Toxicological profile for

Ethyl maltol

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

2-Ethyl-3-hydroxypyran-4-one (PubChem)

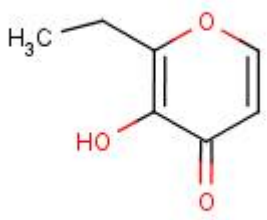
1.2. Synonyms

2-Ethyl pyromeconic acid; 2-Ethyl-3-hydroxy-4-pyrone; 2-Ethyl-3-hydroxy-4H-pyran-4-one; 2-Ethylpyromeconic acid; 3-Hydroxy-2-ethyl-1,4-pyrone; 3-Hydroxy-2-ethyl-4-pyrone; 3-Hydroxy-2-ethyl-4H-pyran-4-one; 3-Hydroxy-2-ethyl-gamma-pyrone; 4H-Pyran-4-one, 2-ethyl-3-hydroxy-; 5-18-01-00135 (Beilstein Handbook Reference); BRN 1618110; EINECS 225-582-5; Ethyl maltol; FEMA No. 3487; UNII-L6Q8K29L05; Veltol plus (ChemIDplus)

1.3. Molecular formula

C7H8O3

1.4. Structural Formula



1.5. Molecular weight (g/mol)

140.14

1.6. CAS registration number

4940-11-8

1.7. Properties

1.7.1. Melting point

(°C): 85-95 or 161-165 (ChemSpider); 90-91 (PubChem); 90.3-90.4

1.7.2. Boiling point

(°C): 170 at 100 mmHg (254.3 at 760 mmHg) or 290–290.3 (ChemSpider)

1.7.3. Solubility

2.423E+04 mg/L at 25°C (estimated) (ChemIDplus; EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 125 (ChemSpider)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

0.0±1.4 mmHg at 25°C (estimated) (ChemSpider); 0.000145 mmHg at 25°C (estimated) (ChemIDplus; EPISuite, 2017)

1.7.11. log Kow

0.63 (ChemIDplus; EPISuite, 2017); 1.16 (ChemSpider)

2. General information

2.1. Exposure

OCCURRENCE IN TOBACCO PRODUCTS			
In the burned part	Yes		
In tobacco naturally	No evidence (Stedman 1968 & Lloyd et al 1976)		
OTHER SOURCES OF EXPOSURE			
Cosmetics	Yes (Cosing)	Food	Yes (Burdock GA, 2010)
Environment	No evidence (Merck 2013)	Pharmaceuticals	No evidence (Martindale 1993; Merck 2013)

The estimated intake from its use as a flavouring is 1580 and 6692 µg/person/day in the EU and US, respectively (EFSA, 2010, 2015).

Ethyl hydroxypyrone (CAS RN 4940-11-8) is used as a fragrance and perfuming ingredient in cosmetics in the EU. As taken from CosIng (undated).

Reported uses (ppm): (FEMA, 1994)

Food Category	**Usual**	**Max.**	**Food Category**	**Usual**	**Max.**
Alcoholic beverages	30.00	100.00	Imitation dairy	0.0025	0.0025
Baked Goods	38.00	100.00	Jams, jellies	13.00	100.00
Breakfast cereals	12.00	100.00	Meat products	9.82	19.65
Cheese	14.00	14.00	Milk products	45.00	50.00
Chewing gum	54.00	59.00	Nonalcoholic beverages	160.00	1000.00
Confection, frosting	18.00	45.00	Reconstitued vegetables	6.40	100.00
Frozen dairy	15.00	100.00	Seasonings, flavourings	100.00	1000.00
Fruit ices	10.00	100.00	Snack foods	19.00	30.00
Fruit juice	7.90	32.00	Soft candy	62.00	130.00
Gelatins, puddings	81.00	220.00	Soups	0.50	1.0
Gravies	23.00	23.00	Sweet sauce	21.00	100.00
Hard candy	5.06	27.93			

Reported individual intake from use as a flavouring: 0.08333 mg/kg bw/day.

As taken from Burdock, 2010.

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

2-Ethyl-3-hydroxy-4-pyrone (CAS RN 4940-11-8) is listed (at given concentrations where specified) as an ingredient in an “old” auto (1-5%) and inside the home (1-5%) products by the CPID.

“Ethyl maltol is an organic compound that is a common flavourant in some confectioneries.” As taken from Human Metabolome Database, 2019.“Sugars are commonly added to American-blended cigarettes, and the presence of sugars in cigarettes increases the appeal, toxicity, and addictive potential of smoking. The purpose of this study was to identify the types and relative quantities of added sugars in the tobacco of popular American cigarette brands. Methods: We reviewed the company websites of Philip Morris USA (PMUSA) and RJ Reynolds Tobacco

Company (RJR) for brand-specific ingredient lists for all PMUSA (n = 179) and RJR (n = 162) cigarette brand styles (combined 79% of US cigarette sales in 2016) and composite lists of all cigarette tobacco ingredients for both companies. From these lists, we identified known forms of saccharides (mono-, di-, and oligosaccharides). Results: All PMUSA and RJR cigarette brands contained at least one type of added sugar, except one RJR brand (6 brand styles), which contained no additives. By weight, sugars were the number one ingredient (excluding tobacco and water) in all PMUSA brands (e.g., Marlboro, Parliament, Virginia Slims). Examples of sugars added to PMUSA brands included high fructose corn syrup, sucrose, maltol, and ethyl maltol. Among RJR brands, sugar was the number two ingredient by weight (excluding tobacco and water) in most brands (e.g., Camel, Newport, Pall Mall). In some RJR brands, quantities of added sugar relative to other ingredients were more variable, ranging from the first to fourth most used ingredient by weight (e.g., Carlton, Doral, Kent, More). Types of sugars added to RJR brands included high fructose corn syrup, brown sugar, honey, glucose, and a variety of fruit juice concentrates (e.g., apple, fig, pineapple). Interestingly, many menthol cigarette brands (e.g., Newport, Marlboro Menthol, Camel Menthol) contained greater quantities of added sugar than menthol. Conclusions: A variety of sugars, including sugars routinely added to processed foods and beverages, are added to American cigarettes. Further, by weight, added sugars were the number one or number two ingredient in most cigarette brands. Given that added sugars increase the appeal, toxicity, and addictive potential of smoking, regulatory actions should be considered (e.g., a product standard for sugar) for the protection of public health." As taken from Seidenberg AB et al. 2018. Cancer Epidemiology Biomarkers & Prevention 27(3), 357. Available at http://cebp.aacrjournals.org/content/27/3/357.1

"BACKGROUND: Flavoring chemicals, or flavorants, have been used in electronic cigarettes (e-cigarettes) since their inception; however, little is known about their toxicological effects. Free radicals present in e-cigarette aerosols have been shown to induce oxidative stress resulting in damage to proliferation, survival, and inflammation pathways in the cell. Aerosols generated from e-liquid solvents alone contain high levels of free radicals but few studies have looked at how these toxins are modulated by flavorants. OBJECTIVES: We investigated the effects of different flavorants on free radical production in e-cigarette aerosols. METHODS: Free radicals generated from 49 commercially available e-liquid flavors were captured and analyzed using electron paramagnetic resonance (EPR). The flavorant composition of each e-liquid was analyzed by gas chromatography mass spectroscopy (GCMS). Radical production was correlated with flavorant abundance. Ten compounds were identified and analyzed for their impact on free radical generation. RESULTS: Nearly half of the flavors modulated free radical generation. Flavorants with strong correlations included β-damascone, δ-tetradecalactone, γ-decalactone, citral, dipentene, ethyl maltol, ethyl vanillin, ethyl vanillin PG acetal, linalool, and piperonal. Dipentene, ethyl maltol, citral, linalool, and piperonal promoted radical formation in a concentration-dependent manner. Ethyl vanillin inhibited the radical formation in a concentration dependent manner. Free radical production was closely linked with the capacity to oxidize biologically-relevant lipids. CONCLUSIONS: Our results suggest that flavoring agents play an important role in either enhancing or inhibiting the production of free radicals in flavored e-cigarette aerosols. This information is important for developing regulatory strategies aimed at reducing potential harm from e-cigarettes." As taken from Bitzer ZT et al. 2018. Free Radic. Biol. Med. 120, 72-79. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29548792

Chemical ingredients in e-cigarette emissions

Chemical name	CAS number	Maximum amount reported	Reference
Ethyl maltol	4940-11-8	-	(Eddingsaas et al. 2018; Garcia-Gomez et al. 2016)

As taken from NICNAS, 2019

Ethyl maltol (CAS RN 4940-11-8) is used as a flavour enhancer and fragrance ingredient in non-medicinal natural health products (Health Canada, 2022).

2.2. Combustion products

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

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
ethyl maltol	3264431359	94.55	3283472862	95.25
unknown	47970181	1.39	34916136	1.01
Total ion chromatogram	3451092158	100	3457043168	100

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

Ingredients CAS Number	Chemical Class	Mol. Wt. (M) Bp or Mp (^{0}C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
Ethyl maltol CAS 49940-11-8	Hydroxy delta-pyrrone (Unsaturated cyclic ketone, ether alcohol)	M=140 mp 89-93	100	99	Ethyl maltol 92.8 Ethyl maltol isomer 6.2 2 Unidentified compounds 4.1	46 3 0.1

In a pyrolysis study, 100% of ethyl maltol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

According to Burdock, GA (2010), ethyl maltol does not occur naturally in food.

Ethyl maltol has been isolated from the passion flower plant (taken from Khan IA and Abourashed EA, 2010).

3. Status in legislation and other official guidance

Food	**UK**	Yes	EU	Yes	**USA**	172.515
ADI / TDI	ADI: 0-2 mg/kg bw (JECFA, 1975, 2005). The ADI established in 1975 was maintained at the 2005 meeting. ADI: 1 mg/kg bw (SCF, 1991).					

	Category A (may be used in foodstuffs as a flavouring) (COE 2000).		
Codex Alim.	637		
C of E no.	692	FEMA no.	3487
TLV / OEL	Not listed		
Cosmetics (UK)	Not listed in Schedule 1		

An EFSA Panel “agrees with the JECFA conclusion of ‘No safety concern at estimated level of intake as flavouring substance’ based on the MSDI (Maximised Survey-derived Daily Intake) approach” (EFSA, 2010, 2015).

Ethyl maltol is included on the FDA’s inventory of “Substances Added to Food (formerly EAFUS)” as a color or coloring adjunct, a flavoring agent or adjuvant and a processing aid and is included under 21 CFR section 172.515 (Synthetic flavoring substances and adjuvants) (FDA, 2022a,b).

There is a REACH dossier on 2-ethyl-3-hydroxy-4-pyrone (CAS RN 4940-11-8) (ECHA, undated).

2-Ethyl-3-hydroxy-4-pyrone (CAS RN 4940-11-8) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Ethyl maltol is listed on the US EPA InertFinder Database (2022) as approved for food, non-food and fragrance use pesticide products. For food use it is covered under 40 CFR sections 180.910 (Inert ingredients used pre- and post-harvest; exemptions from the requirement of a tolerance) and 180.930 (Inert ingredients applied to animals; exemptions from the requirement of a tolerance), with limits in both of not more than 0.2% of the pesticide formulation (US EPA, 2022).

4H-Pyran-4-one, 2-ethyl-3-hydroxy- (CAS RN 4940-11-8) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). US EPA 2020 CDR List. US EPA TSCA inventory

Ethyl maltol (CAS RN 4940-11-8) is authorised for use as a flavouring substance in all categories of flavoured foods in the EU under (EU) legislation no 872/2012 (European Commission, 2012).

Ethyl maltol (FEMA no. 3487) has been designated as GRAS (generally recognized as safe) for use in food by FEMA (Oser BL and Ford RA, 1977).

4H-Pyran-4-one, 2-ethyl-3-hydroxy- (CAS RN 4940-11-8) is included on the New Zealand Inventory of Chemicals does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006).

Ethyl maltol (CAS RN 4940-11-8) is included on the US FDA’s list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose and maximum daily exposures:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)	Record Updated
ETHYL MALTOL	ORAL	ELIXIR	1110651	L6Q8K29L05	0.6mg/15ml		
ETHYL MALTOL	ORAL	POWDER, FOR SUSPENSION	1110651	L6Q8K29L05	10mg/140ml		

ETHYL MALTOL	ORAL	SOLUTION	1110651	L6Q8K29L05		90mg	
ETHYL MALTOL	ORAL	SUSPENSION, EXTENDED RELEASE	1110651	L6Q8K29L05		20mg	Y
ETHYL MALTOL	ORAL	SYRUP	1110651	L6Q8K29L05		122mg	

As taken from FDA, 2022c

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“The safety assessment of a flavour substance examines several factors, including metabolic and physiological disposition data. The present article provides an overview of the metabolism and disposition of flavour substances by identifying general applicable principles of metabolism to illustrate how information on metabolic fate is taken into account in their safety evaluation. The metabolism of the majority of flavour substances involves a series both of enzymatic and non-enzymatic biotransformation that often results in products that are more hydrophilic and more readily excretable than their precursors. Flavours can undergo metabolic reactions, such as oxidation, reduction, or hydrolysis that alter a functional group relative to the parent compound. The altered functional group may serve as a reaction site for a subsequent metabolic transformation. Metabolic intermediates undergo conjugation with an endogenous agent such as glucuronic acid, sulphate, glutathione, amino acids, or acetate. Such conjugates are typically readily excreted through the kidneys and liver. This paper summarizes the types of metabolic reactions that have been documented for flavour substances that are added to the human food chain, the methodologies available for metabolic studies, and the factors that affect the metabolic fate of a flavour substance..” As taken from Smith RL et al. 2018. Toxicol. Res. 7(4), 618-646. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30090611

4.2. Absorption, distribution and excretion

“65-70% of ethyl maltol absorbed appears in the urine as gluconomide or sulfate within 2 hours. […] None was detected in the faeces.” As taken from FAO Nutrition Meetings Report Series 48a, 205, available at: http://www.inchem.org/documents/jecfa/jecmono/v48aje07.htm

“Ethyl maltol was rapidly and extensively absorbed and eliminated when given orally to dogs” (BIBRA, 1996)

The metabolism of ethyl maltol and maltol have been studied in the dog [no abstract available] (Rennhard HH 1971. J. Agric. Fd Chem. 19, 152-154. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/5540749)

“Oral administration of ethyl maltol is almost completely absorbed from the gut.” As taken from FAO Nutrition Meetings Report Series 48a, 205, available at: http://www.inchem.org/documents/jecfa/jecmono/v48aje07.htm

“The pyrones, 3-hydroxy-2-methyl-4-pyrone (maltol) and 3-hydroxy-2-ethyl-4-pyrone (ethyl maltol) chelate iron with a high affinity and selectivity. The resulting 1:3 (metal-ligand) complexes, being neutral, are able to partition readily across cell membranes and thus may facilitate iron transport across the intestinal wall. Absorption of radioactive iron (59Fe) in the presence of these pyrones was investigated in male rats 1, 2, 4 and 6 h after intraduodenal administration of a 7 micrograms dose and compared with that of 59Fe given as the sulphate, gluconate, fumarate or complexed to EDTA. Total body absorption and distribution were calculated from the 59Fe content of various

tissue samples. With all the iron preparations used, blood levels of 59Fe were highest 1 h after injection whilst the 59Fe content at the major site of deposition, i.e. the bone marrow, increased up to 6 h. No 59Fe was found in the urine. Total body absorption of 59Fe was significantly higher from the pyrones than from the other four preparations. Over the dose range 0.7-700 micrograms, the proportion of 59Fe absorbed from both iron maltol and iron sulphate decreased with increasing dose. Enhanced 59Fe uptake from maltol was evident at 0.7-70 micrograms but not at 700 micrograms suggesting that use of these pyrones will not result in iron overload. Absorption of 59Fe given into the stomach was slower in onset but was sustained longer presumably via a steady delivery of iron to the duodenum from the gastric reservoir". As taken from Barrand MA et al. 1987. J. Pharm. Pharmacol. 39(3), 203-11. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/2883285?dopt=Abstract

"Only minute amounts of free ethyl maltol are detected in the urine of rats or dogs given high doses; most of an administered dose of ethyl maltol is rapidly excreted as glucoronic acid and sulphate conjugates in urine" (EFSA, 2010).

