, cocoas)[c]	764	0.211	<LOD–1.03	6.21
Sugar and honey	1 962	0.032	<LOD–0.082	4.10
Spices	86	0.027	<LOD–0.11	0.44
Alcoholic beverages	2 304	0.070	<LOD–0.38	5.80
Cocoa & chocolate & products[c]	206	0.692	<LOD–0.69	45.4

Food category	*n*	Weighted mean lead concentration (mg/kg)[a]	Range of national mean concentrations (mg/kg)[b]	Maximum lead concentration (mg/kg)
Cocoa butter	34	<LOD	<LOD	<LOD
Muscle meat excluding poultry	1 817	0.047	0.0001–0.013	1.36
Meat not included elsewhere	131	0.420	0.22–0.25	10.10
Organ meats except kidney	102	0.140	0.10–0.18	1.44
Muscle meat and poultry combined	40 313	0.134	0.004–0.25	867
Muscle minced	69	0.001	0.001	0.078
Kidney	537	0.067	0.013–0.14	1.24
Muscle poultry	1 589	0.098	0.003–0.021	0.075
Offal	73	0.018	0.006–0.042	0.008
Miscellaneous	9 224	0.035	<LOD–0.20	155
Total	**110 899**	—	—	—

[a] The means were weighted to adjust for different numbers of samples for foods within a category.
[b] Range includes means from the 2007 Chinese TDS and the 20th Australian TDS; maximum lead values were not available from the Chinese TDS and the 20th Australian TDS.
[c] In some cases, cocoas were included in a stimulants category, and in others, they were separately categorized.

Food consumption and dietary exposure assessment

The Committee obtained estimates of exposure to lead based on TDSs for nine countries (Australia, Canada, Chile, China, France, Lebanon, New Zealand, the United Kingdom and the USA) or from other evaluations that had considered levels in foods as consumed (Egypt, India and EFSA). EFSA conducted assessments for 19 European countries, and those are presented together.

The guidelines for conducting exposure assessments for contaminants in foods recommend that dietary exposure estimates should be calculated using regional average contaminant concentration data and the GEMS/Food consumption cluster diets. The WHO GEMS/Food consumption cluster diets contain limited information on the forms of the foods that are considered. Dietary exposure estimates were available to the Committee for 28 countries, mostly based on food as consumed. Lead is taken up from soil into food crops, and the sources of lead in food may also include soil remaining in or on the food, atmospheric deposition, water, contact with lead-containing processing equipment and packaging. It is important to estimate lead levels in food that is as close as possible to the form of the food that is consumed, as levels in raw agricultural commodities do not necessarily reflect levels in foods as they

are consumed. The Committee concluded that the submitted data reflected lead exposures in foods as consumed and were more appropriate than the GEMS/Food consumption cluster diets to use in the lead exposure assessment. Limited information was available describing lead levels in foods or estimating dietary exposures in developing countries.

The Committee included estimates of children's exposure wherever possible. The GEMS/Food consumption cluster diets do not include estimates of children's consumption. Estimates of children's exposure were available for 19 European countries (in the EFSA assessment) and for Australia, Canada, China, New Zealand and the USA. Where exposure assessments were available for the adult population but not for children, the Committee assumed that children's exposure would be 2–3 times that of the general population on a body weight basis, based on the general observation that children consume 2–3 times more food than adults relative to their body weight, and included those values in this report.

Estimates of dietary exposure for individual countries are presented below. Each region/country made its own decisions as to the appropriate matching of food lead levels to food consumption data and also in the treatment of samples without detectable lead levels.

The Committee selected a representative dietary exposure value for each country in order to allow comparisons across countries and across regions for the total/adult population (Table 22) and for children (Table 23). Unfortunately, estimates for the same population subgroup were not always available. In particular, estimates were provided for different age groups by different countries. The Committee selected subgroups that were as similar as possible for comparison purposes. In order to improve comparability, the Committee adjusted some data by standard body weight assumptions. For the total/adult population, mean exposures ranged from 0.02 to 3 μg/kg bw per day (Table 22). Some of the countries also provided estimates of high exposure for consumers. The definition of a consumer with high exposure ranged from the 90th to 97.5th percentile for the population, depending on the country. The estimated high exposures ranged from 0.06 to 2.43 μg/kg bw per day (Table 22). Children's mean exposures ranged from 0.03 to 9 μg/kg bw per day (Table 23). Some countries also provided estimates of high exposures for children. The definition of a consumer with high exposure ranged from the 90th to 97.5th percentile exposures for children. The estimated exposures for children who were defined by the country as consumers with high exposure ranged from 0.2 to 8.2 μg/kg bw per day (Table 23).

Table 22
National lead dietary exposure estimates for total/adult population

Country/region	Population group	Mean exposure (µg/kg bw per day)	High exposure (µg/kg bw per day)
Australia	Adult males 25–34 years	0.06–0.40[a]	—
	Adult females 25–34 years	0.02–0.35[a]	—
Canada	All (2002 study)	0.11[b]	—
Chile	Adults in Santiago	3[c]	—
China	Adults	0.9[d]	1.8 (97.5th)
Egypt	All (exposures measured for selected crops only)	0.74	—
Europe	Adults (individual estimates by country)	0.36–1.24[e]	0.73–2.43 (95th)
India	Adults in Mumbai (Bombay)	0.44[d]	—
Lebanon	All	0.27[f]	—
New Zealand	Adult males	0.13[g]	—
USA	All	0.03[h]	0.06 (90th)

[a] The lower end of the range of reported exposures assumed that results less than the limit of reporting (LOR) are equal to zero, and the upper end of the range assumed that results less than the LOR are the same as the LOR.
[b] LOD/LOQ not provided; mean values were specified for all but a few foods.
[c] Assuming a body weight of 68 kg.
[d] Assuming a body weight of 63 kg.
[e] Range between country with lowest mean exposure and country with highest mean exposure. For lowest mean exposure, values <LOQ = zero (lower-bound approach); for highest mean exposure, values <LOQ = LOQ (upper-bound approach).
[f] Assuming a body weight of 68 kg; foods with concentrations less than the LOQ were assigned a concentration of ½ LOQ.
[g] Concentrations less than the LOD were set to ½ LOD.
[h] Concentrations less than the LOQ were set to zero.

Food category contributions to exposure

The most important contributors to overall dietary exposure were reported by some countries. EFSA evaluated the categories of foods contributing most to exposure and reported large differences between countries. EFSA reported that

> the largest contributors to the calculated overall lead exposure are vegetables, nuts and pulses contributing 19 % to the lower bound and 14 % to the upper bound estimates. Cereals and cereal products contributed 13 % to the lower bound and 14 % to the upper bound. For the lower bound miscellaneous products and food for special uses contributed 12 %, starchy roots and potatoes 8%, meat and meat products 8 %, alcoholic beverages 7 % and milk and dairy

Table 23
National lead dietary exposure estimates for children

Country/region	Age	Mean exposure (µg/kg bw per day)	High exposure (µg/kg bw per day)
Australia	Toddlers 2 years	0.03–0.93[a]	—
Canada	4 years	0.19[b]	—
	2–3 years	0.26[b]	—
Chile	Children	6–9[c]	—
China	2–7 years	3.1	8.2 (97.5th percentile)
Europe	Children 1–3 years	1.10–3.10[d]	1 year 2.1–5.5 (95th percentile)[e] 3 years 1.7–5.2 (95th percentile)
	Children 4–7 years	0.80–2.61[d]	4 years 1.5–4.4 (95th percentile) 7 years 1.4–4.4 (95th percentile)
India	Children	0.9–1.3[c]	—
Lebanon	Children	0.5–0.8[c]	—
New Zealand	Infants	0.34[f]	—
	Children 1–3 years	0.31[f]	—
USA	Infants 6–11 months	0.13[g]	0.3 (90th percentile)
	Children 2 years	0.11[g]	0.2 (90th percentile)

[a] The lower end of the range of reported exposures assumed that results less than the LOR are equal to zero, and the upper end of the range assumed that results less than the LOR are the same as the LOR.
[b] LOD/LOQ not provided; mean values were specified for all but a few foods.
[c] Assuming that children have 2–3 times the adult exposure per unit body weight, respectively.
[d] Means for the country with the lowest exposure and highest exposure. Lowest mean exposure calculated with values less than the LOQ assigned to zero; highest mean exposure calculated with values less than the LOQ set at the LOQ.
[e] Children's high consumer estimates are based on EFSA's combination of estimates from multiple surveys (depending upon the age group; 8–13 surveys were combined).
[f] Concentrations less than the LOD were set to ½ LOD.
[g] Concentrations less than the LOQ were set to zero.

products 6 %. For the upper bound the contributions were: juices, soft drinks and bottled water (11 %), alcoholic beverages (9%) meat and meat products including offal (9 %), milk and dairy products (8 %), miscellaneous products and food for special uses (7 %) and starchy roots and potatoes (6 %).