4.3. Interactions

"Accumulation of radioactive iron (59Fe) into isolated fragments of rat small intestine in the presence of two hydroxypyrones, maltol and ethyl maltol, was compared with that in the presence of another chelator of iron(III), nitrilotriacetic acid (NTA). The characteristics of uptake were similar with all three ligands. Between 10(-6) and 10(-4) M, iron uptake showed saturable kinetics. The uptake was partially inhibited by metabolic inhibitors. Above 10(-4) M a non-saturable uptake, unaffected by metabolic inhibitors became evident in the presence of the pyrones. The distribution of 59Fe after uptake was determined by gel filtration. At low iron concentrations (10(-6) M), 35-40% of absorbed iron was associated with proteins of molecular weights similar to those of ferritin and transferrin. At high concentrations (10(-3) M), the majority of 59Fe was found in a low molecular weight fraction. At each concentration, a small amount of 59Fe was bound to a membrane fraction. 5% Polyethylene glycol, which reduces glycocalyx viscosity enhanced uptake at low iron concentrations (10(-6) M) but did not affect the non-saturable diffusion seen at higher concentrations (10(-3) M). The iron(II) chelator, bathophenanthroline sulphonate (10(-3) M), decreased uptake at low iron concentrations but did not affect the non-saturable uptake. It is suggested that conversion of iron(III) to iron(II) may take place at the mucosal cell surface before uptake via the saturable system. Apparent Km values for iron uptake via the saturable system were higher in the presence of maltol and ethyl maltol than in the presence of NTA, presumably since the iron binds more avidly to the hydroxypyrones and so is less readily donated. Excess ligand, either pyrone or NTA, reduced the rate at which 59Fe was donated to the uptake system. The Vmax value for uptake from the pyrones was greater than from NTA. It is concluded that maltol, ethyl maltol and NTA can hold iron(III) in solution and donate it to an endogenous uptake system. But, the hydroxypyrones may be more suitable ligands for the oral administration of iron since, when complexed with iron, they lack the toxic effects associated with iron(III)-NTA and with iron(II) preparations." As taken from Levey JA et al. 1988. Biochem Pharmacol. 37(10), 2051-7. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/3377810?dopt=AbstractPlus

"When used separately, 20 mmol l-1 maltol or 1600 AU ml-1 nisin resulted in a 0-0.6 log10 reduction in viable counts of Escherichia coli in a buffer system. However, when added in combination they yielded a 1.8-5.5-log-cycle reduction in viable counts of E. coli at pH 5.0 and 6.8 respectively. It is postulated that maltol (and ethyl maltol) destabilizes the cell outer membrane by chelation of Mg2+ and/or Ca2+, thus permeabilizing the E. coli cell to nisin." As taken from Schved F et al. 1996. Lett Appl Microbiol. 22(3), 189-91. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/8852344?dopt=Abstract

"Ethyl maltol (EM) is a flavoring agent commonly used in foods that falls under the generally recognized as safe category. It is added to many commercial e-cigarette vaping fluids and has been detected in the aerosols. Considering that EM facilitates heavy metal transport across plasma

membranes, and that heavy metals have been detected in aerosols generated from e-cigarettes, this study examines whether EM enhances heavy metal mediated toxicity. A decrease in viability was observed in the Calu-6 and A549 lung epithelial cell lines co-exposed to EM and copper (Cu) but no decrease was observed after co-exposure to EM with iron (Fe). Interestingly, co-exposure to EM and Fe decreased viability of the HEK293 and IMR-90 fibroblast cell lines but co-exposure to EM and Cu did not. Increases in the apoptotic markers Annexin V staining and fragmented nuclei were observed in Calu-6 cells co-exposed to EM and Cu. Co-exposure to EM and Cu in Calu-6 cells resulted in DNA damage as indicated by activation of ATM and expression of γH2A.x foci. Finally, co-exposure to EM and Cu caused oxidative stress as indicated by increases in the generation of reactive oxygen species and the expression of ferritin light chain mRNA and hemeoxygenase-1 mRNA and protein. These data show that co-exposure to EM and Cu, at concentrations that are not toxic for either chemical individually, induce apoptosis and evoke oxidative stress and DNA damage in lung epithelial cells. We suggest that there is a greater risk of lung damage in users of c-cigarette who vape with vaping fluid containing EM." As taken from Durrani K et al. 2020. Toxicol. Appl. Pharmacol. 410, 115354. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33271249/

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
chicken	LD50	oral	1270mg/kg (1270mg/kg)		Toxicology and Applied Pharmacology. Vol. 15, Pg. 604, 1969.
mouse	LD50	oral	780mg/kg (780mg/kg)		Toxicology and Applied Pharmacology. Vol. 15, Pg. 604, 1969.
mouse	LD50	subcutaneous	910mg/kg (910mg/kg)		Chemical and Pharmaceutical Bulletin. Vol. 22, Pg. 1008, 1974.
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 13, Pg. 805, 1975.
rat	LD50	oral	1150mg/kg (1150mg/kg)		Toxicology and Applied Pharmacology. Vol. 15, Pg. 604, 1969.

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

Intraperitoneal LD50 mice	980 mg/kg

As taken from BIBRA, 1996.

Acute inhalation:

"Exposure of mice to an unspecified concentration of the vapour for 1hr caused slightly increased physical activity." (BIBRA, 1996)

5.2. Repeated dose toxicity

No toxic effects were observed in a two-year study in which groups of rats received up to 200 mg/kg bw/day in the diet (BIBRA, 1996).

In a similar study, rats were fed up to 1000 mg/kg bw/day for 90 days, survival, growth and organ weights were not significantly affected. However, animals at the highest dose demonstrated kidney damage, and even at the lowest dose (250 mg/kg bw/day) slight changes in the blood composition were observed (BIBRA, 1996).

Oral TDLo Rat: 90 g/kg/90D (intermittent); changes in tubules (including acute renal failure, acute tubular necrosis)

As taken from RTECS, 2006.

“Short-term studies in rat and dog indicated no abnormalities upon repeated oral exposure (90 days). A long-term exposure study in rat also did not indicate any treatment-related effect reference.”

“…in addition, a chronic study was available in which groups of 25 male and female rats were fed for two years on diets containing ethyl maltol calculated to deliver 0, 50, 100 and 200 mg ethyl maltol/kg bw/day. No abnormalities were seen for survival, clinical appearance, growth rate or food consumption, clinical chemistry, haematology and urinalysis. No histopathological changes and no increases in neoplasms were seen after treatment with ethyl maltol, thus, concluding that ethylmaltol is not carcinogenic via the oral route (EFSA, 2015).”

As taken from EFSA, 2015.

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use – Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - NOAEL

POD Value – 167.0

POD Owner - COSMOS TTC (NON-CANCER)

Adjustment factors used in calculations: Adjustment factor: Study: Dose Duration: 3 (3)

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

NOEL/LEL Owner - PAFA

Original NOEL - 500.0 mg/kg bw/day

Original LEL - 1000.0 mg/kg bw/day

Critical Sites - Kidney

Critical Effects - CLINICAL SIGNS – CONDITION; KIDNEY - PATHOLOGY

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

5.3. Reproduction toxicity

In a reproductive study in which rats were fed diets providing up to 200 mg/kg bw/day, no clear effects on fertility, foetal development, pregnancy and birth, litter size, pup survival and pup weight at weaning were observed (BIBRA, 1996).

Species	Test conditions	Effects	Reference
Rat (10/sex per dose)	Rats (from the long-term study described above) were fed diets providing 0, 50, 100 or 200 mg/kg bw/day, and mated between weeks 15-21. They were then retained on the same diets and mated for a second time at weeks 30-36. Fertility, foetal development, pregnancy and birth, litter size, pup survival and weight at weaning, were monitored.	No evidence of any effects on reproduction	Gralla et al. 1969
Dog (4 per sex per group)	When showing signs of oestrus, female dogs (from the 2-yr study described above) given 0, 50, 100 or 200 mg/kg bw/day by oral capsule were mated with males given similar treatment. The results were not described in detail but the report refers to the experience of two female dogs (given 100 or 200 mg/kg bw/day, 5 days/week) that were mated after 6-20 months of treatment.	Although breeding efforts were reported as "not remarkably successful", 15 normal pups were delivered	Gralla et al. 1969

POD Method	POD Value	POD Owner
NOEL	66.7	MUNRO

Lowest-observed effect

Owner	Type	Value	Sites	Effects
MUNRO	LOEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect MUNRO: NOEL: 200.0 mg/kg bw/day

Adjustment factors Domain: Adjustment factor - Type: Study - Subtype: Dose Duration - Value: 3 - Comments: 3

>Critical study: Reproductive/Developmental Toxicity > Reproductive Toxicity (Rat) for 90 day (exposure route not reported)

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

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which ethyl maltol was part of the test mixture include EMT960820 and EMT970621 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

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

Ethyl maltol was weakly mutagenic in Salmonella typhimurium both in the presence and absence of a metabolic activation system. However, it did not induce in vivo micronuclei in mice, and it did not cause lethal heritable mutations when fed to fruit flies (BIBRA, 1996).

In vivo

Species	**Test conditions**	**Endpoint**	**Result**	**Reference**
Mouse (groups of 4, included males and females)	Two intraperitoneal injections of 0, 420, 700 or 980 mg/kg bw, given 24 hr apart. Mice killed at 30 hr and bone marrow examined for micronuclei.	Chromosome damage	-ve	Wild et al. 1983
Mouse (groups of 4, included males and females)	Single intraperitoneal injection of 0 or 980 mg/kg bw. Mice killed at 24, 48 or 72 hr and bone marrow examined for micronuclei.	Chromosome damage	-ve	Wild et al. 1983
Drosophila melanogaster	In 4 “basc” studies for sex-linked recessive lethal mutations, it was fed to male fruit flies (at concentrations of 14-50 mM), and treated males were mated with untreated females to produce three successive broods.	Germ cell mutation	-ve	Wild et al. 1983
+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation				

In vitro

Test system	Test conditions	Endpoint	Activation	Result	Reference
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test at up to 2 mg/plate.	Mutation	with and without S9	+ve weak in TA100	Bjeldanes & Chew, 1979
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test at up to 3.6 mg/plate.	Mutation	with and without S9	? weak effect in TA100, not repro-ducible so concluded to be -ve. The EPA agreed.	Wild et al. 1983 EPA, 2000
+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation					

EFSA concluded that a “genotoxicity concern could be ruled out” for ethyl maltol

“….Ethyl maltol …. were predicted positive with the MultiCASE model on chromosomal aberrations in CHL cells. All other predictions were negative or the substances were out of domain.”

As taken from EFSA, 2015.

“Electronic nicotine delivery systems (ENDS) are regulated tobacco products and often contain flavor compounds. Given the concern of increased use and the appeal of ENDS by young people, evaluating the potential of flavors to induce DNA damage is important for health hazard

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

5.5. Cytotoxicity

"We identified the most popular electronic cigarette (EC) refill fluids using an Internet survey and local and online sales information, quantified their flavor chemicals, and evaluated cytotoxicities of the fluids and flavor chemicals. "Berries/Fruits/Citrus" was the most popular EC refill fluid flavor category. Twenty popular EC refill fluids were purchased from local shops, and the ingredient flavor chemicals were identified and quantified by gas chromatography-mass spectrometry. Total flavor chemical concentrations ranged from 0.6 to 27.9 mg/ml, and in 95% of the fluids, total flavor concentration was greater than nicotine concentration. The 20 most popular refill fluids contained 99 quantifiable flavor chemicals; each refill fluid contained 22 to 47 flavor chemicals, most being esters. Some chemicals were found frequently, and several were present in most products. At a 1% concentration, 80% of the refill fluids were cytotoxic in the MTT assay. Six pure standards of the flavor chemicals found at the highest concentrations in the two most cytotoxic refill fluids were effective in the MTT assay, and ethyl maltol, which was in over 50% of the products, was the most cytotoxic. These data show that the cytotoxicity of some popular refill fluids can be attributed to their high concentrations of flavor chemicals." As taken from Hua M et al. 2019. Sci. Rep. 9(1), 2782. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30808901

"Whereas JUUL electronic cigarettes (ECs) have captured the majority of the EC market, with a large fraction of their sales going to adolescents, little is known about their cytotoxicity and potential effects on health. The purpose of this study was to determine flavor chemical and nicotine concentrations in the eight currently marketed prefilled JUUL EC cartridges ("pods") and to evaluate the cytotoxicity of the different variants (e.g., "Cool Mint" and "Crème Brulee") using in vitro assays. Nicotine and flavor chemicals were analyzed using gas chromatography-mass spectrometry in pod fluid before and after vaping and in the corresponding aerosols. 59 flavor chemicals were identified in JUUL pod fluids, and 3 were >1 mg/mL. Duplicate pods were similar in flavor chemical composition and concentration. Nicotine concentrations (average 60.9 mg/mL) were significantly higher than those of any EC products we have previously analyzed. The transfer efficiency of individual flavor chemicals that were >1 mg/mL and nicotine from the pod fluid into aerosols was generally 35-80%. All pod fluids were cytotoxic at a 1:10 dilution (10%) in the MTT and neutral red uptake assays when tested with BEAS-2B lung epithelial cells. Most aerosols were cytotoxic in these assays at concentrations between 0.2 and 1.8%. The cytotoxicity of collected aerosol materials was highly correlated with nicotine and ethyl maltol concentrations and moderately to weakly correlated with total flavor chemical concentration and menthol concentration. Our study demonstrates that (1) some JUUL flavor pods have sufficiently high concentrations of flavor chemicals that may make them attractive to youth and (2) the concentrations of nicotine and

some flavor chemicals (e.g., ethyl maltol) are high enough to be cytotoxic in acute in vitro assays, emphasizing the need to determine if JUUL products will lead to adverse health effects with chronic use. .” As taken form Omaiye EE et al. 2019a. Chem Res Toxicol. 32(6), 1058-1069. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30896936/

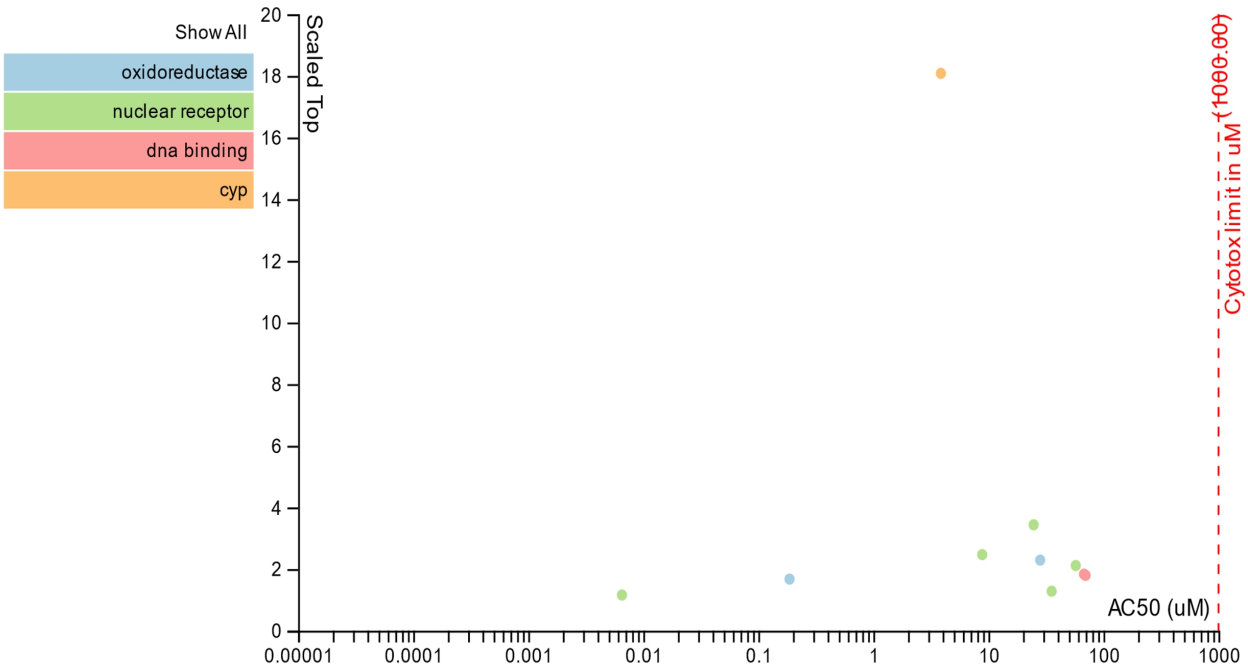
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 2-ethyl-3-hydroxy-4H-pyran-4-one (CAS RN 4940-11-8) 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 2-ethyl-3-hydroxy-4H-pyran-4-one is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA’s CompTox Dashboard.