Milk and milk products, fruits, breads and sugars contributed most to the dietary exposure in a published Chilean TDS. In the 2007 Chinese TDS, the food categories making the largest contributions were cereals (34%) and vegetables (21%). The Lebanese TDS included water and food, water contributing the most to exposure. The foods contributing most to Lebanese exposure were bread and toast, fruits, pizza and pies, and vegetables (raw and

cooked). In the New Zealand TDS, grains contributed 24–27% of dietary lead for adults and 36–39% for children. Chicken, eggs, fish and meat contributed 12–16% of adult dietary lead, and takeaways contributed 9–24%; for children, the corresponding contributions were 7–12% and 10–15%. New Zealand also identified the main food groups contributing to weekly dietary exposure to lead for infants: grains (18%), chicken, eggs, fish and meat (4%), takeaways (6%), fruit (18%) and infant formula and weaning foods (38%).

The relative contribution of diet to total lead exposure is not well known but will probably vary depending upon locale and the contribution from non-dietary sources. Estimates from EFSA suggest that at least half of children's exposure may be due to non-dietary sources of exposure and that soil and dust are major contributors to the non-dietary exposures.

Temporal changes in estimates of dietary exposure to lead since the 1980s

Lead levels in foods have declined over time in many developed countries. The Committee had access to data from five countries (Canada, France, New Zealand, the United Kingdom and the USA) that allowed the trends in lead exposure to be estimated. New Zealand reported changes in dietary exposure to lead since 1982 in its 2003–2004 TDS report. Lead exposure estimates for 19- to 24-year-old males were 3.6 μg/kg bw per day in 1982 and 0.13 μg/kg bw per day in 2003–2004. This represents an apparent decline in exposure to lead of approximately 75%. Dietary exposure estimates for the general population in the United Kingdom declined by approximately 95% between 1980 and 2006, from 0.12 mg/day estimated in the 1980 TDS to 0.006 mg/day in the 2006 TDS. Canada and France have also reported a 50% decline in exposure to lead over the past 10–15 years. The USA reported declines in lead exposure for all age groups, with the greatest decline in teenage males (from 70 μg/day in 1976 to 3.45 μg/day in 2000). During the time periods reported by these countries, there were changes in the food supply that likely contributed to actual declines in dietary exposures. However, some of the apparent decline in exposure may actually be due to improved sensitivity of the analytical methods and the corresponding selection of less conservative values for those samples without detectable levels of lead.

Dose–response analysis

The dose–response modelling for blood lead levels and children's IQ is based on estimates in the Lanphear et al. (*33*) pooled analysis, which includes several newer studies that were not included in the meta-analysis used by the Committee at its fifty-third meeting (Annex 1, reference *143*). The Lanphear et al. (*33*) analysis included 1333 children enrolled in seven longitudinal

Table 24
Estimated dietary lead exposures associated with IQ decreases in children using the combined outputs of the bilinear and Hill models

IQ decrease in children	Dietary exposure (μg/day)[a]	Dietary exposure (μg/kg bw per day) for 20 kg child[a]
0.5	17 (2–194)	0.8 (0.1–9.7)
1	30 (4–208)	1.5 (0.2–10.4)
1.5	40 (5–224)	2.0 (0.3–11.2)
2	48 (7–241)	2.4 (0.4–12.0)
2.5	55 (9–261)	2.8 (0.4–13.1)
3	63 (11–296)	3.1 (0.5–14.8)

[a] Median estimate with 5th–95th percentile CI in parentheses.

cohort studies conducted in the USA, Mexico, Kosovo and Australia, who were followed from birth or early infancy to 5–10 years of age. In this analysis, use of a log-linear model produced an estimated IQ decline of 6.9 points in concurrent blood lead level over a range of 2.4–30 μg/dl. The slope of the inverse association between IQ and concurrent blood lead level was steeper among children with a maximum observed (at any time point) blood lead level below 7.5 μg/dl than it was among children with a maximum blood lead level of 7.5 μg/dl or higher. After initial consideration of six different dose–response models, the bilinear and Hill models were selected for use in characterizing the dose–response relationship between blood lead level and IQ because they provided the best fit.

The relationship between blood lead levels and dietary exposure to lead was estimated to be between 0.052 and 0.16 μg/dl of lead in blood per 1 μg/day of dietary lead exposure. This range was based on toxicokinetic analyses of data on Scottish infants exposed to lead in drinking-water. These analyses were used by the Committee previously.

Dietary exposures associated with a range of decreases in IQ (i.e. 0.5–3 IQ points) were calculated by combining the dose–response models with the toxicokinetic data, using a Monte Carlo simulation. The resulting CIs reflect the uncertainties in both the dose–response modelling of blood lead levels and the extrapolation to dietary exposure. When the outputs from the Monte Carlo simulation of the alternative bilinear and Hill models were combined, the chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 30 μg of lead per day, with a 5th to 95th percentile CI ranging from 4 to 208 μg/day (Table 24). This is equivalent to 1.5 μg/kg bw per day (5th–95th percentiles 0.2–10.4 μg/kg bw per day) for a 20 kg child.

Although the combined outputs of the bilinear and Hill models provide a more complete accounting of the uncertainties associated with the dose–response relationship of lead and IQ, the bilinear model may be more useful in circumstances where other, non-dietary exposures are highly variable or unknown, because the incremental effect of any given lead source/exposure is theoretically independent of other exposures (i.e. the impact of a given dietary exposure will be about the same, regardless of other exposures). Using the bilinear model alone, the chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 12 μg/day, with a 5th–95th percentile CI ranging from 4 to 145 μg/day (Table 25). This is equivalent to 0.6 μg/kg bw per day (5th–95th percentiles 0.2–7.2 μg/kg bw per day) for a 20 kg child. The Committee decided to use the results of the bilinear model in its evaluation because it represents a more conservative approach at low doses and allows non-dietary sources of exposure to be considered independently. However, application of the results of the combined model outputs might be more appropriate in situations where non-dietary exposure is minimal.

Table 25
Estimated dietary lead exposures associated with IQ decrease in children using the bilinear model only

IQ decrease in children	Dietary exposure (μg/day)[a]	Dietary exposure (μg/kg bw per day) for 20 kg child[a]
0.5	6 (2–124)	0.3 (0.1–6.2)
1	12 (4–145)	0.6 (0.2–7.2)
1.5	19 (6–170)	0.9 (0.3–8.5)
2	25 (8–193)	1.3 (0.4–9.7)
2.5	31 (9–217)	1.6 (0.5–10.9)
3	38 (11–237)	1.9 (0.6–11.8)

[a] Median estimate with 5th–95th percentile CI in parentheses.

For adults, increased systolic blood pressure was selected as the most sensitive end-point. A linear slope relating increases in systolic blood pressure as a function of blood lead level was derived by averaging the estimates from four different studies: 0.28 mmHg (0.037 kPa) per 1 μg/dl (5th–95th percentiles 0.03–0.53 mmHg [0.004–0.071 kPa] per 1 μg/dl). Blood lead levels were converted to dietary exposures using the range of values previously used by the Committee for adults (blood lead level of 0.023–0.07 μg/dl per 1 μg/day of dietary lead exposure). Dietary exposure corresponding to an increase in systolic blood pressure of 1 mmHg (0.013 33 kPa) was estimated to be 80 (5th–95th percentiles 34–1700) μg/day, or about 1.3 (5th–95th percentiles 0.6–28) μg/kg bw per day. As the

relationship is linear, the increases in blood pressure associated with other dietary exposures are proportional. Published studies used by WHO in estimating the global burden of disease attributable to lead indicate that relative risks of ischaemic heart disease and cerebrovascular stroke associated with small increases in blood pressure (0.4–3.7 mmHg [0.053–0.49 kPa] systolic blood pressure) have been estimated to be in the range of 1.01–1.4, with higher relative risks at younger ages.

Evaluation

Exposure to lead is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes. Impaired neurodevelopment in children is generally associated with lower blood lead concentrations than the other effects, the weight of evidence is greater for neurodevelopmental effects than for other health effects and the results across studies are more consistent than those for other effects. For adults, the adverse effect associated with lowest blood lead concentrations for which the weight of evidence is greatest and most consistent is a lead-associated increase in systolic blood pressure. Therefore, the Committee concluded that the effects on neurodevelopment and systolic blood pressure provided the appropriate bases for dose–response analyses.

Based on the dose–response analyses, the Committee estimated that the previously established PTWI of 25 μg/kg bw is associated with a decrease of at least 3 IQ points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults. These changes are important when viewed as a shift in the distribution of IQ or blood pressure within a population. The Committee therefore concluded that the PTWI could no longer be considered health protective, and it was withdrawn.

Because the dose–response analyses do not provide any indication of a threshold for the key effects of lead, the Committee concluded that it was not possible to establish a new PTWI that would be considered to be health protective. The dose–response analyses conducted by the Committee should be used to identify the magnitude of effect associated with identified levels of dietary lead exposure in different populations.

The Committee reaffirmed that because of the neurodevelopmental effects, fetuses, infants and children are the subgroups that are most sensitive to lead. The mean dietary exposure estimates for children aged about 1–4 years range from 0.03 to 9 μg/kg bw per day. The health impact at the lower end of this range is considered negligible by the Committee, because it is below the exposure level of 0.3 μg/kg bw per day calculated to be associated with a

population decrease of 0.5 IQ point. The higher end of the exposure range is higher than the level of 1.9 μg/kg bw per day calculated to be associated with a population decrease of 3 IQ points, which is deemed by the Committee to be a concern. For adults, the mean dietary lead exposure estimates range from 0.02 to 3 μg/kg bw per day. The lower end of this range (0.02 μg/kg bw per day) is considerably below the exposure level of 1.2 μg/kg bw per day calculated by the Committee to be associated with a population increase in systolic blood pressure of 1 mmHg (0.1333 kPa). The Committee considered that any health risk that would be expected to occur at this exposure level is negligible. At the higher end of the range (3 μg/kg bw per day), a population increase of approximately 2 mmHg (0.3 kPa) in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated, in a large meta-analysis, with modest increases in the risks of ischaemic heart disease and cerebrovascular stroke. The Committee considered this to be of some concern, but less than that for the neurodevelopmental effects observed in children.