Figure 1



Flavouring compounds in e-cigarettes with human health concerns

Chemical	CAS number	Concern	References
Ethyl maltol	4940-11-8	Respiratory irritation, cytotoxicity	(Sherwood and Boitano 2016; Vardavas et al. 2017)

As taken from NICNAS, 2019

“The development of reduced-risk products aims to provide alternatives to cigarettes that present less risk of harm for adult smokers. Responsible use of flavoring substances in these products may fulfill an important role in product acceptance. While most flavoring substances used in such products are also used by the food industry and are considered safe when ingested, their impact when inhaled may require further assessment. To aid in such an assessment, a three-step approach combining real-time cellular analysis, phenotypic high-content screening assays, and gene expression analysis was developed and tested in normal human bronchial epithelial cells with 28 flavoring substances commonly used in e-liquid formulations, dissolved individually or as a mixture in a base solution composed of propylene glycol, vegetable glycerin, and 0.6% nicotine. By employing this approach, we identified individual flavoring substances that potentially contribute greatly to the overall mixture effect (citronellol and alpha-pinene). By assessing modified mixtures, we showed that, although cytotoxic effects were found when assessed individually, alpha-pinene did not contribute to the overall mixture cytotoxicity. Most of the cytotoxic effect appeared to be attributable to citronellol, with the remaining substances contributing due to synergistic effects. We developed and used different scoring methods (Tox-Score, Phenotypic Score, and Biological Impact Factor/Network Perturbation Amplitude), ultimately enabling a ranking based on cytotoxicity, phenotypic outcome, and molecular network perturbations. This case study highlights the benefits of testing both individual flavoring substances and mixtures for e-liquid flavor assessment and emphasized the importance of data sharing for the benefit of consumer safety.” “Considering the limited stability of butyric acid, ethyl maltol, and isobutyl alcohol, the flavored stock solutions containing these ingredients were used within five days.” As taken from Marescotti D et al. 2020. Toxicol. Rep. 7, 67-80. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31886136/

“E-cigarette-related hospitalizations and deaths across the U.S. continue to increase. A high percentage of patients have elevated liver function tests indicative of systemic toxicity. This study was designed to determine the effect of e-cigarette chemicals on liver cell toxicity. HepG2 cells were exposed to flavoring chemicals (isoamyl acetate, vanillin, ethyl vanillin, ethyl maltol, l-menthol, and trans-cinnamaldehyde), propylene glycol, and vegetable glycerin mixtures, and cell viability was measured. Data revealed that vanillin, ethyl vanillin, and ethyl maltol decreased HepG2 cell viability; repeated exposure caused increased cytotoxicity relative to single exposure, consistent with the hypothesis that frequent vaping can cause hepatotoxicity.” As taken from Rickard BP et al. 2021. ACS Omega 6(10), 6708-6713. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33748584/

“E-cigarettes utilize a wide range of flavoring chemicals with respiratory health effects that are not well understood. In this study, we used pulmonary-associated cell lines to assess the in vitro cytotoxic effects of 30 flavoring chemicals. Human bronchial epithelial cells (BEAS-2B) and both naïve and activated macrophages (THP-1) were treated with 10, 100, and 1000 μM of flavoring chemicals and analyzed for changes in viability, cell membrane damage, reactive oxygen species (ROS) production, and inflammatory cytokine release. Viability was unaffected for all chemicals at the 10 and 100 μM concentrations. At 1000 μM, the greatest reductions in viability were seen with decanal, hexanal, nonanal, cinnamaldehyde, eugenol, vanillin, alpha-pinene, and limonene. High amounts of ROS were elicited by vanillin, ethyl maltol, and the diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione) from both cell lines. Naïve THP-1 cells produced significantly elevated levels of IL-1β, IL-8, and TNF-α when exposed to ethyl maltol and hexanal. Activated THP-1 cells released increased IL-1β and TNF-α when exposed to ethyl maltol, but many flavoring chemicals had an apparent suppressive effect on inflammatory cytokines released by activated macrophages, some with varying degrees of accompanying cytotoxicity. The diketones, L-carvone, and linalool suppressed cytokine release in the absence of cytotoxicity. These findings provide insight into lung cell cytotoxicity and inflammatory cytokine release in response to flavorings commonly used in e-cigarettes.”

Morris AM et al. (2021) Effects of E-Cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells.

“E-cigarette-related hospitalizations and deaths across the U.S. continue to increase. A high percentage of patients have elevated liver function tests indicative of systemic toxicity. This study was designed to determine the effect of e-cigarette chemicals on liver cell toxicity. HepG2 cells were exposed to flavoring chemicals (isoamyl acetate, vanillin, ethyl vanillin, ethyl maltol, l-menthol, and *trans*-cinnamaldehyde), propylene glycol, and vegetable glycerin mixtures, and cell viability was measured. Data revealed that vanillin, ethyl vanillin, and ethyl maltol decreased HepG2 cell viability; repeated exposure caused increased cytotoxicity relative to single exposure, consistent with the hypothesis that frequent vaping can cause hepatotoxicity.”

Pickard BP et al. (2021) E-Cigarette Flavoring Chemicals Induce Cytotoxicity in HepG2 Cells.

5.6. Carcinogenicity

Limited studies have been carried out, although they would not meet current regulatory guidelines. There was no increase in tumour incidence, compared to untreated controls, in a study in which rats were fed diets containing up to 200 mg/kg bw/day for two years. Similar results were obtained in a study in which dogs were fed capsules containing up to 200 mg/kg bw/day for up to two years (BIBRA, 1996).

Carcinogenicity			
Species	Test conditions	Evidence of carcinogenicity	Reference
Rat (25/sex per dose)	Rats were fed diets providing 0, 50, 100 or 200 mg/kg bw/day for up to 2 years (5/sex/group were killed after 1 yr). A microscopic examination of a comprehensive range of tissues was carried out. Limited study, modern protocols recommend treating about 50 animals/sex/group	None	Gralla et al. 1969
Dog (4/sex per dose)	Dogs were fed capsules providing 0, 50, 100 or 200 mg/kg bw/day, 5 days/week, for up to 2 years (2/group were killed after 1 yr). Detailed gross and microscopic examination of a comprehensive range of organs and tissues was undertaken This study would have had limited ability to detect carcinogenic activity owing to the small number of animals tested and the relatively short duration.	None	Gralla et al. 1969

“There is a carcinogenicity study on ethyl maltol [FL-no: 07.047] in rats. Although the number of animals per group were lower than suggested in OECD guidelines they were in accordance with the standards at the time the study was performed and the Panel concluded that the result could overrule the mutagenicity observed with ethyl maltol in bacteria. The data available do not indicate a genotoxic or carcinogenic potential for ethyl maltol” (EFSA, 2010).

POD Method	POD Value	POD Owner
HNEL	Not calculated	PAFA

Lowest-observed effect

Owner	Type	Value	Sites	Effects
PAFA	LOAEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect PAFA: HNEL: 200.0 mg/kg bw/day

Adjustment factors

>Critical study: Carcinogenicity > Chronic/Carcinogenicity (Rat, Oral exposure) for 730 day

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

5.7. Irritation/immunotoxicity

Ethyl maltol was neither an irritant nor sensitizer of human skin when tested at 10 % in petrolatum (BIBRA, 1996).

Sensitization

No skin sensitization reactions were induced in 25 volunteers following a maximization test at a concentration of 10% in petrolatum (Kligman, 1974). [This procedure usually involves five 48-hr covered patch tests (often separated by 24 hr) followed 10-14 days later by a 48-hr covered challenge patch test using the same concentration].

A patch test with ethyl maltol at 10% in petrolatum [presumably 24/48-hr covered contact] failed to produce a response in a women who had developed eczema around the lips following long-term use of a strawberry lip salve containing ethyl maltol (Taylor et al. 1996).

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing ethyl maltol was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of ethyl maltol when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	19.5	JTI KB Study Report(s)
In vitro cytotoxicity	19.5	JTI KB Study Report(s)

High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 2-ethyl-3-hydroxy-4H-pyran-4-one (CAS RN 4940-11-8) 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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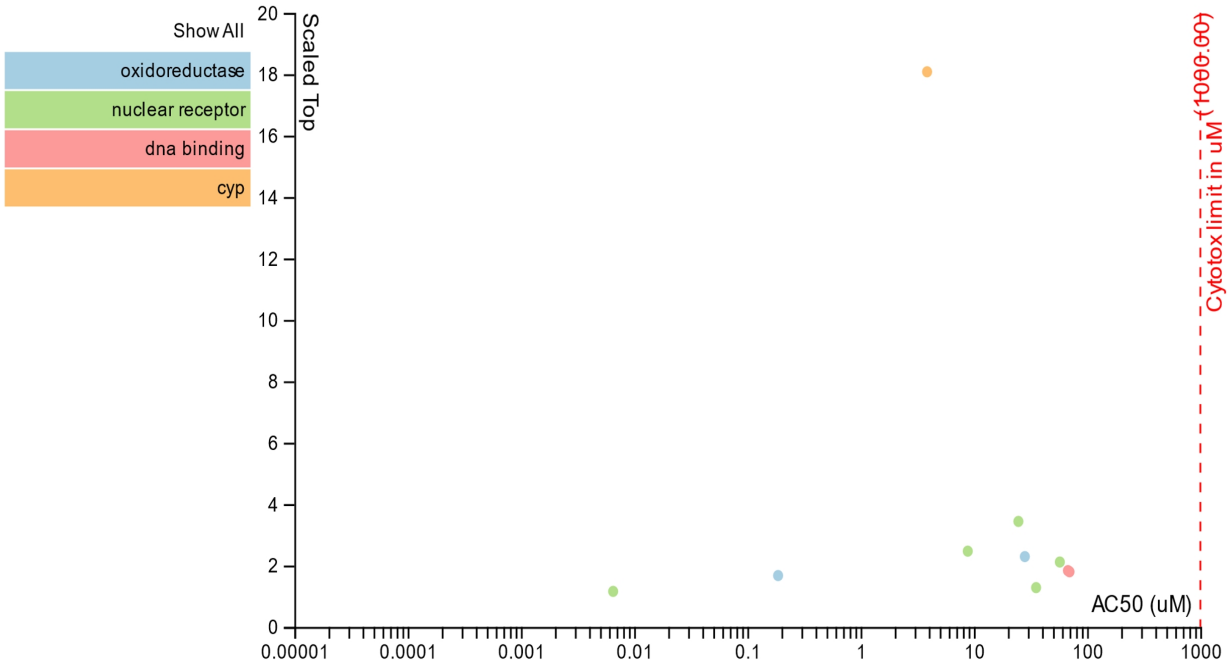
A summary of the ToxCast assay data on 2-ethyl-3-hydroxy-4H-pyran-4-one is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

“Tobacco products containing flavorings, such as electronic nicotine delivery devices (ENDS) or e-cigarettes, cigars/cigarillos, waterpipes, and heat-not-burn devices (iQOS) are continuously evolving. In addition to increasing the exposure of teenagers and adults to nicotine containing flavoring products and flavoring enhancers, chances of nicotine addiction through chronic use and abuse also increase. These flavorings are believed to be safe for ingestion, but little information is available about their effects on the lungs. In this review, we have discussed the in vitro and in vivo data on toxicity of flavoring chemicals in lung cells. We have further discussed the common flavoring agents, such as diacetyl and menthol, currently available detection methods, and the toxicological mechanisms associated with oxidative stress, inflammation, mucociliary clearance, and DNA damage in cells, mice, and humans. Finally, we present potential biomarkers that could be utilized for future risk assessment. This review provides crucial parameters important for evaluation of risk associated with flavouring agents and flavoring enhancers used in tobacco products and ENDS. Future studies can be designed to address the potential toxicity of inhaled flavorings and their biomarkers in users as well as in chronic exposure studies.” As taken from Kaur G et al. 2018. Toxicology Letters 288, 143-155. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29481849

6.2. Cardiovascular system

“The nature of the blood changes in rats repeatedly fed ethyl maltol has led to the suggestion that exposure may be a particular problem for patients suffering from one of the thalassaemia disorders (hereditary abnormalities of the red blood cells).” (BIBRA, 1996)

6.3. Nervous system

“Central nervous system depression was seen in mice given 150 mg/kg bw or greater. In addition, the investigators reported an increased hexabarbital sleeping time, which may also indicate an effect on liver enzymes.” (BIBRA, 1996).

Evidence of central nervous system depression (including [transient] inhibition of spontaneous motor activity, anti-convulsant action and loss of righting reflex) were seen in mice given ethyl maltol by subcutaneous injection at 150 mg/kg bw and above (Aoyagi et al. 1974; Kimura et al. 1980).

Exposure of mice (apparently groups of 4 females) to an unspecified concentration of the vapour for 1 hour caused slightly increased physical activity in one group (Buchbauer et al. 1993).

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

“3-hydroxy-2-ethyl-4-pyrone (ethyl maltol) chelate iron with a high affinity and selectivity. The resulting 1:3 (metal-ligand) complexes, being neutral, are able to partition readily across cell membranes and thus may facilitate iron transport across the intestinal wall.” As taken from Barrand MA et al. 1987. J Pharm Pharmacol. 39(3), 203-11. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/2883285?dopt=Abstract

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

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	288	Carmines, 2002 & Rustemeier et al. 2002
	117	Baker et al. 2004a
	9.1 65 240 (cigar)	JTI KB Study Report(s)
	3,870	Gaworski et al. 2011 & Coggins et al. 2011f
	291	Roemer et al, 2014
In vitro genotoxicity	288	Carmines, 2002 & Roemer et al. 2002
	117	Baker et al. 2004c
	9.1	Renne et al. 2006

	9.1 65 240 (cigar)	JTI KB Study Report(s)
	161	fGLH Study Report (2010)
	3,870	Gaworski et al. 2011 & Coggins et al. 2011f
	291	Roemer et al, 2014
In vitro cytotoxicity	288	Carmines, 2002 & Roemer et al. 2002
	117	Baker et al. 2004c
	9.1 65 240 (cigar)	JTI KB Study Report(s)
	161	fGLH Study Report (2010)
	3,870	Gaworski et al. 2011 & Coggins et al. 2011f
	291	Roemer et al, 2014
Inhalation study	4	Gaworski et al. 1998
	288	Carmines, 2002 & Vanscheeuwijck et al. 2002
	117	Baker et al. 2004c
	9.1	Renne et al. 2006
	9.1 65 240 (cigar)	JTI KB Study Report(s)
	291	Schramke et al, 2014
Skin painting	4	Gaworski et al. 1999
	9.1 65	JTI KB Study Report(s)
In vivo genotoxicity	291 240 (cigar)	Schrmake et al, 2014 JTI KB Study Report(s)

In a pyrolysis study, 100% of ethyl maltol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing ethyl maltol was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of ethyl maltol when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
Aerosol Chemistry	1000	Crooks et al; 2018

In vitro genotoxicity	19.5	JTI KB Study Report(s)
	1000	Crooks et al; 2018
In vitro cytotoxicity	19.5	JTI KB Study Report(s)
	1000	Crooks et al; 2018
In vitro carcinogenicity (Cell Transformation Assay)	1000	Crooks et al; 2018

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

Endpoint	**Tested level (ppm)**	**Reference**
Aerosol chemistry	3,000	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	3,000	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	3,000	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	750	Logic (2019a)
Inhalation study	750	Logic (2019a)

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

Endpoint	**Tested level**	**Reference**
Aerosol chemistry	0.0866 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.0866 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.0866 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.0866 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.0866 mg/(tobacco portion; 310 mg)	Logic (2019b)

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

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	1.41	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	1.41	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	1.41	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

“BACKGROUND: Flavoring chemicals, or flavorants, have been used in electronic cigarettes (e-cigarettes) since their inception; however, little is known about their toxicological effects. Free radicals present in e-cigarette aerosols have been shown to induce oxidative stress resulting in damage to proliferation, survival, and inflammation pathways in the cell. Aerosols generated from e-liquid solvents alone contain high levels of free radicals but few studies have looked at how these toxins are modulated by flavorants. OBJECTIVES: We investigated the effects of different flavorants on free radical production in e-cigarette aerosols. METHODS: Free radicals generated from 49 commercially available e-liquid flavors were captured and analyzed using electron paramagnetic resonance (EPR). The flavorant composition of each e-liquid was analyzed by gas chromatography mass spectroscopy (GCMS). Radical production was correlated with flavorant abundance. Ten compounds were identified and analyzed for their impact on free radical generation. RESULTS: Nearly half of the flavors modulated free radical generation. Flavorants with strong correlations included β-damascone, δ-tetradecalactone, γ-decalactone, citral, dipentene, ethyl maltol, ethyl vanillin, ethyl vanillin PG acetal, linalool, and piperonal. Dipentene, ethyl maltol, citral, linalool, and piperonal promoted radical formation in a concentration-dependent manner. Ethyl vanillin inhibited the radical formation in a concentration dependent manner. Free radical production was closely linked with the capacity to oxidize biologically-relevant lipids. CONCLUSIONS: Our results suggest that flavoring agents play an important role in either enhancing or inhibiting the production of free radicals in flavored e-cigarette aerosols. This information is important for developing regulatory strategies aimed at reducing potential harm from e-cigarettes.” As taken from Bitzer ZT et al. 2018. Free Radic. Biol. Med. 120, 72-79. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29548792

“Tobacco products containing flavorings, such as electronic nicotine delivery devices (ENDS) or e-cigarettes, cigars/cigarillos, waterpipes, and heat-not-burn devices (iQOS) are continuously evolving. In addition to increasing the exposure of teenagers and adults to nicotine containing flavoring products and flavoring enhancers, chances of nicotine addiction through chronic use and abuse also increase. These flavorings are believed to be safe for ingestion, but little information is available about their effects on the lungs. In this review, we have discussed the in vitro and in vivo data on toxicity of flavoring chemicals in lung cells. We have further discussed the common flavoring agents, such as diacetyl and menthol, currently available detection methods, and the toxicological mechanisms associated with oxidative stress, inflammation, mucociliary clearance, and DNA damage in cells, mice, and humans. Finally, we present potential biomarkers that could be utilized for future risk assessment. This review provides crucial parameters important for evaluation of risk associated with flavouring agents and flavoring enhancers used in tobacco products and ENDS. Future studies can be designed to address the potential toxicity of inhaled flavorings and their biomarkers in users as well as in chronic exposure studies.” As taken from Kaur G et al. 2018. Toxicology Letters 288, 143-155. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29481849

“Whereas JUUL electronic cigarettes (ECs) have captured the majority of the EC market, with a large fraction of their sales going to adolescents, little is known about their cytotoxicity and potential effects on health. The purpose of this study was to determine flavor chemical and nicotine concentrations in the eight currently marketed prefilled JUUL EC cartridges ("pods") and to evaluate the cytotoxicity of the different variants (e.g., "Cool Mint" and "Crème Brulee") using in vitro assays. Nicotine and flavor chemicals were analyzed using gas chromatography-mass spectrometry in pod fluid before and after vaping and in the corresponding aerosols. 59 flavor chemicals were identified in JUUL pod fluids, and 3 were >1 mg/mL. Duplicate pods were similar in flavor chemical composition and concentration. Nicotine concentrations (average 60.9 mg/mL) were significantly higher than those of any EC products we have previously analyzed. The transfer efficiency of individual flavor chemicals that were >1 mg/mL and nicotine from the pod fluid into aerosols was generally 35-80%. All pod fluids were cytotoxic at a 1:10 dilution (10%) in the MTT and neutral red uptake assays when tested with BEAS-2B lung epithelial cells. Most aerosols were cytotoxic in these assays at concentrations between 0.2 and 1.8%. The cytotoxicity of collected aerosol materials was highly correlated with nicotine and ethyl maltol concentrations and moderately to weakly correlated with total flavor chemical concentration and menthol concentration. Our study demonstrates that (1) some JUUL flavor pods have sufficiently high concentrations of flavor chemicals that may make them attractive to youth and (2) the concentrations of nicotine and some flavor chemicals (e.g., ethyl maltol) are high enough to be cytotoxic in acute in vitro assays, emphasizing the need to determine if JUUL products will lead to adverse health effects with chronic use..” As taken form Omaiye EE et al. 2019a. Chem Res Toxicol. 32(6), 1058-1069. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30896936/

“BACKGROUND: Most electronic-cigarette liquids contain propylene glycol, glycerin, nicotine and a wide variety of flavors of which many are sweet. Sweet flavors are classified as saccharides, esters, acids or aldehydes. This study investigates changes in cariogenic potential when tooth surfaces are exposed to e-cigarette aerosols generated from well-characterized reference e-liquids with sweet flavors. METHODS: eference e-liquids were prepared by combining 20/80 propylene glycol/glycerin (by volume fraction), 10 mg/mL nicotine, and flavors. Aerosols were generated by a Universal Electronic-Cigarette Testing Device (49.2 W, 0.2 Ω). Streptococcus mutans (UA159) were exposed to aerosols on tooth enamel and the biological and physiochemical parameters were measured. RESULTS: E-cigarette aerosols produced four-fold increase in microbial adhesion to enamel. Exposure to flavored aerosols led to two-fold increase in biofilm formation and up to a 27% decrease in enamel hardness compared to unflavored controls. Esters (ethyl butyrate, hexyl acetate, and triacetin) in e-liquids were associated with consistent bacteria-initiated enamel demineralization, whereas sugar alcohol (ethyl maltol) inhibited S. mutans growth and adhesion. The viscosity of the e-liquid allowed S. mutans to adhere to pits and fissures. Aerosols contained five metals (mean ± standard deviation): calcium (0.409 ± 0.002) mg/L, copper (0.011 ± 0.001) mg/L, iron (0.0051 ± 0.0003) mg/L, magnesium (0.017 ± 0.002) mg/L, and silicon (0.166 ± 0.005) mg/L. CONCLUSIONS: This study systematically evaluated e-cigarette aerosols and found that the aerosols have similar physio-chemical properties as high-sucrose, gelatinous candies and acidic drinks. Our data suggest that the combination of the viscosity of e-liquids and some classes of chemicals in sweet flavors may increase the risk of cariogenic potential. Clinical investigation is warranted to confirm the data shown here.” As taken from Kim SA et al. 2018. PLoS One 13(9), e0203717. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30192874

“Thousands of electronic cigarette refill fluids are commercially available. The concentrations of nicotine and the solvents, but not the flavor chemicals, are often disclosed on product labels. The purpose of this study was to identify and quantify flavor chemicals in 39 commercial refill fluids that were previously evaluated for toxicity. Twelve flavor chemicals were identified with concentrations ≥1 mg/ml: cinnamaldehyde, menthol, benzyl alcohol, vanillin, eugenol, p-anisaldehyde, ethyl cinnamate, maltol, ethyl maltol, triacetin, benzaldehyde, and menthone. Transfer of these flavor chemicals into aerosols made at 3V and 5V was efficient (mean transfer = 98%). We produced lab-made refill fluids containing authentic standards of each flavor chemical and analyzed the toxicity of

their aerosols produced at 3V and 5V using a tank Box Mod device. Over 50% of the refill fluids in our sample contained high concentrations of flavor chemicals that transferred efficiently to aerosols at concentrations that produce cytotoxicity. When tested with two types of human lung cells, the aerosols made at 5V were generally more toxic than those made at 3V. These data will be valuable for consumers, physicians, public health officials, and regulatory agencies when discussing potential health concerns relating to flavor chemicals in electronic cigarette products." As taken from Behar RZ et al. 2018. Sci. Rep. 8(1), 8288. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29844439

"We characterized the flavor chemicals in a broad sample of commercially available electronic cigarette (EC) refill fluids that were purchased in four different countries. Flavor chemicals in 277 refill fluids were identified and quantified by gas chromatography-mass spectrometry, and two commonly used flavor chemicals were tested for cytotoxicity with the MTT assay using human lung fibroblasts and epithelial cells. About 85% of the refill fluids had total flavor concentrations >1 mg/ml, and 37% were >10 mg/ml (1% by weight). Of the 155 flavor chemicals identified in the 277 refill fluids, 50 were present at ≥1 mg/ml in at least one sample and 11 were ≥10 mg/ml in 54 of the refill fluids. Sixty-one% (170 out of 277) of the samples contained nicotine, and of these, 56% had a total flavor chemical/nicotine ratio >2. Four chemicals were present in 50% (menthol, triacetin, and cinnamaldehyde) to 80% (ethyl maltol) of the samples. Some products had concentrations of menthol ("Menthol Arctic") and ethyl maltol ("No. 64") that were 30 times (menthol) and 100 times (ethyl maltol) their cytotoxic concentration. One refill fluid contained cinnamaldehyde at ~34% (343 mg/ml), more than 100,000 times its cytotoxic level. High concentrations of some flavor chemicals in EC refill fluids are potentially harmful to users, and continued absence of any regulations regarding flavor chemicals in EC fluids will likely be detrimental to human health." As taken from Omaiye EE et al. 2019b. Sci. Rep. 9(1), 2468. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30792477

"Electronic cigarettes (e-cigs) deliver nicotine in an aerosol to the user that simulates the smoke of traditional cigarettes purportedly without the pathology of inhaling tobacco smoke due to the absence of combustion. Advanced versions of e-cigs enable the user to potentially moderate the concentration of drug in the aerosol by selecting from a range of voltages on the power supply. A method was developed to trap the aerosol produced by a KangerTech AeroTank, 1.8 Ω preassembled atomizer in order to analyze the concentration of nicotine and to evaluate the constituents of the aerosol at various voltages on the power supply. A 12-mg/mL formulation of nicotine in 50:50 propylene glycol (PG):vegetable glycerin (VG) was used to produce aerosol at 3.9, 4.3 and 4.7 V. The aerosol was trapped in a simple glass assemblage and analyzed by a 3200 Q Trap HPLC-MS-MS. The dose of nicotine delivered in the aerosol at 3.9, 4.3 and 4.7 V was determined to be 88 ± 12 µg, 91 ± 15 µg and 125 ± 22 µg. The average recovery of nicotine in the trap across the voltages was 99.8%. The glass trap system was an effective device for collecting the aerosol for analysis and an increase in drug yield was observed with increasing voltage from the power supply on the e-cig. The glass trap system was also used in combination with a 100-µm solid-phase microextraction fiber to capture the aerosol and analyze it via DART-MS and GC-MS. Four commercial e-liquids labeled to contain nicotine were aerosolized at 4.3 V. The pharmacologically active ingredient, nicotine, as well as PG, VG and a number of flavoring agents found in these formulations were identified." As taken from Peace MR et al. 2018. J. Anal. Toxicol. 42(8), 537-543. Available at https://www.ncbi.nlm.nih.gov/pubmed/30371842

"The in vitro mutagenic and genotoxic potential of Heated Tobacco Products (HTPs) has already been studied with the particulate phase and reported previously. This study has been designed to complement the in vitro assessment of the HTP and to determine whether the inclusion of potential flavourings would alter the in vitro response by testing the other phase of the aerosol, the gas-vapour phase (GVP). Both flavoured and unflavoured Neostik GVP samples did not show any sign of mutagenic activity in the Ames test but induced a mutagenic response in the mouse lymphoma assay (MLA), however, these responses were significantly less than those of the reference

cigarette, 3R4F. The results demonstrated that GVP emissions of this HTP did not induce either new qualitative or quantitative mutagenic hazards compared to 3R4F, as assessed by the Ames test (no new responsive strains) and MLA (a lower mutagenic response), respectively. A statistical comparative analysis of the responses showed that the addition of flavourings that may thermally decompose under the conditions of use did not add to the in vitro baseline responses of the unflavoured Neostik." As taken from Le Godec T et al. 2019. Toxicol. Rep. 6, 1155-1163. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31737489/

"Introduction: A recent study raised concerns about e-cigarette liquids toxicity by reporting the presence of 14 flavouring chemicals with toxicity classification. However, the relevant toxicity classification was not estimated according to the measured concentrations. The purpose of this study was to calculate the toxicity classification for different health hazards for all the flavouring chemicals at the maximum concentrations reported. Methods: The analysis was based on the European Union Classification Labelling and Packaging regulation. The concentration of each flavouring chemical was compared with the minimum concentration needed to classify it as toxic. Additionally, toxicity classification was examined for a theoretical e-cigarette liquid containing all flavouring chemicals at the maximum concentrations reported. Results: There was at least one toxicity classification for all the flavouring chemicals, with the most prevalent classifications related to skin, oral, eye and respiratory toxicities. One chemical (methyl cyclopentenolone) was found at a maximum concentration 150.7% higher than that needed to be classified as toxic. For the rest, the maximum reported concentrations were 71.6 to > 99.9% lower than toxicity concentrations. A liquid containing all flavouring compounds at the maximum concentrations would be classified as toxic for one category only due to the presence of methyl cyclopentenolone; a liquid without methyl cyclopentenolone would have 66.7 to > 99.9% lower concentrations of flavourings than those needed to be classified as toxic. Conclusions: The vast majority of flavouring compounds in e-cigarette liquids as reported in a recent study were present at levels far lower than needed to classify them as toxic. Since exceptions exist, regulatory monitoring of liquid composition is warranted." "The difference between the minimum concentrations needed to classify a solution as toxic and the maximum concentrations reported by Vardavas et al. ranged from approximately 72% (for ethyl maltol) to > 99.9% (250.000-fold lower concentration for limonene)." As taken from Farsalinos K and Lagoumintzis G. 2019. Harm. Reduct. J. 16(1), 48. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31345235/