The Committee stressed that these estimates are based on dietary exposure (mainly food) and that other sources of exposure to lead also need to be considered.

The Committee concluded that, in populations with prolonged dietary exposures to lead that are at the higher end of the ranges identified above, measures should be taken to identify major contributing sources and foods and, if appropriate, to identify methods of reducing dietary exposure that are commensurate with the level of risk reduction.

A detailed monograph addendum was prepared.

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Annex 1

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Annex 2

Tolerable intakes, other toxicological information and information on specifications

Food additives considered for specifications only

Food additive	Specifications[a]
Activated carbon	R
Annatto extract (oil-processed bixin)	W
Cassia gum	R
Indigotine	R
Steviol glycosides	R
Sucrose esters of fatty acids	R
Sucrose monoesters of lauric, palmitic or stearic acid	N, T
Titanium dioxide	R

[a] N, new specifications; R, existing specifications revised; T, tentative specifications; W, existing specifications withdrawn.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents[1]

A. Alicyclic ketones, secondary alcohols and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Cyclohexanone diethyl ketal	2051	N	No safety concern
3,3,5-Trimethylcyclohexyl acetate	2053	N	No safety concern
Structural class II			
2-(*trans*-2-Pentenyl)cyclopentanone	2049	N	No safety concern
2-Cyclopentylcyclopentanone	2050	N	No safety concern

[1] The flavouring agent 2-aminoacetophenone (No. 2043) was on the agenda to be evaluated in the group of aromatic substituted secondary alcohols, ketones and related esters. Although the compound fulfils some of the structural requirements for this group, the main toxicologically relevant structural feature is the amino group; hence, the compound was not evaluated and should be evaluated in the future in the group of aliphatic and aromatic amines and amides. The flavouring agent (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) was on the agenda to be evaluated in the group of benzyl derivatives. However, as this compound did not meet the structural requirements for this group, the compound was not evaluated at this meeting.

(continued)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
2-Cyclohexenone	2052	N	No safety concern
2,6,6-Trimethyl-2-hydroxycyclohexanone	2054	N	No safety concern
Cyclotene propionate	2055	N	No safety concern
Cyclotene butyrate	2056	N	No safety concern
4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (mixture of isomers)	2057	N	No safety concern
4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (mixture of isomers)	2058	N	No safety concern
Structural class III			
(–)-8,9-Dehydrotheaspirone	2059	N	No safety concern
(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	2060	N	No safety concern

[a] N, new specifications.

B. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
cis-4-(2,2,3-Trimethylcyclopentyl)-butanoic acid	1899	N	No safety concern
Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	1900	N	No safety concern
(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	1902	N	No safety concern
d-Limonen-10-ol	1903	N	No safety concern
p-Menthan-7-ol	1904	N	No safety concern
p-Menth-1-en-9-ol	1905	N	No safety concern
1,3-*p*-Menthadien-7-al	1906	N	No safety concern
Structural class II			
Methyl dihydrojasmonate	1898	N	No safety concern
cis- and *trans*-2-Heptylcyclopropanecarboxylic acid	1907	N	No safety concern
(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl)cyclopropanecarbaldehyde	1908	N	No safety concern
Structural class III		N	
Perillaldehyde propyleneglycol acetal	1901	N	No safety concern

[a] N, new specifications.

C. Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
3-Methyl-2,4-nonedione	2032	N	No safety concern
Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	2034	N	No safety concern
3-Hydroxy-2-octanone	2035	N	No safety concern
2,3-Octanedione	2036	N	No safety concern
4,5-Octanedione	2037	N	No safety concern
(±)-2-Hydroxypiperitone	2038	N	No safety concern
Structural class III			
Acetoin propyleneglycol ketal	2033	N	No safety concern
1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	2039	N	No safety concern

[a] N, new specifications.

D. Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Dimethylbenzyl carbinyl crotonate	2025	N	No safety concern
Dimethylbenzyl carbinyl hexanoate	2026	N	No safety concern
Caryophyllene alcohol	2027	N	No safety concern
Cubebol	2028	N	No safety concern
(–)-Sclareol	2029	N	No safety concern
(+)-Cedrol	2030	N	No safety concern
α-Bisabolol	2031	N	No safety concern

[a] N, new specifications.

E. Aliphatic and aromatic amines and amides

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Choline chloride	2003	N	No safety concern
3-(Methylthio)propylamine	2004	N	No safety concern

(continued)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class III			
N-Ethyl-2,2-diisopropylbutanamide	2005	N	Additional data required to complete evaluation
Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	2006	N	No safety concern
(±)-*N*-Lactoyl tyramine	2007	N	Additional data required to complete evaluation
N-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	2008	N	No safety concern
N-*p*-Benzeneacetonitrile menthanecarboxamide	2009	N	No safety concern
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	N	Additional data required to complete evaluation
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	N	Additional data required to complete evaluation

[a] N, new specifications.

F. Aliphatic lactones

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
5-Pentyl-3H-furan-2-one	1989	N	No safety concern
5-Hydroxy-4-methylhexanoic acid δ-lactone	1990	N	No safety concern
Isoambrettolide	1991	N	No safety concern
7-Decen-4-olide	1992	N	No safety concern
9-Decen-5-olide	1993	N	No safety concern
8-Decen-5-olide	1994	N	No safety concern
Orin lactone	1995	N	No safety concern
9-Dodecen-5-olide	1996	N	No safety concern
9-Tetradecen-5-olide	1997	N	No safety concern
γ-Octadecalactone	1998	N	No safety concern
δ-Octadecalactone	1999	N	No safety concern
Structural class III			
4-Hydroxy-2-butenoic acid γ-lactone	2000	N	No safety concern
2-Nonenoic acid γ-lactone	2001	N	No safety concern
4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	2002	N	No safety concern

[a] N, new specifications.

G. Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Hydroxyacetone	1945	N	No safety concern
Propyl pyruvate	1946	N	No safety concern
Methyl 3-hydroxybutyrate	1947	N	No safety concern
Dodecyl lactate	1948	N	No safety concern
(±)-Ethyl 3-hydroxy-2-methylbutyrate	1949	N	No safety concern
Hexadecyl lactate	1950	N	No safety concern
Methyl 3-acetoxy-2-methylbutyrate	1951	N	No safety concern
1-Hydroxy-4-methyl-2-pentanone	1952	N	No safety concern
Ethyl 2-acetylhexanoate	1953	N	No safety concern
3-Isopropenyl-6-oxoheptanoic acid	1954	N	No safety concern
Ethyl 3-hydroxyoctanoate	1955	N	No safety concern
Methyl 3-acetoxyoctanoate	1956	N	No safety concern
5-Oxooctanoic acid	1957	N	No safety concern
Ethyl 2-acetyloctanoate	1958	N	No safety concern
Ethyl 5-acetoxyoctanoate	1959	N	No safety concern
5-Oxodecanoic acid	1960	N	No safety concern
Ethyl 5-oxodecanoate	1961	N	No safety concern
Ethyl 5-hydroxydecanoate	1962	N	No safety concern
5-Oxododecanoic acid	1963	N	No safety concern
Dimethyl adipate	1964	N	No safety concern
Dipropyl adipate	1965	N	No safety concern
Diisopropyl adipate	1966	N	No safety concern
Diisobutyl adipate	1967	N	No safety concern
Dioctyl adipate	1968	N	No safety concern
Methyl levulinate	1970	N	No safety concern
Propyl levulinate	1971	N	No safety concern
Isoamyl levulinate	1972	N	No safety concern
cis-3-Hexenyl acetoacetate	1974	N	No safety concern
Propyleneglycol diacetate	1976	N	No safety concern
Mixture of 6-(5-Decenoyloxy)-decanoic acid and 6-(6-Decenoyloxy)decanoic acid	1977	N	No safety concern
Propyleneglycol dipropionate	1978	N	No safety concern
Propyleneglycol monobutyrate (mixture of isomers)	1979	N	No safety concern
Propyleneglycol dibutyrate	1980	N	No safety concern
Propyleneglycol mono-2-methylbutyrate (mixture of isomers)	1981	N	No safety concern
Propyleneglycol di-2-methylbutyrate	1982	N	No safety concern
Propyleneglycol monohexanoate (mixture of isomers)	1983	N	No safety concern
Propyleneglycol dihexanoate	1984	N	No safety concern

(continued)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Propyleneglycol dioctanoate	1985	N	No safety concern
2-Oxo-3-ethyl-4-butanolide	1986	N	No safety concern
Ethyl 5-hydroxyoctanoate	1987	N	No safety concern
Structural class III			
Ethyl acetoacetate ethyleneglycol ketal	1969	N	No safety concern
Ethyl levulinate propyleneglycol ketal	1973	N	Additional data required to complete evaluation
Hydroxycitronellal propyleneglycol acetal	1975	N	No safety concern
Mixture of Isopropylideneglyceryl 5-hydroxyoctanoate and δ-Decalactone (No. 232)	1988	N	Additional data required to complete evaluation

[a] N, new specifications.