"The aerosols generated from e-cigarettes are composed of liquid and gas phases resulting from vapourized e-liquid. The apportioning of substances from e-liquid into the liquid and gas phases during e-cigarette use has not been extensively studied. Partitioning of e-liquid components between the gas and the liquid phase of the aerosol influences the substances inhaled and exhaled by the users, leading to second-hand exposure. It seems important to determine which compounds and how much of them are transferred into the gas phase and may immediately enter the bloodstream. For this purpose, a method based on thermal desorption followed by gas chromatography coupled with tandem mass spectrometry (GC–MS/MS) in electron ionization mode was developed. As in a previous study, an automatic generator of an aerosol from an e-cigarette with a collection tube filled with melt-blown non-woven fabric discs and equipped with Tenax TA sorption tubes was used. The melt-blown non-woven fabric is designed to capture liquid phase compounds, while sorption tubes are meant to sorb compounds in the gas phase of the aerosol. To control the e-liquid mass changes before and after a puff session, quantitation based on the mass change tracking approach (MCT) was applied. Accuracy of the developed method ranged between 91% and 110% regardless of the spiking level, with precision and reproducibility better than 10%. The limits of detection (LODs) ranged from 0.015 to 0.076 ng of substance emitted/mg of consumed e-liquid, while limits of quantitation (LOQs) ranged from 0.045 to 0.23 ng of substance emitted/mg of consumed e-liquid. Most of the compounds are deposited in the liquid phase of the aerosol, while only trace levels of some substances may be observed in an actual, non-condensed gas phase." As taken from Aszyk J et al. 2019. Microchemical Journal 148, 717-724. Available at https://www.sciencedirect.com/science/article/abs/pii/S0026265X19311634

“Flavor chemicals in electronic cigarette fluids (ECs), which may negatively impact human health, have been studied in a limited number of countries/locations. To gain an understanding of how the composition and concentrations of flavor chemicals in ECs are influenced by product sale location, we evaluated refill fluids manufactured by one company (Ritchy LTD) and purchased worldwide. Flavor chemicals were identified and quantified using gas chromatography-mass spectrometry (GC-MS). We then screened the fluids for their effects on cytotoxicity (MTT assay) and proliferation (live-cell imaging) and tested authentic standards of specific flavor chemicals to identify those that were cytotoxic at concentrations found in refill fluids. One hundred twenty-six flavor chemicals were detected in 103 bottles of refill fluid, and their number per/bottle ranged from 1 – 50 based on our target list. Two products had none of the flavor chemicals on our target list, nor did they have any non-targeted flavor chemicals. Twenty-eight flavor chemicals were present at concentrations ≥ 1 mg/mL in at least one product, and 6 of these were present at concentrations ≥ 10 mg/mL. The total flavor chemical concentration was ≥ 1 mg/mL in 70% of the refill fluids and ≥ 10 mg/mL in 26%. For sub-brand duplicate bottles purchased in different countries, flavor chemical concentrations were similar and induced similar responses in the in vitro assays (cytotoxicity and cell growth inhibition). The levels of furaneol, benzyl alcohol, ethyl maltol, ethyl vanillin, corylone, and vanillin were significantly correlated with cytotoxicity. The margin of exposure calculations showed that pulegone and estragole levels were high enough in some products to present a non-trivial calculated risk for cancer. Flavor chemical concentrations in refill fluids often exceeded concentrations permitted in other consumer products. These data support the regulation of flavor chemicals in EC products to reduce their potential for producing both cancer and non-cancer toxicological effects.”
Omaiye EE et al. (2021) Electronic Cigarette Refill Fluids Sold Worldwide: Flavor Chemical Composition, Toxicity and Hazard Analysis.

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that 4H-pyran-4-one, 2-ethyl-3-hydroxy- (CAS RN 4940-11-8) 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	0.6718
TOPKAT probability of biodegradation	0.814
EPI Predicted Ozone reaction half-life (days)	0.8731
EPI Predicted Atmospheric Oxidation half-life (days)	0.2096

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 :	8.69E-006 atm-m3/mole (8.80E-001 Pa-

	m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.103E-009 atm-m3/mole (1.118E-004 Pa-m3/mole) VP: 0.000145 mm Hg (source: MPBPVP) WS: 2.42E+004 mg/L (source: WSKOWWIN)

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

Log Kow used:	0.63 (exp database)
Log Kaw used:	-3.449 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	4.079
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.4990 0.1486 3.0183 (weeks) 3.7430 (days-weeks) 0.6378 0.6718 0.1191
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	0.052 Pa (0.00039 mm Hg)
Log Koa (Koawin est):	4.079
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	5.77E-005 2.94E-009

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.00208
Mackay model:	0.00459

Octanol/air (Koa) model:	2.36E-007

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	51.0213 E-12 cm3/molecule-sec
Half-Life =	0.210 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	2.516 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	1.312500 E-17 cm3/molecule-sec
Half-Life =	0.873 Days (at 7E11 mol/cm3)
Half-Life =	20.955 Hrs
Fraction sorbed to airborne particulates (phi): 0.00334 (Junge-Pankow, Mackay avg) 2.36E-007 (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 :	9.271 L/kg (Kow method)
Log Koc:	0.967 (Kow method)

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

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 8.69E-006 atm-m3/mole (estimated by Bond SAR Method)

Half-Life from Model River:	80.97 hours (3.374 days)
Half-Life from Model Lake:	982.5 hours (40.94 days)

Removal In Wastewater Treatment:

Total removal:	2.34 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.76 percent

Total to Air:	0.49 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.584	4.06	1000
Water	40.2	360	1000
Soil	59.1	720	1000
Sediment	0.0905	3.24e+003	0

Persistence Time: 357 hr

10.2. Aquatic toxicity

According to the Ecological Categorization Results from the Canadian Domestic Substances List, 4H-pyran-4-one, 2-ethyl-3-hydroxy- (CAS RN 4940-11-8) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	1127.877
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	1,127.877
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	11,118.1514
Toxicity to fish (LC50 in mg/l) as predicted by Aster	1,813.137375
Toxicity to fish (LC50 in mg/l) as predicted by PNN	6,207.24801
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	5,691.959
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	4.12E+001

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

ECOSAR Version 1.11 reports the following aquatic toxicity data for CAS RN 4940-11-8:

Values used to Generate ECOSAR Profile

Log Kow: 0.296 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2.423E+004 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations Vinyl/Allyl Ketones Vinyl/Allyl Ethers

Vinyl/Allyl Alcohols

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	
Vinyl/Allyl Ketones :	Fish	96-hr	LC50	2841.477	
Vinyl/Allyl Ketones :	Daphnid	48-hr	LC50	3212.065	
Vinyl/Allyl Ketones :	Green Algae	96-hr	EC50	1349.848	
Vinyl/Allyl Ketones :	Fish		ChV	1752.268	
Vinyl/Allyl Ketones :	Daphnid		ChV	342.061 !	
Vinyl/Allyl Ketones :	Green Algae		ChV	211.703 !	
Vinyl/Allyl Ketones :	Fish (SW)	96-hr	LC50	2.3e+005 *	
Vinyl/Allyl Ketones :	Mysid (SW)	96-hr	LC50	5311.270	
Vinyl/Allyl Ketones :	Fish (SW)		ChV	10566.357	
Vinyl/Allyl Ketones :	Mysid (SW)		ChV	1498.552 !	
Vinyl/Allyl Ethers :	Fish	96-hr	LC50	5.638	
Vinyl/Allyl Ethers :	Daphnid	48-hr	LC50	909.284	
Vinyl/Allyl Ethers :	Green Algae	96-hr	EC50	576.029	
Vinyl/Allyl Ethers :	Fish		ChV	0.498 !	
Vinyl/Allyl Ethers :	Daphnid		ChV	99.064 !	
Vinyl/Allyl Ethers :	Green Algae		ChV	107.875	
Vinyl/Allyl Alcohols :	Fish	96-hr	LC50	4.880	
Vinyl/Allyl Alcohols :	Daphnid	48-hr	LC50	0.591	
Vinyl/Allyl Alcohols :	Green Algae	96-hr	EC50	205.375	
Vinyl/Allyl Alcohols :	Fish		ChV	0.391 !	
Vinyl/Allyl Alcohols :	Daphnid		ChV	0.057 !	
Vinyl/Allyl Alcohols :	Green Algae		ChV	21.486	
Neutral Organic SAR :	Fish	96-hr	LC50	3901.708	
(Baseline Toxicity) :	Daphnid	48-hr	LC50	1892.938	

	Green Algae	96-hr	EC50	735.805	
	Fish		ChV	316.789	
	Daphnid		ChV	119.153	
	Green Algae		ChV	135.745	

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.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR Version 1.11 reports the following terrestrial toxicity data for CAS RN 4940-11-8:

Values used to Generate ECOSAR Profile

Log Kow: 0.296 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2.423E+004 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Vinyl/Allyl Ethers

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Vinyl/ Allyl Ethers :	Earthworm	14-day	LC50	1504.713

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.5007 days (HL = 0.03157 days)
Log BCF Arnot-Gobas method (upper trophic):	0.058 (BCF = 1.143)
Log BAF Arnot-Gobas method (upper trophic):	0.058 (BAF = 1.143)

log Kow used:	0.63 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List state that 4H-pyran-4-one, 2-ethyl-3-hydroxy- (CAS RN 4940-11-8) is not bioaccumulative in the environment:

Empirical Log Kow	0.63
Log Kow predicted by KowWin	0.3
Log BAF T2MTL predicted by Gobas	0.0814528276491076
Log BCF 5% T2LTL predicted by Gobas	0.0669011438021939
Log BCF Max predicted by OASIS	1.09964230364523
Log BCF predicted by BCFWIN	0.5

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

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13. Last audited

April 2022

FAO Nutrition Meetings
Report Series No. 48A
WHO/FOOD ADD/70.39

TOXICOLOGICAL EVALUATION OF SOME EXTRACTION SOLVENTS AND CERTAIN OTHER SUBSTANCES

The content of this document is the result of the deliberations of the Joint FAO/WHO Expert Committee on Food Additives which met in Geneva, 24 June -2 July 1970[1]

Food and Agriculture Organization of the United Nations
World Health Organization

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[1] Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives, FAO Nutrition Meetings Report Series in press; Wld Hlth Org. techn. Rep. Ser., in press.

ETHYL MALTOL

Biological data

Biochemical aspects

Oral administration of ethyl maltol is almost completely absorbed from the gut. 65-70% appears in the urine as gluconomide or sulfate within 2 hours. None was detected in the faeces (Gralla et al., 1969).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body weight)	References
Mouse	oral	780	Gralla et al., 1969
Rat	oral	1150	Gralla et al., 1969
Chick	oral	1270	Gralla et al., 1969

Short-term studies

Rat. Four groups of 10 male and 10 female rats were fed for 90 days on diets containing 0, 250, 500 or 1000 mg/kg body-weight of ethyl maltol. No abnormalities were detected with regard to survival, growth, organ weight, haematology, urinalysis, gross-and histopathology with the exception of some anaemia and icterus at the 250 mg/kg dose level. There was slight depression of body-weight only in females at the 500 and 1000 mg/kg level and very slight reduction at the 250 mg/kg level. The only pathological abnormality was noted at the highest level and consisted of dilatation of the glomerular tuft with protein loss and casts in Bowman's space and renal tubules (Gralla et al., 1969).

Dog. Four groups of beagles each received ethyl maltol in oral capsules at 0, 125, 250 and 500 mg/kg body-weight/day for 90 days. No deleterious effects were noted on mortality, body-weight gain, haematology, urinalysis, clinical chemistry and gross-and histopathology. Slight icterus was noted in the serum of animals at the two highest levels tested but this colour change may have been due to an iron complex formed by ethyl maltol. Vomiting occurred at the highest dose level (Gralla et al., 1969).

In another experiment four groups of 8 dogs each received orally capsules containing ethyl maltol at 0, 50, 100 and 200 mg/kg body-weight/day for 2 years. No abnormalities were found as regards mortality, body-weight, organ weight, haematology. urinalysis, clinical chemistry. gross-and histopathology except for slight myeloid

hyperplasia of the sternal marrow in 2 females at the 200 mg/kg level (Gralla et al., 1969).

Long-term studies

Rat. Groups of 25 male and female rats were fed for 2 years on

diets containing ethyl maltol at the following dose levels: 0, 50, 100 and 200 mg/kg body-weight. No abnormalities were seen as regards growth rate or food consumption, urinalysis and haematology. Five male and five female rats were sacrificed after one year and the remainder after two years. There was no significant difference between controls and test animals with respect to growth, rate weight, survival, urinalysis, haematology, clinical chemistry, tumour incidence, gross-and histopathology (Gralla et al., 1969).

Special studies

Rat. Four groups of 20 rats given 0, 50, 100 and 200 mg/kg bodyweight/day were mated after 90 days feeding for 18 days and allowed-to produce a first litter. After a rest period they were mated again for 18 days and produced a second litter. No difference was seen between controls and test animals as regards conception, litter size, survival of pups, weight at weaning and teratology at 21 days of age (Gralla et al., 1969).

Comments

No data are available on the metabolic behaviour of ethyl maltol and studies on this aspect would be desirable. Adequate two year studies have been provided in rat and dog, in addition to a reproduction study in rats.

Evaluation

Level causing no toxicological effect in the rat

0.4 per cent (= 4000 ppm) equivalent to 200 mg/kg bodyweight in the rat.

Estimate of acceptable daily intake for man

	mg/kg body-weight
Unconditional acceptance	0-2

REFERENCES

Gralla, E. et al. (1969) Toxicol. Appl. Pharmacol., 15, 604.

See Also:

Toxicological Abbreviations

ETHYL MALTOL (JECFA Evaluation)

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

TOXICOLOGICAL EVALUATION OF SOME FOOD COLOURS, ENZYMES, FLAVOUR ENHANCERS, THICKENING AGENTS, AND CERTAIN FOOD ADDITIVES

WHO FOOD ADDITIVES SERIES 6

The evaluations contained in this publication were prepared by the Joint FAO/WHO Expert Committee on Food Additives which met in Rome, 4-13 June 1974[1]

World Health Organization Geneva 1975

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[1] Eighteenth Report of the Joint FAO/WHO Expert Committee on Food Additives, Wld Hlth Org. techn. Rep. Ser., 1974, No. 557. FAO Nutrition Meetings Report Series, 1974, No. 54.

ETHYLMALTOL

Explanation

This compound has been evaluated for acceptable daily intake by the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1,

Ref. No. 22) in 1970. No toxicological monograph has been published at that time.

In the Fourteenth Report[1] it was noted that ethylmaltol is intended as an alternative to its homologue, meltol. Data were sufficient to assign an acceptable daily intake of 0-2 mg/kg bw.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Orally administered ethylmaltol is almost completely absorbed from the gut. 65-70% appears in the urine as glucuronide/or sulfate within two hours. None was detected in the faeces (Gralla et al., 1969). Following oral (200 mg/kg) and i.v. (10 mg/kg) administration to dogs showed that orally given ethylmaltol is rapidly and extensively absorbed from the gut. 65-70% are excreted in the urine as sulfate and glucuronide after oral and 30-96% after i.v. administration. Faecal excretion varied from 0.3-4% (Rennhard).