H. Aliphatic secondary alcohols, ketones and related esters and acetals

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
(±)-Octan-3-yl formate	2070	N	No safety concern
2-Pentyl 2-methylpentanoate	2072	N	No safety concern
3-Octyl butyrate	2073	N	No safety concern
Structural class II			
(*R*)-(–)-1-Octen-3-ol	2071	N	No safety concern
2-Decanone	2074	N	No safety concern
Structural class III			
6-Methyl-5-hepten-2-one propyleneglycol acetal	2075	N	No safety concern
2-Nonanone propyleneglycol acetal	2076	N	No safety concern

[a] N, new specifications.

I. Aromatic substituted secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
4-Hydroxyacetophenone	2040	N	No safety concern
3-Hydroxy-4-phenylbutan-2-one	2041	N	No safety concern
2-Methoxyacetophenone	2042	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
2-Methylacetophenone	2044	N	No safety concern
2-Hydroxy-5-methylacetophenone	2045	N	No safety concern
Dihydrogalangal acetate	2046	N	Additional data required to complete evaluation
2,3,3-Trimethylindan-1-one	2047	N	No safety concern
Structural class III			
4-(3,4-Methylenedioxyphenyl)-2-butanone	2048	N	No safety concern

[a] N, new specifications.

J. Benzyl derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Benzyl hexanoate	2061	N	No safety concern
o-Anisaldehyde	2062	N	No safety concern
Prenyl benzoate	2063	N	No safety concern
Benzyl levulinate	2064	N	No safety concern
4-Methylbenzyl alcohol	2065	N	No safety concern
Benzyl nonanoate	2066	N	No safety concern
Structural class II			
2-Ethylhexyl benzoate	2068	N	No safety concern
Structural class III			
4-Methylbenzaldehyde propyleneglycol acetal	2067	N	No safety concern

[a] N, new specifications.

K. Phenol and phenol derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
4-Propenylphenol	2012	N	No safety concern
2,4,6-Trimethylphenol	2013	N	No safety concern
Sodium 3-methoxy-4-hydroxycinnamate	2014	N	No safety concern
Guaicol butyrate	2015	N	No safety concern
Guaicol isobutyrate	2016	N	No safety concern

(continued)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Guaicol propionate	2017	N	No safety concern
4-(2-Propenyl)phenyl-β-D-glucopyranoside	2018	N	No safety concern
Phenyl butyrate	2019	N	No safety concern
Hydroxy(4-hydroxy-3-methoxyphenyl)acetic acid	2020	N	No safety concern
Structural class II			
1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	2021	N	No safety concern
Structural class III			
3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	2022	N	No safety concern
Magnolol	2023	N	No safety concern
5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	2024	N	No safety concern

[a] N, new specifications.

L. Simple aliphatic and aromatic sulfides and thiols

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Subgroup i: Simple sulfides			
Structural class I			
Methyl octyl sulfide	1909	N	No safety concern
Methyl 1-propenyl sulfide	1910	N	No safety concern
Di-(1-propenyl)-sulfide (mixture of isomers)	1911	N	No safety concern
Structural class III			
Butanal dibenzyl thioacetal	1939	N	Additional data required to complete evaluation
Subgroup ii: Acyclic sulfides with oxidized side-chains			
Structural class I			
Ethyl 2-hydroxyethyl sulfide	1912	N	No safety concern
2-(Methylthio)ethyl acetate	1913	N	No safety concern
Ethyl 3-(methylthio)-(2*Z*)-propenoate	1915	N	No safety concern
Ethyl 3-(methylthio)-(2*E*)-propenoate	1916	N	No safety concern
Ethyl 3-(methylthio)-2-propenoate (mixture of isomers)	1917	N	No safety concern
4-Methyl-2-(methylthiomethyl)-2-pentenal	1918	N	No safety concern
4-Methyl-2-(methylthiomethyl)-2-hexenal	1919	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
5-Methyl-2-(methylthiomethyl)-2-hexenal	1920	N	No safety concern
Butyl β-(methylthio)acrylate	1921	N	No safety concern
Ethyl 3-(ethylthio)butyrate	1922	N	No safety concern
Methional diethyl acetal	1940	N	No safety concern
3-(Methylthio)propyl hexanoate	1941	N	Additional data required to complete evaluation
Structural class III			
1-(3-(Methylthio)-butyryl)-2,6,6-trimethylcyclohexene	1942	N	No safety concern
Subgroup iii: Cyclic sulfides			
Structural class II			
2-Oxothiolane	1923	N	No safety concern
Structural class III			
(±)-*cis*- and *trans*-2-Pentyl-4-propyl-1,3-oxathiane	1943	N	Additional data required to complete evaluation
2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	1944	N	Additional data required to complete evaluation
Subgroup iv: Simple thiols			
Structural class I			
Dodecanethiol	1924	N	No safety concern
Subgroup v: Thiols with oxidized side-chains			
Structural class I			
2-Hydroxyethanethiol	1925	N	No safety concern
4-Mercapto-4-methyl-2-hexanone	1926	N	No safety concern
3-Mercapto-3-methylbutyl isovalerate	1927	N	No safety concern
(±)-Ethyl 3-mercapto-2-methylbutanoate	1928	N	No safety concern
3-Mercaptohexanal	1929	N	No safety concern
3-Mercaptopropionic acid	1936	N	No safety concern
2-Ethylhexyl 3-mercaptopropionate	1938	N	No safety concern
Structural class III			
3-(Methylthio)propyl mercaptoacetate	1914	N	Additional data required to complete evaluation
Subgroup vii: Simple disulfides			
Structural class I			
Diisoamyl disulfide	1930	N	No safety concern
Butyl propyl disulfide	1932	N	No safety concern
di-*sec*-Butyl disulfide	1933	N	No safety concern
Structural class III			
Bis(2-methylphenyl) disulfide	1931	N	Additional data required to complete evaluation
Methyl 2-methylphenyl disulfide	1935	N	No safety concern

(continued)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Subgroup ix: Trisulfides			
Structural class I			
Diisoamyl trisulfide	1934	N	No safety concern
Subgroup xi: Thioesters			
Structural class I			
Methyl isobutanethioate	1937	N	No safety concern

[a] N, new specifications.

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
439	4-Carvomenthenol	R
952	5,6,7,8-Tetrahydroquinoxaline	R

[a] R, revised specifications.

Contaminants evaluated toxicologically

Cadmium

Since cadmium was last considered by the Committee, there have been a number of new epidemiological studies that have reported cadmium-related biomarkers in urine following environmental exposure. The Committee noted that a large meta-analysis of studies that measured the dose–response relationship between the excretion of β_2-microglobulin and cadmium in urine was available. As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved after 45–60 years of exposure. Therefore, data relating β_2-microglobulin excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older provided the most reliable basis on which to determine a critical concentration of cadmium in the urine. An analysis of the group mean data from individuals who were 50 years of age and older showed that the urinary excretion of less than 5.24 (confidence interval 4.94–5.57) μg of cadmium per gram creatinine was not associated with an increased excretion of β_2-microglobulin. Higher urinary cadmium levels were associated with a steep increase in β_2-microglobulin excretion.

To determine a corresponding dietary exposure that would result in a urinary cadmium concentration at the breakpoint of 5.24 (confidence interval 4.94–5.57) μg of cadmium per gram creatinine, a one-compartment

toxicokinetic model was used. The lower bound of the 5th population percentile dietary cadmium exposure that equates to the breakpoint was estimated to be 0.8 µg/kg body weight per day or 25 µg/kg body weight per month.

The Committee noted that the existing health-based guidance value for cadmium was expressed on a weekly basis (provisional tolerable weekly intake, or PTWI), but, owing to cadmium's exceptionally long half-life, considered that a monthly value was more appropriate. **The Committee therefore withdrew the PTWI of 7 µg/kg body weight.**

In view of the long half-life of cadmium, daily ingestion in food has a small or even a negligible effect on overall exposure. In order to assess long- or short-term risks to health due to cadmium exposure, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a provisional tolerable monthly intake (PTMI). **The Committee established a PTMI of 25 µg/kg body weight.**

The estimates of exposure to cadmium through the diet for all age groups, including consumers with high exposure and subgroups with special dietary habits (e.g. vegetarians), examined by the Committee at this meeting are below the PTMI.

Lead

Exposure to lead is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes. For children, impaired neurodevelopment is generally associated with lower blood lead concentrations than the other effects, the weight of evidence is greater for neurodevelopmental effects than for other health effects, and the results across studies are more consistent than those for other effects. For adults, the adverse effect associated with lowest blood lead concentrations for which the weight of evidence is greatest and most consistent is a lead-associated increase in systolic blood pressure. Therefore, the Committee concluded that the effects on neurodevelopment and increase in systolic blood pressure provided the appropriate bases for dose–response analyses.