TOXICOLOGICAL STUDIES

Special studies on reproduction

Rat

Four groups of 20 rats given 0, 50, 100 and 200 mg/kg bw/day were mated after 90 days feeding for 18 days and allowed to produce a first litter. After a rest period they were mated again for 18 days and produced a second litter. No difference was seen between controls and test animals as regards conception, litter size, survival of pups, weight at weaning and teratology at 21 days of age (Gralla et al., 1969).

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[1] Wld Hlth Org. techn. Rep. Ser., 1971, No. 462

Acute toxicity

Animal	Route	LD_{50} (mg/kg bw)	References
Mouse	Oral	780	Gralla et al., 1969

Rat	Oral	1150	Gralla et al., 1969
Chick	Oral	1270	Gralla et al., 1969

Short-term studies

Rat

Four groups of 10 male and 10 female rats were fed for 90 days on diets containing 0, 250, 500 or 1000 mg/kg bw of ethylmaltol. No abnormalities were detected with regard to survival, growth, organ weight, haematology, urinalysis, gross- and histopathology with the exception of some anaemia and icterus at the 250 mg/kg dose level. There was slight depression of body weight only in females at the 500 and 1000 mg/kg level and very slight reduction at the 250 mg/kg level. The only pathological abnormality was noted at the highest level and consisted of dilation of the glomerular tuft with protein loss and casts in Bowman's space and renal tubules (Gralla et al., 1969).

Dog

Four groups of beagles each received ethylmaltol in oral capsules at 0, 125, 250 and 500 mg/kg bw/day for 90 days. No deleterious effects were noted on mortality, body weight gain, haematology, urinalysis, clinical chemistry and gross- and histopathology. Slight icterus was noted in the serum of animals at the two highest levels tested but this colour change may have been due to an iron complex formed by ethylmaltol. Vomiting occurred at the highest dose level (Gralla et al., 1969).

In another experiment four groups of eight dogs each received orally capsules containing ethylmaltol at 0, 50, 100 and 200 mg/kg bw/day for two years. No abnormalities were found as regards mortality, body weight, organ weight, haematology, urinalysis, clinical chemistry, gross- and histopathology except for slight myeloid hyperplasia of the sternal marrow in two females at the 200 mg/kg level (Gralla et al., 1969).

Long-term studies

Rat

Groups of 25 male and female rats were fed for two years on diets containing ethylmaltol at the following dose levels: 0, 50, 100 and 200 mg/kg bw. No abnormalities were seen as regards growth rate or food consumption, urinalysis and haematology. Five male and five female rats were sacrificed after one year and the remainder after two

years. There was no significant difference between controls and test animals with respect to growth, organ weight, survival, urinalysis, haematology, clinical chemistry, tumour incidence, gross- and histopathology (Gralla et al., 1969).

Comments:

The metabolic data point to rapid absorption and excretion as sulfate and glucuronide, Adequate two-year studies have been provided in rat and dog in addition to a reproduction study in rats.

EVALUATION

Level causing no toxicological effect

Rat: 0.4% (=4000 ppm) in the diet equivalent to 200 mg/kg bw

Estimate of acceptable daily intake for man

0-2 mg/kg bw

REFERENCES

Gralla, E. et al. (1969) Toxicol. appl. Pharmacol., 15, 604

Rennhard, H. H. (1971) J. Agr. Food Chem., Vol. 19, 152

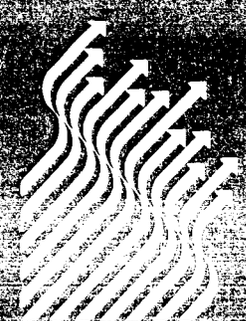
See Also:

Toxicological Abbreviations

Commission of the European Communities

Reports of the Scientific Committee for Food

(Twenty-fifth series)



Report

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Reports of the Scientific Committee for Food

(Twenty-fifth series)

First series of food additives of various technological functions

(Opinion expressed on 18 May 1990)

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Report of the Scientific Committee for Food on a First Series of Food Additives of Various Technological Functions

(Opinion expressed 18 May 1990)

Terms of Reference

To advise on the safety of a first series of additives not yet covered by current Community provisions and included in the categories in the framework directive on food additives [1].

Background

The framework directive lists 24 categories of food additives falling within the scope of the directive (see Annex I).

Community provisions already exist for 7 of these 24 categories: colouring matters [2], preservatives [3], antioxidants [4] and emulsifiers, stabilizers, thickeners and gelling agents [5].

The Committee has already advised on additives in these categories on several occasions, and these opinions have been published in the series of SCF reports:

Colouring matters: 27/6/75 (1st Series), 27/2/76 (2nd Series), 16/9/77 (4th Series), 23/3/79 (8th Series), 7/7/83 (14th Series) and 10/12/87 (21st Series).

Preservatives: 15/11/74 (1st Series), 2/4/76 (2nd Series), 20/10/77 (4th Series), 23/6/78 (6th Series), 31/10/79 (9th Series) and 15/1/81 (11th Series).

Antioxidants: 13/11/75 (1st Series), 2/7/76 (2nd Series), 24/6/77 (4th Series), 29/4/83 (14th Series) and 11/12/87 (22nd Series).

Emulsifiers etc: 30/11/78 (7th Series), 7/2/79 (8th Series), 12/6/81 (13th Series), 8/7/83 (15th series) and 11/11/88 (21st Series).

On 1st May 1978 (5th Series) the Committee advised on the specific use of a series of additives in **fine bakers wares.** In many cases, only temporary evaluations were made. For many of these additives a full evaluation is now included in this report.

In 1976 the Committee initially obtained data on **"acids, bases and salts"**. During the process of evaluation other additives were also considered. The title was therefore changed to "Miscellaneous additives". This expression has, however, led to misunderstandings and has therefore been eliminated from this report.

In the intervening period additives belonging to a number of categories covered by the framework directive were allocated temporary EEC numbers in order to facilitate their identification on food labels [6]. The list provided useful information to the Committee on the number of substances included for evaluation.

With the adoption of the Single European Act it became increasingly necessary to prepare Community provisions for all remaining additives. The Commission services therefore requested industry to establish a complete inventory of all food additives in use in the Member States covered by all the categories listed in the framework directive.

Substances covered in this review

Although this review was intended to cover all remaining additives from the inventory falling within the categories listed in Annex I of the framework directive and not covered by the existing directives, some categories have been omitted from this report:

Sweeteners and modified starches have already been evaluated by the Committee in separate reports: 14/9/84 (16th Series), and 10/12/87 as well as10/11/88, (21st Series) (sweeteners), 27/2/76 (2nd Series) and 12/6/81 (13th Series) (modified starches).

Glazing agents, flour treatment agents, bulking agents and a few other substances will be the subject of separate reports. The Committee also decided that the safety assessment of **enzymes** which are used both as processing aids and as food additives, as well as **novel foods**, require a special approach. They will therefore be evaluated later.

Furthermore, additives falling out of the scope of the directive, such as **processing aids** and **macro- and micro-nutrients**, have not been included in this report and will be dealt with separately.

In some cases a substance, or group of substances, has been included in this review although it was already included in existing directives. These are substances which have technological functions additional to those covered by the existing directives and are therefore included in this review (e.g. citric acid has antioxidant activity but may be also used as an acidifying agent).

The order in which most of the substances have been considered in this review is not based, as in other Committee reports, on technological function but on chemical relationships, as many of the substances have multiple technological applications. The summary table, on pp. 7-8, as well as the table of contents of Annex II on p.10, should enable the reader to locate comments on individual substances.

Principles used in the evaluation

In its 10th report the Committee laid down the guidelines for the safety assessment of food additives. The Commission document "Presentation of an application for assessment of a food additive prior to its authorization" [7] outlines the general procedures for the presentation of biological data and other relevant information.
The available data for many substances in this review are limited. Most of these substances are known to occur naturally in food and/or as intermediate metabolites and to have well established biological properties. Although *natural occurrence per se is not a guarantee of safety* to health, the Committee nevertheless considers further toxicological testing unnecessary as long as the uses are restricted to those indicated in this report. In some cases the Committee made its own review of the available toxicological data. In other cases it was satisfied with the summary information provided by the Commission, interested parties or other international review bodies, e.g. JECFA.

The Committee attaches great importance to the provision of information on the known uses of these additives. The reasons for the evaluations now established by the Committee may be gleaned from the background information collated in Annex II. Any new uses which could alter significantly the total intake of an additive will necessitate a new evaluation. The Committee recommends that any relevant Community legislation adopted in the future pay particular attention to this aspect.

In some cases, only one salt of a group relating to a single anion has been tested. Where appropriate, other ionizable salts of that anion have been included in the group ADI established for this anion.

The cations and anions evaluated in this manner have been listed in the summary table, pp. 7-8. It should be noted, however, that not all possible combinations of these ions have similar toxicological properties. Furthermore the Committee has only evaluated those substances specifically requested for use as food additives. Therefore only those combinations specifically mentioned in the summary table are to be considered acceptable under this evaluation procedure.

Unless otherwise specified, any ADI is expressed as trelating to the named cation or anion

The Committee notes that the framework directive on food additives (art. 3.1) provides that individual conditions of use of food additives should be specified eventually, the extent of these provisions depending on the toxicological evaluation. The Committee supports these principles.

Although the Committee did not systematically assess the actual technological need for these additives, it deems it necessary nevertheless to specify on some occasions that certain additives should be restricted to particular food items. Thus whenever the expression "acceptable" is used it denotes an evaluation of the safety in use under the conditions specified.

The evaluations in this report only cover substances used with a satisfactory food quality specification.

Definition of terms used in this report

ADI (Acceptable Daily Intake) is the amount of a food additive, expressed on a mg/kg body weight basis, that can be ingested daily over a lifetime without incurring any appreciable health risk, and is based on an evaluation of available toxicological data.

ADI not specified is a term used when, on the basis of the available toxicological, biochemical and clinical data, the total daily intake of the substance, arising from its natural occurrence and/or its present use or uses in food at the levels necessary to achieve the desired technological effect, will not represent a hazard to health. For this reason, the establishment of a numerical limit for the ADI is not considered necessary for these substances.

It should be noted that any amount of such substances would not necessarily be toxicologically acceptable. Any additive allocated an "ADI not specified" must be used according to good manufacturing practice, i.e. it should be technologically efficacious, should be used at the lowest level necessary to achieve its technological effect, should not conceal inferior food quality or adulteration, and should not create a nutritional imbalance.

(Toxicologically) acceptable is an evaluation used in those cases where the Committee has not been able to allocate an ADI. If, however, the limited and defined use of the additive is judged to create no health problems, this specific use may be regarded as acceptable from a toxicological point of view.

Not (toxicologically) acceptable is a term used when a substance may be suspected of having undesirable health effects at the proposed level of use, or when the available data are inadequate to assess the safety in use.

(P)MTDI ((Provisional) maximum tolerable daily intake) is the terminology used by JECFA for some nutrients and contaminants without cumulative properties. Its value represents permissible human exposure as a result of the natural or accidental occurrence of the substance in food and in drinking water.

PTWI (Provisional tolerable weekly intake) is the terminology used by JECFA for food contaminants, such as heavy metals, with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods and drinking water.

The designations (P)MTDI and PTWI are normally used for unavoidable contaminants not acceptable as food additives. In this report, however, they have been used in some cases for food additives. In those circumstances it shall be understood that the substances specifically mentioned are acceptable as food additives as long as the total intake from all sources of the element in question lies within the specified limits.

References

1. Council Directive of 21 December 1988 on the approximation of the laws of the Member States concerning **food additives** authorized for use in foodstuffs intended for human consumption.

   Directive 89/107/EEC O.J. L4O of 11.2.89, p.27

2. Council Directive of 23 October 1962 on the approximation of the rules of the Member States concerning the **colouring matters** authorised for use in foodstuffs intended for human consumption (amended 7 times).

   Directive 62/23.10EEC O.J. N° 115 of 11.11.62, p.2645

3. Council Directive of 5 November 1963 on the approximation of the laws of the Member States concerning the **preservatives** authorised for use in foodstuffs intended for human consumption (amended 24 times).

   Directive 64/54/EEC O.J. N° 12 of 27.1.64, p.161

4. Council Directive of 13 July 1970 on the approximation of the laws of the Member States concerning the **antioxidants** authorised for use in foodstuffs intended for human consumption (amended 7 times).

   Directive 70/357/EEC O.J. L157 of 18.7.70, p.31

5. Council Directive of 18 June 1974 on the approximation of the laws of the Member States relating to **emulsifiers, stabilizers, thickeners and gelling agents** for use in foodstuffs (amended 4 times).

   Directive 74/329/EEC O.J. L189 of 12.7.74, p.1

6. Commission Directive of 22 July 1983 introducing temporary measures for the designation of certain ingredients in the **labelling of foodstuffs** for sale to the ultimate consumer.

   Directive 83/463/EEC O.J. L255 of 15.9.83, p.1

7. Presentation of an application for assessment of a food additive prior to its authorization (Catalogue number CB 57-89-370-EN-C (also available in DE and FR)

Introduction to the Summary Table of Evaluations

The table on pages 7 and 8 gives information on those substances included in the evaluations in Annex II. It is divided into two parts:

Part 1 (p. 7) summarizes in tabular form the majority of the substances. The first column lists the anions (vertical) and the most common cations (horizontal) included in the evaluations. The next column (vertical and horizontal) gives the evaluation allocated by the Scientific Committee for Food and the section in Annex II where the specific ion is evaluated. The remaining columns give the EEC numbers of the specific additives:

- Numbers with an "E" prefix refer to existing directives on food additives (refs. 2,3,4 and 5).
- Numbers without "E" refer to the temporary numbers allocated for labelling purposes (ref. 6).
- Numbers in round brackets "( )"refer to substances with no EEC number, but where a number has been assigned by Codex in the International Numbering System (INS).
- Numbers marked with an asterisk "*" are the present, official numbers of these substances. The new, replacement number being allocated to them is indicated in square brackets "[ ]".
- No number: the specific substance is not included in the evaluation either because a technological need has not been established or because the substance is not commercially available.
- "+": no number p.t., but included in the evaluation.
- "–": not acceptable.

Part 2 (p. 8) lists other substances evaluated in this report.

Notes to the Summary Table

1 Large doses of magnesium ions cause diarrhœa and should be avoided.
2 For definition of ADI not specified, see p. 4.
3 Evaluation includes E262 sodium diacetate.
4 The use of ammonium chloride in large amounts in licorice products is currently under evaluation.
5 Evaluation includes 575 glucono-delta lactone.
6 Evaluation includes 370 1,4-heptonolactone.
7 Evaluation includes 529 calcium oxide.
8 Evaluation includes 530 magnesium oxide.
9 Evaluation includes 635 disodium 5'-ribonucleotides.
10 For food specially prepared for small children, only the L(+) isomer should be used.
11 Includes myristic, palmitic, stearic and oleic acid.
12 Evaluation includes 551 silicium dioxide.
13 For aluminium compounds see evaluation in part 2 of the summary table (p. 8).
14 DL-tartaric acid: not acceptable.
353 Metatartric acid: acceptable in wine up to 100 mg/l.