Based on the dose–response analyses, the Committee estimated that the previously established PTWI of 25 µg/kg body weight is associated with a decrease of at least 3 intelligence quotient (IQ) points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults. These changes are important when viewed as a shift in the distribution

of IQ or blood pressure within a population. **The Committee therefore concluded that the PTWI could no longer be considered health protective and withdrew it.**

Because the dose–response analyses do not provide any indication of a threshold for the key effects of lead, the Committee concluded that it was not possible to establish a new PTWI that would be considered to be health protective. The dose–response analyses conducted by the Committee should be used to identify the magnitude of effect associated with identified levels of dietary lead exposure in different populations.

The Committee reaffirmed that because of the neurodevelopmental effects, fetuses, infants and children are the subgroups that are most sensitive to lead. The mean dietary exposure estimates of children aged about 1–4 years range from 0.03 to 9 μg/kg body weight per day. The health impact at the lower end of this range (0.03 μg/kg body weight per day) is considered negligible by the Committee, because it is below the exposure level of 0.3 μg/kg body weight per day calculated to be associated with a population decrease of 0.5 IQ points. The higher end of the exposure range (9 μg/kg body weight per day) is higher than the level of 1.9 μg/kg body weight per day calculated to be associated with a population decrease of 3 IQ points, which is deemed by the Committee to be a concern. For adults, the mean dietary lead exposure estimates range from 0.02 to 3.0 μg/kg body weight per day. The lower end of this range (0.02 μg/kg body weight per day) is considerably below the exposure level of 1.2 μg/kg body weight per day calculated by the Committee to be associated with a population increase in systolic blood pressure of 1 mmHg (0.1 kPa). The Committee considered that any health risk that would be expected to occur at this exposure level is negligible. At the higher end of the range (3.0 μg/kg body weight per day), a population increase of approximately 2 mmHg (0.3 kPa) in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated, in a large meta-analysis, with modest increases in the risks of ischaemic heart disease and cerebrovascular stroke. The Committee considered this to be of some concern, but less than that for the neurodevelopmental effects observed in children.

The Committee stressed that these estimates are based on dietary exposure (mainly food) and that other sources of exposure to lead also need to be considered.

The Committee concluded that, in populations with prolonged dietary exposures to lead that are in the higher end of the ranges identified above, measures should be taken to identify major contributing sources and foods and, if appropriate, to identify methods of reducing dietary exposure that are commensurate with the level of risk reduction.

Annex 3

Further information required or desired

β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester and β-carotene (synthetic)

The revision of the specifications monographs of β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester and β-carotene (synthetic) was deferred to a future meeting, pending submission of the data necessary for revision of purity tests for carotenoids and subsidiary colouring matter.

Sucrose monoesters of lauric, palmitic or stearic acid

A test method capable of distinguishing sucrose monoesters of lauric, palmitic or stearic acid from sucrose esters of fatty acids is needed. The tentative specifications for sucrose monoesters of lauric, palmitic or stearic acid will be withdrawn if the requested data are not received by the end of 2011.

Additional data required to complete the evaluation according to the Procedure for the Safety Evaluation of Flavouring Agents

Additional data are required to complete the toxicological evaluations of 13 flavouring agents (Nos 1914, 1931, 1939, 1941, 1943, 1944, 1973, 1988, 2005, 2007, 2010, 2011 and 2046).

HPLC methods for subsidiary dyes and isomers in food colours

The Committee noted the need for high-performance liquid chromatographic (HPLC) methods for the separation and quantification of subsidiary dyes and isomers in food colours to replace the paper chromatographic method in Volume 4 of the *Combined Compendium of Food Additive Specifications* (FAO JECFA Monographs 1, 2006) (Annex 1, reference *180*). To this end, producers of food colours, industries and organizations are encouraged to notify the FAO JECFA Secretariat of the availability of appropriate methods.

Annex 4

Summary of the safety evaluation of the secondary components for flavouring agents with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Alicyclic ketones, secondary alcohols and related esters				
2053	3,3,5-Trimethylcyclohexyl acetate	90	6–7% 3,3,5-trimethylcyclohexanol	3,3,5-Trimethylcyclohexanol (No. 1099) was evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*) and found to be of no safety concern at estimated dietary exposures as a flavouring agent.
2055	Cyclotene propionate	92	4–5% cyclotene	Cyclotene (No. 418) was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and was concluded to be of no safety concern at estimated dietary exposures as a flavouring agent.
Alicyclic primary alcohols, aldehydes, acids and related esters				
1898	Methyl dihydrojasmonate	85	9–11% methyl epi-dihydrojasmonate	Methyl epi-dihydrojasmonate is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1901	Perillaldehyde propyleneglycol acetal	91	3–4% perillaldehyde; 2–3% propylene glycol	Perillaldehyde (No. 973) and propylene glycol are metabolites of the primary substance and are considered not to present a safety concern at current estimated dietary exposures.
1902	(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	80	10–11% *trans*-4-isopropyl-cyclohexane-1-carboxaldehyde; 4–5% *cis*-4-isopropyl-	*Trans*-4-Isopropyl-cyclohexane-1-carboxaldehyde, *cis*-4-isopropyl-cyclohexane-1-carboxaldehyde and 4-isopropenyl-cyclohex-1-enecarboxaldehyde are expected to share the same metabolic fate as the primary substance, i.e. oxidation of the aldehyde to the carboxylic acid,

			cyclohexane-1-carboxaldehyde; 1–2% 4-isopropenyl-cyclohex-1-enecarboxaldehyde	followed by glucuronic acid conjugation. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
1906	1,3-*p*-Menthadien-7-al	91	5–6% cumin aldehyde	Cumin aldehyde (No. 868) was evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.
1908	(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl) cyclopropanecarbaldehyde	90	5–10% [2-methyl-2-(4-methylpent-3-en-1-yl)-cyclopropyl]methanol	[2-Methyl-2-(4-methylpent-3-en-1-yl)cyclopropyl]-methanol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. oxidation to the carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances				
2027	Caryophyllene alcohol	92	3–6% dihydrocloven-9-ol	Dihydrocloven-9-ol is expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
2031	α-Bisabolol	93	1–2% β-bisabolol	β-Bisabolol is expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(continued)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Aliphatic and aromatic amines and amides				
2007	(±)-*N*-Lactoyl tyramine	90	2–3% lactic acid; 2–3% ethyl lactate	Lactic acid (No. 930) and ethyl lactate (No. 931) were evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and were concluded to be of no safety concern at estimated dietary exposures as flavouring agents.
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	94	2–5% *N*-*p*-benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*); neo-isomer	*N*-*p*-Benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*) is expected to share the same metabolic fate as the primary substance, i.e. oxidation followed by elimination. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic lactones				
2002	4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	93	1–2% 3,4-dimethyl 5-ketobutanoic acid γ-lactone	3,4-Dimethyl 5-ketobutanoic acid γ-lactone is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups				
1948	Dodecyl lactate	88	10% dodecanol	Dodecanol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

1950	Hexadecyl lactate	88	15% 1-hexadecanol	1-Hexadecanol (No. 114) was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.
1962	Ethyl 5-hydroxydecanoate	56	40–42% δ-decalactone	δ-Decalactone (No. 232) was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.
1974	*cis*-3-Hexenyl acetoacetate	93	2–3% *cis*-3-hexenol	*cis*-3-Hexenol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1979	Propyleneglycol monobutyrate	88	6–10% propyleneglycol dibutyrate	Propyleneglycol dibutyrate (No. 1980) was evaluated at the current meeting and was considered not to present a safety concern at current estimated dietary exposures to the flavouring agent.
1987	Ethyl 5-hydroxyoctanoate	50	5–6% ethanol; 17–18% 1,5-octanolide; 21–24% 5-hydroxydecanoic acid and ethyl-5-hydroxyoctanoate ester	Ethanol (No. 41) was evaluated by the Committee at its forty-sixth meeting (Annex 1, reference *122*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent. 1,5-Octanolide, 5-hydroxydecanoic acid and ethyl-5-hydroxyoctanoate ester are metabolites of the primary substance and expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.

(continued)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1988	Mixture of Isopropylideneglyceryl 5-hydroxydecanoate and δ-Decalactone (No. 232)	73	The mixture contains 25% isopropylideneglyceryl 5-hydroxydecanoate and 47–49% δ-decalactone (No. 232); other components are 22–24% 2,2-dimethyl-1,3-dioxolane-4-methanol and 1–5% 2-propyl 5-hydroxydecanoate	Isopropylideneglyceryl 5-hydroxydecanoate, δ-decalactone (No. 232), 2,2-dimethyl-1,3-dioxolane-4-methanol and 2-propyl 5-hydroxydecanoate are expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic secondary alcohols, ketones and related esters and acetals				
2075	6-Methyl-5-hepten-2-one propyleneglycol acetal	88	7–9% 6-methyl-6-hepten-2-one propyleneglycol acetal	6-Methyl-6-hepten-2-one propyleneglycol acetal is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding ketone and diol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aromatic substituted secondary alcohols, ketones and related esters				
2041	3-Hydroxy-4-phenylbutan-2-one	93	3–5% 4-hydroxy-4-phenylbutan-2-one	4-Hydroxy-4-phenylbutan-2-one is expected to share the same metabolic fate as the primary substance, i.e. reduction of the ketone to the corresponding secondary alcohol, followed by formation of the glucuronic acid conjugate and elimination in the urine.