Summary Table of Evaluations (with reference to EEC numbers)

Part 1

ANIONS \ CATIONS	ADI with ref. to section in Annex II	Acid (H^+)	Na^+	K^+	Ca^{++}	Mg^{++} [1]	NH_4^+
		Group ADI not specified[2] (NS) (Section 1.1)					
Acetate [2]	NS 1.3.4	E260	262 [3]	E261	E263		(264)
Adipate	5 mg/kg bw 1.3.5	355	(356)	(357)			(359)
Carbonate	NS 1.3.1	E290	500	501	E170	504	503
Chloride	NS 1.3.1	507	Food ingredient	508	509	511	510 [4]
Citrate	NS 1.3.6	E330	E331	E332	E333		380
Fumarate	6 mg/kg bw 1.3.5	297	(365)	(366)	(367)		
Gluconate [5]	NS 1.3.6	574	576	577	578	(580)	
Glutamate	NS 2.1.2	620	621	622	623	625	624
Glycinate	NS 2.1.1	(640)	+	+	+		
Guanylate [9]	NS 2.2	626	627	628	629		
Heptonate [6]	Not acceptable 1.3.6	–	–	–	–	–	–
Hydroxide	Section 1		524	525	526 [7]	528 [8]	527
Inosinate [9]	NS 2.2	630	631	632	633		
Lactate [10]	NS 1.3.6	E270	E325	E326	E327	(329)	(328)
Malate	NS 1.3.6	296	350	351	352	+	
Fatty Acids [11, 13]	NS 1.3.4	570 *	E470	E470 *	E470 *	[470] 572 *	[470]
[13] Phosphate, ortho	MTDI 70 mg/kg bw from all sources expressed as P 1.3.3	E338	E339	E340	E341	343	(342)
", di + tri			E450 *	E450 *	(450)	(450)	
", poly			[452] E450 *	[452] E450 *	[452] 544 *		545
Silicate [13, 12]	NS 1.3.2		550	(560)	552	553	
Succinate	NS 1.3.5	363					
Sulphate [13]	NS 1.3.1	513	514	515	516	518	(517)
L(+)-Tartrate[13]	30 mg/kg bw 1.3.6	E334	E335	E337 / E336	354		

Summary Table of Evaluations

Part 2

EEC Number	Other Accepted Additives	Evaluation
520 521 522 523 (541) 554 555 556 558 559 573	Aluminium sulphate Aluminium sodium sulphate Aluminium potassium sulphate Aluminium ammonium sulphate Sodium aluminium phosphate, acid Sodium aluminium silicate Potassium aluminium silicate Calcium aluminium silicate Bentonite Aluminium silicate (kaolin) Aluminium salts of fatty acids	PTWI: 7 mg/kg bw expressed as Al from all sources (Section 1.1.2) When setting conditions of use for the accepted substances, intake from natural sources should be taken into account.
579 (585)	Ferrous gluconate Ferrous lactate	Acceptable as colour stabilizing agents in olives (Section 1.1.3)
(512)	Stannous chloride	Acceptable as colour stabilizing agent for white vegetables (canned and bottled) (Section 1.2.1)
519	Cupric sulphate	Acceptable as colour stabilizing agent in canned and bottled green beans and cucumbers (Section 1.2.2)
637	Ethyl maltol	ADI: 1 mg/kg bw (Section 4.4)
(387)	Oxystearin	ADI: 25 mg/kg bw (Section 4.1)
900	Dimethylpolysiloxane	ADI: 1.5 mg/kg bw (Section 4.2)
535 536	Sodium ferrocyanide Potassium ferrocyanide	ADI: 0.025 mg/kg bw (Section 4.3)
(946) (941) E290 (942) (945) (947)	Oxygen Nitrogen Carbon dioxide Nitrous oxide Hydrogen Argon	Toxicologically acceptable as packaging gases and propellants (Section 3)

Annex I

Categories of food additives

as listed in the Annex I of Directive 89/107/EEC of 21.12.88, OJ no L40, 11.2.89, p. 27, on the approximation of the laws of the member states concerning food additives authorized for use in foodstuffs intended for human consumption.

Colour

Preservative

Anti-oxidant

Emulsifier

Emulsifying salt

Thickener

Gelling agent

Stabilizer (1)

Flavour enhancer

Acid

Acidity regulator (2)

Anti-caking agent

Modified starch

Sweetener

Raising agent

Anti-foaming agent

Glazing agent (3)

Flour treatment agent

Firming agent

Humectant

Sequestrant (4)

Enzyme (4) (5)

Bulking agent

Propellent gas and packaging gas

---

(1) This category also comprises foam stabilizers.

(2) These can act as two-way acidity regulators. .

(3) These substances include lubricants.

(4) Inclusion of these terms in this list is without prejudice to any future decision or mention thereof in the labelling of foodstuffs intended for the final consumer.

(5) Only those used as additives.

Annex II

to the Report of the Scientific Committee for Food on a First Series of Food Additives of Various Technological Functions

Evaluation of the Additives

Contents

1. Acids, bases and their salts

1.1 Cations

1.1.1 Ammonium, sodium, potassium, calcium, magnesium

1.1.2 Aluminium

1.1.3 Iron

1.2 Other compounds where use is restricted by the cation

1.2.1 Stannous chloride

1.2.2 Cupric sulphate

1.3 Anions (acids)

1.3.1 Chloride, sulphate, carbonate

1.3.2 Silicate and silicon dioxide

1.3.3 Orthophosphate, di-, tri- and polyphosphate

1.3.4 Monocarboxylic acids and their salts (acetate, fatty acids)

1.3.5 Dicarboxylic acids and their salts (succinate, adipate, fumarate)

1.3.6 Hydroxycarboxylic acids and their salts (lactate, citrate, malate, tartrate, gluconate, heptonate)

2. Amino acids and ribonucleotides

2.1 Amino acids

2.1.1 Glycine and its salts

2.1.2 L-Glutamic acid and its salts

2.1.3 L-Cysteine

2.2 5'-Ribonucleotides (inosinate, guanylate)

3. Packaging gases and propellants

3.1 Carbon dioxide, oxygen, nitrogen

3.2 Nitrous oxide

3.3 Hydrogen

3.4 Argon

4. Others

4.1 Oxystearin

4.2 Dimethylpolysiloxane

4.3 Potassium and sodium ferrocyanide

4.4 Ethylmaltol

1. Acids, bases and their salts

The evaluations of ionizable salts in this report have been based on the respective anions and cations listed below. The evaluation of specific salts may result in ADI's that differ from the general evaluation of the respective ions and such ADI's would supersede the general evaluations. Only those salts listed in the summary tables pp. 6-8 are included in the present evaluations. Other salts will need a separate individual assessment.

The ions contributed by mineral acids and bases (hydroxides), when used as acidity regulators according to GMP, cannot be distinguished toxicologically or analytically from the same ions already present in food from other sources. These contributions are therefore included in the evaluation of the respective anions and cations unless stated otherwise. Where appropriate, metal oxides have been included in the evaluation of the respective cations.

1.1 Cations

1.1.1 Ammonium, sodium, potassium, calcium, magnesium

These cations are natural constituents of man, animals and plants, and therefore occur in foodstuffs. They, together with certain anions, constitute the major electrolytes present in all biological materials. The Committee therefore established a **group ADI not specified** for these cations, although exhaustive systematic toxicological studies have not been carried out with the individual ions. No safety problems are likely to arise, provided the contributions from food intake do not disturb the homoeostatic mechanisms controlling the electrolyte balance of the body. For **magnesium**, large single doses taken in bulk are known to produce diarrhoea particularly in children, and should be avoided.

Calcium oxide and **magnesium oxide** are to be considered included in the group ADI of the ions

1.1.2 Aluminium

Intakes from foods containing aluminium-based food additives represent the major route of human exposure except for those individuals who regularly ingest medication containing aluminium. The toxicology of this cation was reviewed by JECFA and forms the basis of the evaluation. Recent estimates of intake range from about 2-6 mg/day for children and from 6-14 mg/day for teenagers and adults. Metabolic studies on selected aluminium compounds indicated poor absorption even at high levels of consumption and no significant accumulation in the short term. Absorbed aluminium deposits preferentially in the heart, spleen and bone marrow, without any associated histopathological lesions. Only individuals with chronic renal disease accumulate aluminium ions. There are no definite studies relating diet to a possible but unproven association between aluminium intake and neurological disorders such as Alzheimer's disease. The Committee agrees with the **PTWI for Al of 7 mg/kg body weight** established by JECFA for all intake sources. The aluminium salts considered acceptable as food additives by the Committee are listed in the summary table p.8.

Although there is reason to believe that only minute amounts of Al are likely to be absorbed from aluminium silicate, the Committee nevertheless considers that this contribution should be included in the PTWI for Al. If evidence of minute bioavailability is presented, the Committee would reconsider its position.

When setting conditions of use for aluminium salts, the intake from other food and drink sources should also be taken into account.

1.1.3 Iron

Iron is an essential nutrient and an unavoidable constituent of foods but may also be present as a contaminant. A considerable body of information about iron is available from biochemical, physiological, toxicological and epidemiological studies. Adequate guidelines for nutritional requirements for iron have been published. There is still some uncertainty with regard to the maximum level of iron that can be tolerated. The body has a considerable capacity to store iron, and chronic toxicity only occurs when stores become overloaded under certain pathological conditions. Normal individuals can tolerate 50 mg of iron/day (ferrous iron) for long periods without adverse effects. The Committee agrees with the JECFA which established a **PMTDI of 0.8 mg/kg body weight** calculated as Fe from all sources except for iron oxides used as colouring agents and iron supplements taken during pregnancy and lactation or for specific clinical requirements. The iron salts considered acceptable as food additives are listed in the summary table p 8.

1.2 Other compounds where use is restricted by the cation

1.2.1 Stannous chloride

This salt is used specifically for stabilizing the white colour of certain vegetable products (e.g. asparagus packed in glass jars) in amounts of up to 25 mg/kg. This use will contribute an intake of tin which lies well below the **PMTDI of 2 mg/kg body weight** established by JECFA. The Committee therefore accepts the continued use of stannous chloride for this purpose.

1.2.2 Cupric sulphate

This salt is used specifically for stabilizing the colour of canned green beans and cucumber salad. The possible intake from this use is unlikely to contribute significantly to the total dietary intake of copper and will lie well below the **PMTDI of 0.5 mg/kg body weight** calculated as Cu from all sources, established by JECFA. The Committee considers the continued use of cupric sulphate for this purpose as toxicologically acceptable at the technological levels proposed.

1.3 Anions (acids)

1.3.1 Chloride, sulphate, carbonate

These anions are natural constituents of man, animals and plants, and therefore occur in foodstuffs. They, together with certain cations constitute the major electrolytes present in all biological

materials. The Committee therefore established a **group ADI not specified** for these anions, although exhaustive systematic toxicological studies have not been carried out with these ions. No safety problems are likely to arise, provided the contributions from food do not disturb the homoeostatic mechanisms controlling the electrolyte balance of the body.

1.3.2 Silicate and silicon dioxide

The available data on orally administered silica and silicates, including amorphous silicon dioxide, appear to substantiate the biological inertness of those compounds. Any silicate absorbed is excreted by the kidneys without evidence of toxic accumulation in the body, except for the reported damage to dog kidney by magnesium trisilicate and sodium silicate. Methods for estimating silica in body tissues have been greatly improved in recent years, making some of the earlier data somewhat less valuable. A number of short-term studies in two species are available.

The effect on the kidney observed with magnesium trisilicate and sodium silicate in the dog was not observed in rats and chickens. There is also a wide experience with magnesium trisilicate as an antacid in man without any observed adverse effects. The Committee established a **group ADI not specified** for silicon dioxide and the silicates listed in the summary table, when used as anticaking agent. The use of aluminium silicates should be restricted to comply with the PTWI of aluminium (see section 1.1.2).

1.3.3 Orthophosphate, di-, tri- and polyphosphate.

Phosphate salts provide a metabolic source of the various cations and the phosphate anion. Of greatest concern is the toxicity arising from calcium, magnesium and phosphate imbalances in the diet. Ingested phosphate from food additive sources should be considered together with that from natural sources.

Polyphosphates are not absorbed to any significant extent as such, but only in the form of monophosphates, into which they are broken down in the intestine. Since the extent of hydrolysis is difficult to predict, the toxicological evaluation must be based on the assumption of complete conversion to monophosphate.

Phosphates are not mutagenic in a number of test systems. Teratogenic effects have not been observed in mammalian systems. Numerous animal studies have shown that excessive dietary phosphorus causes an increase in plasma P and a decrease in serum Ca. There is still uncertainty over the optimal Ca:P ratio and whether this is of any significance for the human dietary pattern.

The Committee agrees with the JECFA estimate of an **MTDI of 70 mg/kg body weight** for man, calculated as P, as the sum of phosphates naturally present in food and derived from additives in diets nutritionally adequate in respect of calcium. If the calcium intake were high, the intake of phosphate could be proportionally higher and the reverse relationship would also apply.

1.3.4 Monocarboxylic acids and their salts

Acetate: human studies determining the maximum metabolic load of acetate are not available. In evaluating the acceptance of acetates emphasis is placed on their established metabolic pathway and the consumption by man as normal constituents of the diet. The Committee established a **group ADI not specified** for acetate including diacetate.

Fatty acids: (myristic, stearic, palmitic, oleic acid) no exhaustive systematic toxicological studies have been carried out with these fatty acids. They are all constituents of biological fat and are therefore present in food generally. They are also produced during the metabolism of fats. The Committee established a **group ADI not specified** for the fatty acids and their salts listed in the summary table.

1.3.5 Dicarboxylic acids and their salts

Succinate: this anion occurs in nature and plays a role as an intermediate metabolite in the citric acid cycle. It also participates in the glucose and fatty acid synthesis. No systematic toxicological studies are available. However, in view of its role as an intermediate metabolite the Committee established a **group ADI not specified** for succinate.

Adipate: the evaluation was based on metabolic studies, acute, short-term and long-term toxicity studies, teratogenicity tests in 4 animal species, and mutagenicity studies. The Committee agrees with the **ADI of 5 mg/kg body weight** established by JECFA.

Fumarate: fumarates are normal components of intermediate metabolism. The testicular atrophy in rabbits reported after intraperitoneal administration of high doses was not seen after oral administration of doses as high as 6-9% in the diet of rabbits and other species. The Committee agrees with the **ADI of 6 mg/kg body weight** previously established by JECFA.

1.3.6 Hydroxycarboxylic acids

Lactate: in evaluating lactates emphasis is placed on the well-established metabolic pathways for the lactate anion in man after normal consumption. Lactate is an important intermediate of carbohydrate metabolism and a natural component of food. However, human studies determining the maximum load of lactate are not available. There is some evidence that babies in their first three months of life have difficulties in utilizing small amounts of DL- and D(-)-lactic acid. Adults metabolize D(-)-lactate without difficulty. The Committee agrees with the **group ADI not specified** established by JECFA. For food specially prepared for small children only the L(+) isomer should be used.

Citrate: in evaluating the acceptability of citrate emphasis is placed on the well-established role of citrate as an intermediate metabolite in the citric acid cycle and as a natural component of food. The Committee agrees with the **group ADI not specified** established by JECFA.

Malate: in evaluating the acceptance of malate emphasis is placed on the well-established metabolic pathway of this anion and the daily consumption of malate-containing food. The malate anion also occurs in D(+) and L(-) forms. The available evidence shows that D(+)-malate is metabolized without difficulty and there is no clear evidence for a need to distinguish between the enantiomers when malate is used in food. The Committee agrees with the **group ADI not specified** established by JECFA.