				It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Phenol and phenol derivatives				
2014	Sodium 3-methoxy-4-hydroxycinnamate	93	2–5% vanillin	An ADI of 0–10 mg/kg bw was established for vanillin by the Committee at its eleventh meeting (Annex, reference *14*). At the fifty-seventh meeting of the Committee, when vanillin (No. 889) was evaluated using the Procedure, vanillin was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent, and the ADI was maintained (Annex 1, reference *154*).
2023	Magnolol	92	3–7% honokiol; 1–2% eudesmol	Honokiol and eudesmol are expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
Simple aliphatic and aromatic sulfides and thiols				
1915	Ethyl 3-(methylthio)-(2*Z*)-propenoate	88	7–9% ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916)	Ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) is expected to share the same metabolic fate as the primary substance, i.e. oxidation of the sulfur to the corresponding sulfoxide or sulfone in addition to ester hydrolysis to the corresponding alcohol and carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(continued)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1916	Ethyl 3-(methylthio)-(2*E*)-propenoate	81	14–16% ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915)	Ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915) is expected to share the same metabolic fate as the primary substance, i.e. oxidation of the sulfur to the corresponding sulfoxide or sulfone in addition to ester hydrolysis to the corresponding alcohol and carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1932	Butyl propyl disulfide	51	24–25% dipropyl disulfide; 23–24% dibutyl disulfide	Dipropyl disulfide and dibutyl disulfide are expected to share the same metabolic fate as the primary substance, i.e. reduction of the disulfide, followed by formation of mixed disulfides with glutathione and cysteine. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
1944	2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	88	5–8% 2-[(2*E*)-pent-2-en-1-yl]-4-propyl-1,3-oxathiane; 2–3% 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane	2-[(2*E*)-Pent-2-en-1-yl]-4-propyl-1,3-oxathiane and 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane are expected to share the same metabolic fate as the primary substance, i.e. oxidization to the sulfoxide and sulfone and hydrolysis to the thioalcohol, which may undergo further oxidation, alkylation or conjugation. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.

Annex 5

Food categories and standard portion sizes to be used in the additional method for making estimates of dietary exposure to flavouring agents

Table A1 contains the food categories and the standard portion sizes (expressed as consumed) to be used in the additional method for making estimates of dietary exposure to flavouring agents. The complete classification can be found online at http://www.codexalimentarius.net/gsfaonline/foods/index.html. The portion sizes were derived from "Reference amounts customarily consumed per eating occasion" in Title 21 of the United States Code of Federal Regulations, Part 101.12(b) (http://cfr.vlex.com/vid/customarily-consumed-eating-occasion-19705320). If specific information were available to indicate that a flavouring agent would be used only in a more refined subcategory, an appropriate estimate of a portion size for that subcategory could be provided by the industry in place of the value for the broader category.

Table A1
Food categorization system for the General Standard for Food Additives (first sublevel only) with standard portion sizes

Food category	Standard portion sizes (g)
01.0 Dairy products, excluding products of category 02.0	
01.1 Milk and dairy-based drinks	200
01.2 Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200
01.3 Condensed milk and analogues	NF
01.4 Cream (plain) and the like	NF
01.5 Milk powder and cream powder and powder analogues (plain)	NF
01.6 Cheese and analogues	40
01.7 Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	125
01.8 Whey and whey products, excluding whey cheese	NF
02.0 Fats and oils and fat emulsions	
02.1 Fats and oils essentially free from water	15

Table A1 (continued)

Food category	Standard portion sizes (g)
02.2 Fat emulsions mainly of type water-in-oil	15
02.3 Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15
02.4 Fat-based desserts excluding dairy-based dessert products of category 01.7	50
03.0 Edible ices, including sherbet and sorbet	50
04.0 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes and aloe vera), seaweeds, and nuts and seeds	
04.1 Fruit	
04.1.2 Processed fruit	125
04.2 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes and aloe vera), seaweeds, and nuts and seeds	
04.2.2 Processed vegetables and nuts and seeds	200
05.0 Confectionery	
05.1 Cocoa products and chocolate products, including imitations and chocolate substitutes	40
05.2 Confectionery including hard and soft candy and nougats etc. other than 5.1, 5.3 and 5.4	30
05.3 Chewing gum	3
05.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35
06.0 Cereals and cereal products derived from cereal grains, from roots and tubers, and pulses and legumes, excluding bakery wares of food category 07.0	
06.1 Whole, broken or flaked grain, including rice	NF
06.2 Flours and starches (including soybean powder)	NF
06.3 Breakfast cereals, including rolled oats	30
06.4 Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soybean pasta and noodles)	200
06.5 Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)	200
06.6 Batters (e.g. for breading or batters for fish or poultry)	30
06.7 Pre-cooked or processed rice products, including rice cakes (Oriental type only)	200
06.8 Soybean products (excluding soybean products of food category 12.9 and fermented soybean products of food category 12.10)	100
07.0 Bakery wares	
07.1 Bread and ordinary bakery wares	50
07.2 Fine bakery wares (sweet, salty, savoury) and mixed	80
08.0 Meat and meat products, including poultry and game	
08.1 Fresh meat, poultry and game	NF
08.2 Processed meat, poultry and game products in whole pieces or cuts	100
08.3 Processed comminuted meat, poultry and game products	100

Food category	Standard portion sizes (g)
08.4 Edible casings (e.g. sausage casings)	NF
09.0 Fish and fish products, including molluscs, crustaceans and echinoderms	
09.1 Fresh fish and fish products, including molluscs, crustaceans and echinoderms	
09.1.1 Fresh fish	NF
09.1.2 Fresh molluscs, crustaceans and echinoderms	NF
09.2 Processed fish and fish products, including molluscs, crustaceans and echinoderms	100
09.3 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	100
09.4 Fully preserved, including canned or fermented fish and fish products, including molluscs, crustaceans and echinoderms	100
10.0 Eggs and egg products	
10.1 Fresh eggs	NF
10.2 Egg products	100
10 3 Preserved eggs, including alkaline, salted and canned eggs	100
10.4 Egg-based desserts (e.g. custard)	125
11.0 Sweeteners, including honey	
11.1 Refined and raw sugar	10
11.2 Brown sugar excluding products of food category 11.1.3	10
11.3 Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle excluding products of food category 11.1.3	30
11.4 Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30
11.5 Honey	15
11.6 Table-top sweeteners, including those containing high-intensity sweeteners	15
12.0 Salts, spices, soups, sauces, salads, protein products (including soybean protein products) and fermented soybean products	
12.1 Salt and salt substitutes	NF
12.2 Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1
12.3 Vinegars	15
12.4 Mustards	15
12.5 Soups and broths	200
12.6 Sauces and like products	30
12.7 Salads (e.g. macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories	120 / 20*
12.8 Yeast and like products	NF
12.9 Protein products	15
12.10 Fermented soybean products	40

Table A1 (continued)

Food category	Standard portion sizes (g)
13.0 Foodstuffs intended for particular nutritional uses	
13.1 Infant formulae and follow-on formulae, and formulae for special medical purposes for infants	NC
13.2 Complementary foods for infants and young children	NC
13.3 Dietetic foods intended for special medical purposes	NC
13.4 Dietetic formulae for slimming purposes and weight reduction	NC
13.5 Dietetic foods other than 13.1–13.4	NC
13.6 Food supplements	5
14.0 Beverages, excluding dairy products	
14.1 Non-alcoholic ("soft") beverages	300
14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	
14.2.1 Beer and malt beverages	300
14.2.3 Grape wines	150
14.2.5 Mead	
14.2.6 Spirituous beverages	30
15.0 Ready-to-eat savouries	
15.1 Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30
15.2 Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)	30
15.3 Snacks – fish based	30
16.0 Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	**NF**

* 120 for salads and 20 for spreads.

NF, Not flavoured; appears in those categories that would not be expected to contain any flavouring agent.

NC, Not considered; appears in those categories that would not be considered in an assessment of dietary exposure to flavour.

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various flavouring agents, with a view to concluding as to safety concerns and to preparing specifications for identity and purity. The Committee also evaluated the risk posed by two food contaminants, with the aim of deriving tolerable intakes where appropriate and advising on risk management options for the purpose of public health protection.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives (particularly flavouring agents) and contaminants. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for 12 groups of flavouring agents (alicyclic ketones, secondary alcohols and related esters; alicyclic primary alcohols, aldehydes, acids and related esters; aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones; aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances; aliphatic and aromatic amines and amides; aliphatic lactones; aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups; aliphatic secondary alcohols, ketones and related esters and acetals; aromatic substituted secondary alcohols, ketones and related esters; benzyl derivatives; phenol and phenol derivatives; and simple aliphatic and aromatic sulfides and thiols) and two food contaminants (cadmium and lead).

Specifications for the following food additives were revised: activated carbon, cassia gum, indigotine, steviol glycosides, sucrose esters of fatty acids, sucrose monoesters of lauric, palmitic or stearic acid and titanium dioxide. Specifications for the following flavouring agents were revised: 4-carvomenthol and 5,6,7,8-tetrahydroquinoxaline.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of the flavouring agents and contaminants considered.

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various flavouring agents, with a view to concluding as to safety concerns and to preparing specifications for identity and purity. The Committee also evaluated the risk posed by two food contaminants, with the aim of deriving tolerable intakes where appropriate and advising on risk management options for the purpose of public health protection.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives (particularly flavouring agents) and contaminants. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for 12 groups of flavouring agents (alicyclic ketones, secondary alcohols and related esters; alicyclic primary alcohols, aldehydes, acids and related esters; aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones; aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances; aliphatic and aromatic amines and amides; aliphatic lactones; aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups; aliphatic secondary alcohols, ketones and related esters and acetals; aromatic substituted secondary alcohols, ketones and related esters; benzyl derivatives; phenol and phenol derivatives; and simple aliphatic and aromatic sulfides and thiols) and two food contaminants (cadmium and lead).

Specifications for the following food additives were revised: activated carbon, cassia gum, indigotine, steviol glycosides, sucrose esters of fatty acids, sucrose monoesters of lauric, palmitic or stearic acid and titanium dioxide. Specifications for the following flavouring agents were revised: 4-carvomenthol and 5,6,7,8-tetrahydroquinoxaline.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of the flavouring agents and contaminants considered.

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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

WHO FOOD ADDITIVES SERIES: 44

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

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

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

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1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 47 flavouring agents that

includes aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups (see Table 1) using the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, p. 122).

The Committee previously evaluated eight members of this group for other functional uses. Fumaric acid (No. 618) was first considered by the Committee at its tenth meeting (Annex 1, reference 13), and at its thirty-fifth meeting (Annex 1, reference 88) the Committee established a group ADI of 'not specified'[1] for fumaric acid and its salts. Triethyl citrate (No. 629) was first considered by the Committee at its twenty-third meeting (Annex 1, reference 50), and at its twenty-eighth meeting (Annex 1, reference 66) the Committee established an ADI of 0-20 mg/kg bw. Diethyl tartrate (No. 622) was first considered by the Committee at its twenty-third meeting (Annex 1, reference 50), but an evaluation was not possible on the basis of the data available at that time. As no additional data were available to the Committee at its twenty-fifth meeting (Annex 1, reference 56), no ADI was allocated. The Committee also evaluated related terpenoid flavouring agents, including linalool, linalyl acetate, citronellol, citral, and geranyl acetate, and established a group ADI of 0-0.5 mg/kg bw at its twenty-third meeting (Annex 1, reference 50).

1.2 Estimated daily per capita intake

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

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

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

1.3 Metabolism

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

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

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

substances are metabolized through the fatty acid ß-oxidation pathway or the tricarboxylic acid cycle.

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

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

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

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

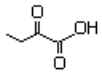
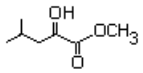
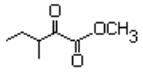
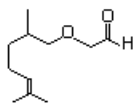
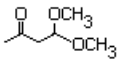
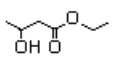
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
2-Oxobutyric acid 	589	600-18-0	No	N/R	N/R	No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

Table 1. (continued)

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



Ethyl 3-hydroxybutyrate	594	5405-41-4	No	N/R	N/R	No safety concern



Ethyl acetoacetate	595	141-97-9	Yes	Yes[b]	N/R	No safety concern

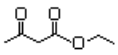
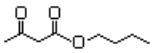
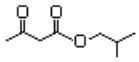


Table 1. (continued)

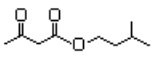
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Butyl acetoacetate	596	591-60-6	No	N/R	N/R	No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



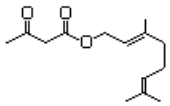
Isobutyl acetoacetate	597	7779-75-1	No	N/R	N/R	No safety concern



Isoamyl acetoacetate	598	2308-18-1	No	N/R	N/R	No safety concern



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



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

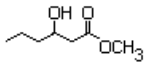
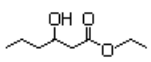
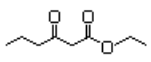


Table 1. (continued)

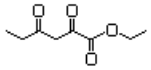
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Ethyl 3-hydroxyhexanoate	601	2305-25-1	No	N/R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



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



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



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

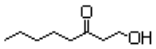
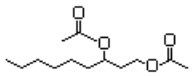
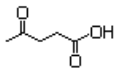


Table 1. (continued)

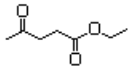
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
1,3-Nonanediol acetate (mixed esters)	605	1322-17-4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



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



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



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

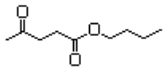
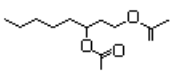
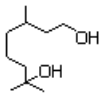


Table 1. (continued)

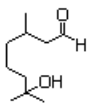
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
1,4-Nonanediol diacetate	609	67715-81-5	No	N/R	N/R	No safety concern



Hydroxycitronellol	610	107-74-4	No	N/R	N/R	No safety concern



Hydroxycitronellal	611	107-75-5	No	N/R	N/R	No safety concern



Hydroxycitronellal dimethyl acetal	612	141-92-4	No	N/R	N/R	No safety concern

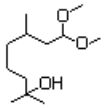


Table 1. (continued)

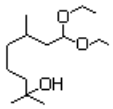
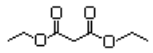
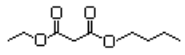
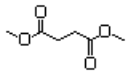
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Hydroxycitronellal diethyl acetal 	613	7779-94-4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

Table 1. (continued)

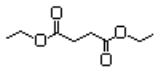
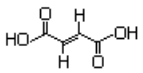
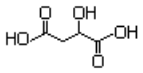
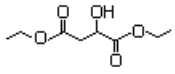
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Diethyl succinate 	617	123-25-1	No	N/R	N/R	No safety concern
Fumaric acid[c] 	618	110-17-8	Yes	Yes[d]	N/R	No safety concern
(-)-Malic acid 	619	97-67-6	Yes	Yes[d]	N/R	No safety concern
Diethyl malate 	620	7554-12-3	No	N/R	N/R	No safety concern

Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake	Step A4 Is the	Step A5 Adequate NOEL	Conclusion based on current intake

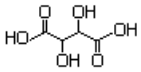
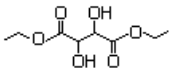
			exceed the threshold for human intake?	substance or are its metabolites endogenous?	for substance or related substance?	
Tartaric acid (+-, --, ±-, meso-)	621	87-69-4	Yes	No	Yes. NOEL was 1200 mg/kg bw per day in a two-year study in rats	No safety concern
						
Diethyl tartrate	622	87-91-2	No	N/R	N/R	No safety concern
						

Table 1. (continued)

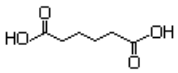
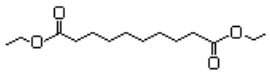
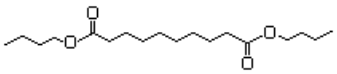
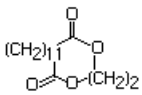
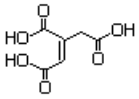
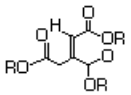
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Adipic acid	623	124-04-9	Yes	No	Yes. The NOEL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

Table 1. (continued)

Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Ethylene brassylate	626	105-95-3	No	N/R	N/R	No safety concern
						
Aconitic acid	627	499-12-7	No	N/R	N/R	No safety concern



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



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

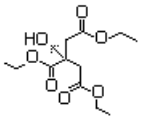
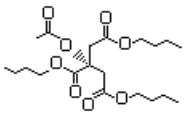
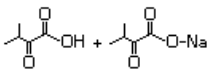


Table 1. (continued)

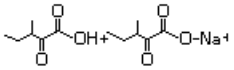
Substance and structure	JECFA No.	CAS No.	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Tributyl acetylcitrate	630	77-90-7	No	N/R	N/R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



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



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



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

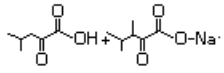
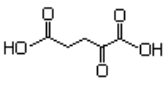
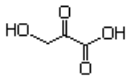


Table 1. (continued)

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



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



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

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

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

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

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

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

1.5 Consideration of combined intake

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

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

1.6 Conclusions

The Committee concluded that the safety of flavouring agents in

this group would not raise concern when they were used at he current levels of estimated intake.

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

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

2.2 Additonal considerations on intake

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

2.3 Biological data

2.3.1 Absorption, metabolism, and elimination

2.3.1.1 Ester and diesters

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

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

The alcohol, aldehyde, and acid components of these esters,

diesters, and cyclic diester are completely metabolized. At higher concentrations, they may be conjugated with glucuronic acid and excreted.

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

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

ultimately enters the tricarboxylic acid cycle as succinyl-coenzyme A. Nos 631-635 are intermediates formed endogenously from the oxidative deamination of valine, isoleucine, leucine, glutamic acid, and serine, respectively (Voet & Voet, 1990).

2.3.1.3 Acetals

Three substances in this group are acetals (Nos 593, 612, and 613), which are likely to undergo uncatalysed hydrolysis *in vivo* to yield their component aldehydes and alcohols. 3-Oxobutanal dimethyl acetal (No. 593) would be expected to undergo hydrolysis to yield methanol and acetoacetaldehyde, which may be oxidized to acetoacetic acid. More than 99% of hydroxycitronellal dimethyl acetal (No. 612) was hydrolysed to the terpenoid hydroxycitronellal and methanol in simulated gastric juice (pH 2.1) after 1 h, and > 6% was hydrolysed in intestinal fluid (pH 7.5) after 2 h (Morgareidge, 1962b). Hydroxy-citronellal diethyl acetal (No. 613) would be expected to undergo similar metabolism.

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

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

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

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

2.3.1.7 omega-Substituted derivatives

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

2.3.1.8 Aliphatic di- and tricarboxylic acids and their esters

The simple aliphatic di- and tricarboxylic acids either occur endogenously in humans (Nos 618, 619, 627, and 634) or are structurally related to endogenous substances (Nos 621-626, and 630). The esters of these acids (616, 617, 620, 628, and 629) are hydrolysed, as discussed above. Succinic acid, derived from the esters (Nos 616 and 617), fumaric acid (No. 618), (-)-malic acid (No. 619), aconitic acid (No. 627), citric acid derived from triethyl citrate (No. 629), and 2-oxopentandioic acid (No. 634) are components of the tricarboxylic acid cycle (Voet & Voet, 1990). Fumaric acid is present in the blood, brain, liver, muscle, and kidney of normal rats (Marshall et al., 1949), and citric, tartaric, malic, aconitic,

fumaric, and adipic acids are present in adult human urine (Osteux & Laturaze, 1954). alpha-Ketoglutaric acid is an intermediate metabolite of citric acid, fumaric acid, and succinic acid and is formed by alpha-oxidation (Krebs et al., 1938; Simola & Krusius, 1938).

Simple aliphatic di-and tricarboxylic acids and their esters (Nos 614-635) are metabolized (after hydrolysis in the case of esters) in the fatty acid beta-oxidation pathway or tricarboxylic acid cycle. When ^{14}C-labelled (-)-malic acid (No. 619) was administered to male albino Wistar rats by gavage at a dose of 2.5 mg/kg bw, 93% of the radiolabel was recovered in expired air, urine, and faeces (Daniel, 1969). Radiolabelled adipic acid fed to rats by stomach tube at a dose of 200-300 mg/kg bw was partially or completely metabolized, and the radiolabelled products identified in the urine included glutamic acid, lactic acid, beta-ketoadipic acid, and citric acid. The presence of the beta-oxidation metabolite beta-ketoadipic acid indicates that adipic acid participates in beta-oxidation in the fatty acid pathway (Rusoff et al., 1960).

The linear and branched-chain aliphatic primary alcohol components would be oxidized in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidized to their corresponding carboxylic acids (Bosron & Ting-Kai, 1980; Levi & Hodgson, 1989; Feldman & Weiner, 1972). The resulting carboxylic acids would be metabolized in the fatty acid pathway and tricarboxylic acid cycle (Voet & Voet, 1990). Branched-chain diols or keto alcohols may undergo oxidation to their corresponding aldehydes and carboxylic acid, which would be further metabolized or excreted.

2.4 Toxicological studies

2.4.1 Acute toxicity

The available data on this group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters which contain additional oxygenated functional groups demonstrate that they have little acute toxicity when given orally. Oral LD_{50} values have been reported for 29 of the 47 substances in the group; these range from 1628 to > 34 000 mg/kg bw in male and female rats and from 1900 to > 31 000 mg/kg bw in male and female mice (Smyth et al., 1949, 1951; Smith, 1953; Smyth et al., 1954; Horn et al., 1957; Finkelstein & Gold, 1959; Wolven & Leverstein, 1962; Jenner et al., 1964;

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

2.4.2 Short-term and long-term studies of toxicity

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

2.4.2.1 Tartaric acid (No. 621)

Rats

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

Rabbits

In a study of the toxicity of citric, fumaric, and tartaric acids, 15 New Zealand rabbits (sex not specified) weighing 1-3 kg were given the sodium salt of tartaric acid in the diet at a concentration of 7.7% for 150 days, equivalent to 2300 mg/kg bw per day. A control group was fed ground diet alone. Each animal was examined daily, and food intake and body weights were determined weekly. Haematological

and urinary analyses were performed after 60 days of treatment on five treated and six control rabbits. Two animals were examined grossly 30 days after treatment, and one animal was examined after 60 days. The testis was examined histologically. At 100 days, half of the surviving rabbits were examined grossly, and the liver, kidney, and testis were examined microscopically. At the end of the study at 150 days, all animals were killed and examined grossly and histologically. Haematological and urinary analyses showed no changes. No significant gross or histopathological changes attributable to tartaric acid were observed (Packman et al., 1963).

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

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

Table 3. (continued)

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

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

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

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

[c] Only one dose tested

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

[e] Administered as the sodium salt

Dogs

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

2.4.2.2 Diethyl sebacate (No. 624)

Rats

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

2.4.2.3 Dibutyl sebacate (No. 625)

Rats

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

Groups of 16 five-to six-week-old male Sprague-Dawley rats were given dibutyl sebacate in the diet at concentrations of 0 (two control groups), 0.01, 0.05, 0.25, 1.25, or 6.25%, equivalent to 0, 10, 50, 250, 1250, and 6250 mg/kg bw per day, for two years. Administration of dibutyl sebacate did not adversely affect the growth or survival of the animals. Body weight and food intake were measured periodically throughout the study. Measurement of haematological parameters and microscopic examination at necropsy revealed no adverse effects. The lesions observed in older control and treated rats at necropsy included inflammatory changes in the lungs, enlarged and discoloured kidneys, and fatty changes in the liver. The incidence of these gross lesions was not considered to be associated with the administration of dibutyl sebacate. The NOEL was 6250 mg/kg bw per day (Smith, 1953).

2.4.4 Genotoxicity

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

2.4.5 Other relevant studies

2.4.5.1 Adipic acid (No. 623)

In a study of teratogenicity, groups of 20-24 pregnant rats were given adipic acid by oral intubation on days 6-15 of gestation at

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

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

2.4.5.2 Tartaric acid (No. 621)

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

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

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

Table 4. (continued)

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

Table 4. (continued)

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

[a] With and without metabolic activation

[b] Calculation based on relative molecular mass of 160.17

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See Also:
Toxicological Abbreviations

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.2
Revision Date 25.04.2022
Print Date 06.11.2022

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Ethyl levulinate Ethyl levulinate
Product Number	:	122629
Brand	:	Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	539-88-8

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Merck Life Science UK Limited New Road The Old Brickyard GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833-000
Fax	:	+44 (0)1747 833-313
E-mail address	:	TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Skin irritation (Category 2), H315
Eye irritation (Category 2), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram	
Signal Word	Warning
Hazard statement(s)	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
Precautionary statement(s)	
P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ eye protection/ face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram	
Signal Word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : Ethyl 4-oxopentanoate

Formula : C7H12O3
Molecular weight : 144.17 g/mol
CAS-No. : 539-88-8
EC-No. : 208-728-2

Component	Classification	Concentration
Ethyl 4-oxovalerate		
CAS-No. 539-88-8 EC-No. 208-728-2	Skin Irrit. 2; Eye Irrit. 2; H315, 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
Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed
After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Water Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Carbon oxides
Combustible.
Vapors are heavier than air and may spread along floors.
Forms explosive mixtures with air on intense heating.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information
Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed.

Storage class
Storage class (TRGS 510): 10: Combustible liquids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
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.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Full contact
Material: butyl-rubber

Minimum layer thickness: 0.3 mm
Break through time: 480 min
Material tested:Butoject® (KCL 897 / Aldrich Z677647, Size M)

Splash contact
Material: Nature latex/chloroprene
Minimum layer thickness: 0.6 mm
Break through time: 30 min
Material tested:Lapren® (KCL 706 / Aldrich Z677558, 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 EC 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
protective clothing

Respiratory protection
required when vapours/aerosols are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.
Recommended Filter type: Filter type ABEK

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	clear, liquid
b)	Color	colorless
c)	Odor	fruity
d)	Melting point/freezing point	Melting point/freezing point: < -60 °C - (ECHA)
e)	Initial boiling point and boiling range	93 - 94 °C at 24 hPa - lit.
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	94 °C - closed cup
i)	Autoignition	460 °C

	temperature	at 1,002.4 hPa
j)	Decomposition temperature	No data available
k)	pH	No data available
l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	170.7 g/l at 20 °C - OECD Test Guideline 105
n)	Partition coefficient: n-octanol/water	log Pow: 0.324 at 20 °C
o)	Vapor pressure	No data available
p)	Density	1.016 g/cm3 at 25 °C - lit.
	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	none

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
Violent reactions possible with:
Strong oxidizing agents

10.4 Conditions to avoid
Strong heating.

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - female - > 2,000 mg/kg
(OECD Test Guideline 423)
Inhalation: No data available
LD50 Dermal - Rabbit - > 5,000 mg/kg
Remarks: (RTECS)

Skin corrosion/irritation
Skin - reconstructed human epidermis (RhE)
Result: Skin irritation - 60 min
(OECD Test Guideline 439)

Serious eye damage/eye irritation
Eyes - In vitro study
Result: Eye irritation - 28 min
(OECD Test Guideline 492)

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: OI1700000
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Hazardous properties cannot be excluded.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to algae	static test ErC50 - Pseudokirchneriella subcapitata - 932.1 mg/l - 72 h (OECD Test Guideline 201)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 28 d Result: 72 % - Readily biodegradable. (OECD Test Guideline 301F)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions. Notice Directive on waste 2008/98/EC.

SECTION 14: Transport information

14.1 UN number

ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name

ADR/RID:	Not dangerous goods
IMDG:	Not dangerous goods
IATA:	Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: - IMDG: - IATA: -

14.4 Packaging group

ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H315	Causes skin irritation.
H319	Causes serious eye irritation.

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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