Tartrate: the long-term study in rats with L(+)-tartrate showed no adverse effects at the highest level tested. Tartrates have been used medicinally for long periods. The evaluation of L(+)-tartrate can therefore be based on experimental data, the metabolic inertness of tartrates and the fact that they are normal constituents of food. Monosodium-L(+)-tartrate also produced no adverse effects in long-term studies. The available data were inadequate to assess the safety of DL-tartrate. The Committee agrees with the **group ADI of 30 mg/kg body weight** established by JECFA for L(+) tartrate, while the **DL-form is not acceptable.**

Metatartaric acid: the Committee could **not establish an ADI** on the basis of the available data. It considered **acceptable,** however, the continued use in wine at a level up to 100 mg/l.

Gluconate and glucono-delta-lactone: consideration of these substances may be based on the metabolic evidence as intermediates of normal glucose metabolism in mammalian species. These is considerable experience with gluconates in man and animals. A single long-term test at one dose level showed no evidence of carcinogenicity for the lactone. Teratogenic tests have shown no abnormalities in 4 species. In view of their role in the glucose metabolism in mammals the Committee agrees with the **group ADI not specified** established by JECFA.

Heptonate and 1,4-heptonolactone: the Committee was unable to evaluate the safety in use of this anion in the absence of adequate data. It considered the use of heptonic acid and its salts as food additives **not acceptable.**

2. Amino acids and ribonucleotides

2.1 Amino acids

These substances are the essential constituents of proteins and are thus present in all foodstuffs containing proteins. Only the L-forms are physiologically important. The Committee considers the

use of L-amino-acids generally **acceptable** provided the addition to food does not give rise to a nutritional imbalance of the amino acids.

2.1.1 Glycine and its salts

The Committee reviewed the nutritional, biochemical and toxicological information on this non-essential amino acid. If used at levels corresponding to good manufacturing practice no nutritional or toxicological hazards arise to man. The Committee **accepted** the use of glycine as an acidity regulator, flavour modifier and humectant, but did not include its use as a sweetening agent in this evaluation.

2.1.2 L-Glutamic acid and its salts

L-Glutamic acid is a component of animal and plant proteins and represents some 20% of ingested protein. Glutamates are claimed to have a taste which is distinct from the basic four physiological tastes and which is recognised by many organisms. Bound glutamate is released during digestion and absorbed comparatively slowly. Infants, including prematures, have been shown to metabolize glutamate as efficiently as adults and therefore do not display any special susceptibility to elevated oral intakes of glutamate.

Acute, subchronic and chronic toxicity studies in mice, rats and dogs have shown no specific toxic effects due to monosodium glutamate (MSG). There was no evidence of carcinogenic or genotoxic potential. Numerous reproduction and teratology studies in mice, rats, rabbits and monkeys revealed no deleterious effects on the offspring.

Some investigations have demonstrated a strain-dependent but variable vulnerability of the developing mouse or rat central nervous system to high levels of glutamate alone or in combination with other amino acids following administration of massive doses. No brain lesions have occurred in numerous studies in the mouse, rat or hamster ingesting high doses of MSG in their diet.

Some of the acute human reactions, reported after ingestion of over 3g of glutamate per person, have also been observed with other foods not containing glutamates. No objective clinical measurements have been associated with the wide variety of symptoms described.

The Committee established a **group ADI not specified** on the basis of the data provided and in view of the large normal dietary intake of glutamates.

2.1.3 L-Cysteine

Both L-cysteine hydrochloride and the monohydrate have been used in bakery processes as dough improvers. L-cysteine is a non-essential amino acid, occurring in a wide variety of foods, especially cereals. The contribution to the total daily dietary intake from the use in bakery processes is insignificant. The Committee therefore considers its use as flour treatment **toxicologically acceptable.**

2.2 5'-Ribonucleotides

Inosinate and guanylate

These substances are widely distributed in all tissues of animals and plants. Their role in purine metabolism as well as their breakdown to uric acid and to allantoin (in the majority of mammals, but not man), is well substantiated. There are extensive biological data available including metabolic, short- and long-term studies in several species, as well as reproduction, teratology and mutagenicity studies. No evidence of carcinogenicity, of adverse effects on reproduction and of teratogenic or genotoxic potential has been observed.

Ingestion of large amounts by man can increase the serum uric acid level and urinary uric acid excretion. This is of importance only for people with gouty diathesis or those taking uric acid-retaining diuretics. The changes in dietary purine intake from the use of these substances as flavour modifiers are no greater than those due to variability in the consumption of the major dietary contributors of purines. The likely intake of these substances from their use as flavour modifiers varies from 10-30 mg/day compared with 400-600 mg/day contributed by the diet. The dietary treatment of gout or hyperuricaemia has been abandoned in favour of more efficient therapy by uricosoric agents. Based on this information, the Committee sees no reason for special warning labels in relation to gout. The Committee established a **group ADI not specified** for ribonucleotides when used as flavour modifiers at the levels proposed according to good manufacturing practice.

3. Packaging gases and propellants

3.1 Carbon dioxide, oxygen, nitrogen

Man is permanently exposed to these atmospheric gases. Additionally, carbon dioxide is a natural metabolite. Compared to this exposure, the intake from their use as packaging gases and propellants is insignificant. The establishment of ADI's for these compounds is unnecessary. The Committee considers these compounds **acceptable** as packaging gases and propellants provided they comply with a food grade specification.

3.2 Nitrous oxide

The pharmacological and pharmacokinetic properties of this gas are known from its wide and established use as an anaesthetic. Although no residue data are available, these are likely to be so low as to present no hazard to the consumer. The Committee considers the establishment of an ADI unnecessary and its use as packaging gas and propellant **acceptable**. The specification should exclude the presence of other oxides of nitrogen.

3.3 Hydrogen

Apart from drawing attention to its explosive properties the Committee considers the establishment of an ADI unnecessary. Its use as a packaging gas is toxicologically **acceptable** provided a food grade specification is available.

3.4 Argon

This rare gas is an elemental constituant of air. It is completely inert chemically. The Committee considers the establishment of an ADI unnecessary. Its use as a packaging gas and propellant is **toxicologically acceptable** provided a food grade specification is available.

4. Others

4.1 Oxystearin

The toxicological data on this substance include metabolic studies, acute, short-term and long-term toxicity studies in mice and rats. The Committee agrees with the evaluation of JECFA establishing an **ADI of 25 mg/kg body weight.**

4.2 Dimethylpolysiloxane

The available toxicological data include studies on the metabolism, acute, short-term and long-term toxicity and observations in man. A recent long-term feeding study in mice showed no evidence of absorption or carcinogenic potential. The Committee agrees with the **ADI of 1.5 mg/kg body weight** established by JECFA.

4.3 Sodium and potassium ferrocyanide

The Committee agrees with the **ADI of 0.025 mg/kg body weight** (calculated as sodium ferrocyanide) established by JECFA. When used as a processing aid in the production of wine only small residues are found, and only small technological levels are needed as anticaking agent in salt. Therefore the Committee has no objection, on toxicological grounds, to the continued use for these purposes.

4.4 Ethylmaltol

The available metabolic data point to rapid absorption, and rapid elimination as conjugate with sulphate or glucuronic acid. The various short-term studies showed no evidence of any serious target organ toxicity nor was there any evidence of interference with reproductive function or of foetal toxicity. Adequate long-term studies in the rat and dog exclude chronic toxicity and carcinogenic potential. Mutagenicity tests are also available. The Committee established an **ADI of 1 mg/kg body weight.**

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The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, p. 1) to advise the Commission on any problem relating to the protection of the health and safety of persons arising from the consumption of food and, in particular, the composition of food processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminants.

The members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

The secretariat of the Committee is provided by the Directorate-General for the Internal Market and Industrial Affairs of the Commission of the European Communities. Recent Council directives require the Commission to consult the Committee on provisions which may have an effect on public health falling within the scope of these directives.

The present report deals with a first series of food additives of various technological functions (Opinion expressed on 18 May 1990).

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

ETHYL MALTOL

INS:	637
COE No.:	7047
FEMA No.:	3487
JECFA No.:	1481
Chemical names:	2-ETHYL-3-HYDROXY-4-PYRONE
Synonyms:	2-ETHYL-3-HYDROXY-4-PYRONE; 2-ETHYL-3-HYDROXY-4H-PYRAN-4-ONE; 2-ETHYLPYROMECONIC ACID
Functional class:	FLAVOURING AGENT; FLAVOUR ENHANCER
Latest evaluation:	2005
ADI:	0-2 mg/kg bw (1974)
Comments:	The ADI established at the eighteenth meeting in 1974 was maintained.
Report:	TRS 934-JECFA 65/36
Specifications:	COMPENDIUM ADDENDUM 13/FNP 52 Add. 13/23,54
Tox monograph:	FAS for JECFA 65 in press
Previous status:	2001, COMPENDIUM ADDENDUM 9/FNP 52 Add.9/192 (METALS LIMITS). S 1974, NMRS 54/TRS 557-JECFA 18/13, FAS 6/NMRS 54A-JECFA 18/19. 0-2. FU 1970, NMRS 48/TRS 462-JECFA 14/23, FAS 70.40/NMRS 48B-JECFA 14/15, FAS 70.39/NMRS 48A-JECFA 14/37. 0-2. FU. N

31 Jan 06

See Also:
Toxicological Abbreviations
Ethyl maltol (FAO Nutrition Meetings Report Series 48a)

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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

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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)
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.

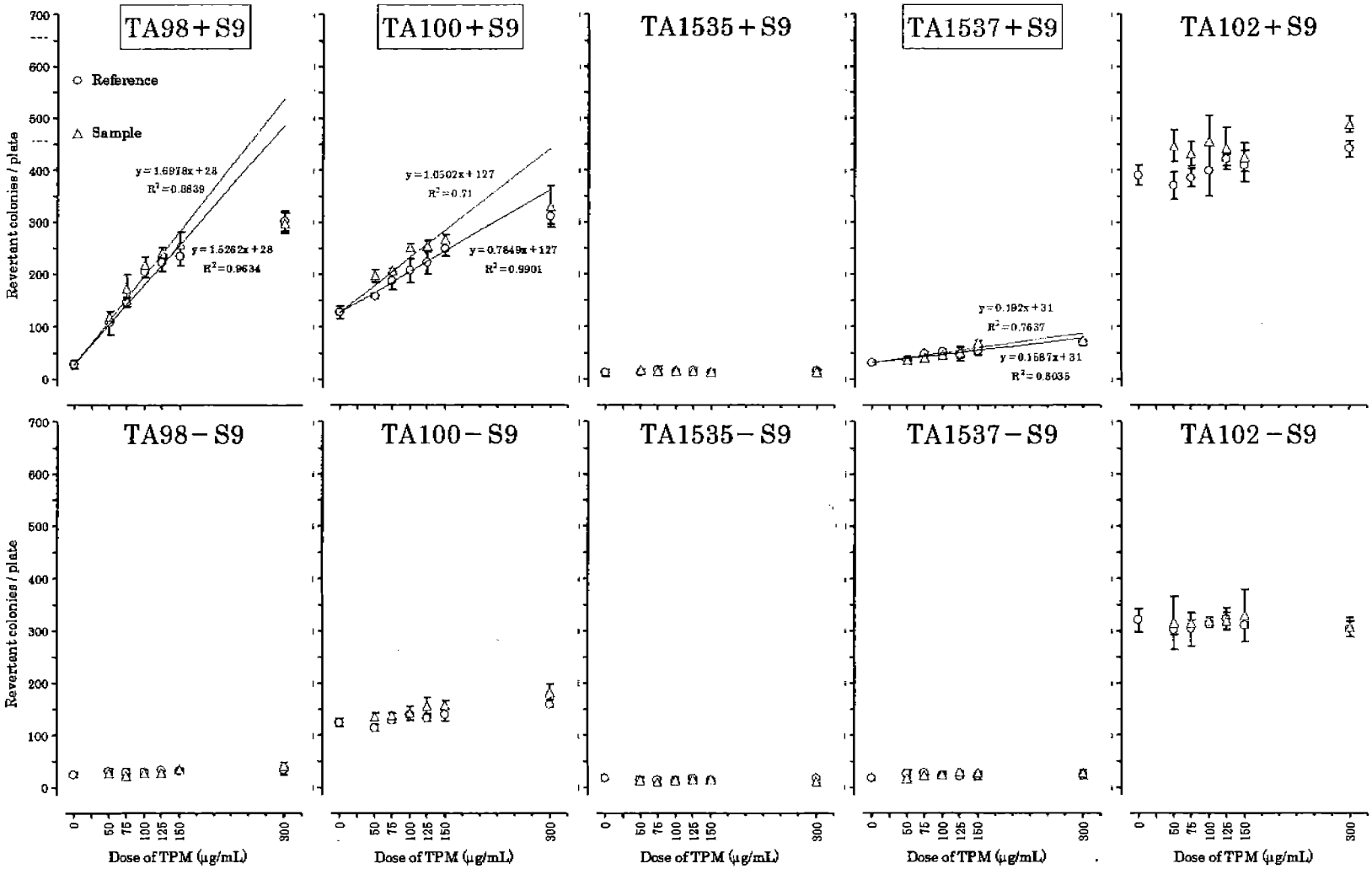
Body Weights and Clinical Observations

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.

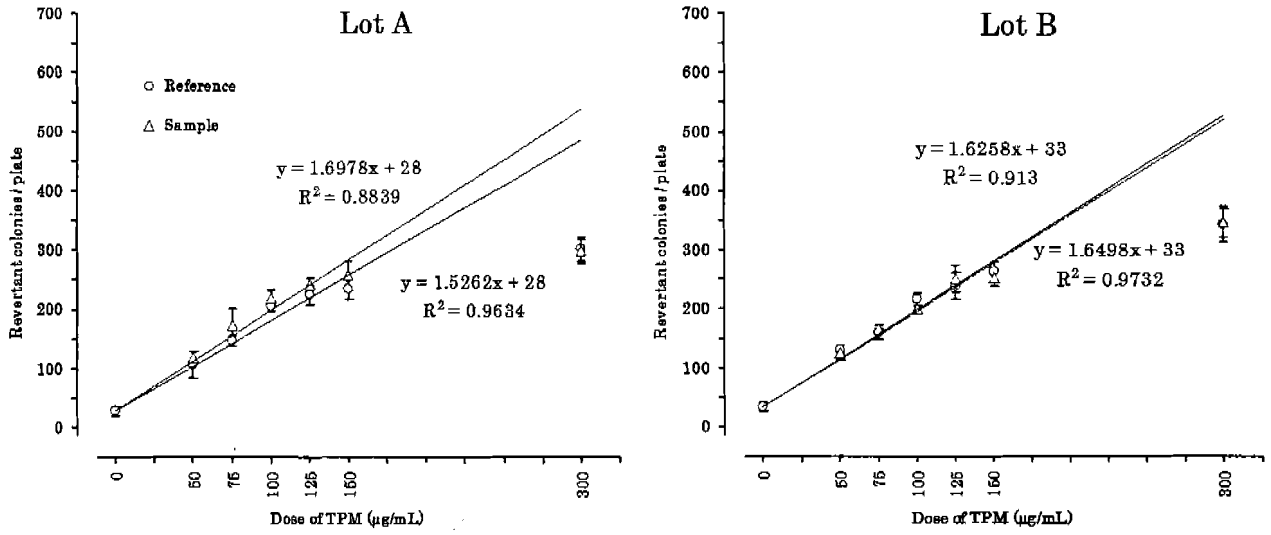
Organ Weights

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

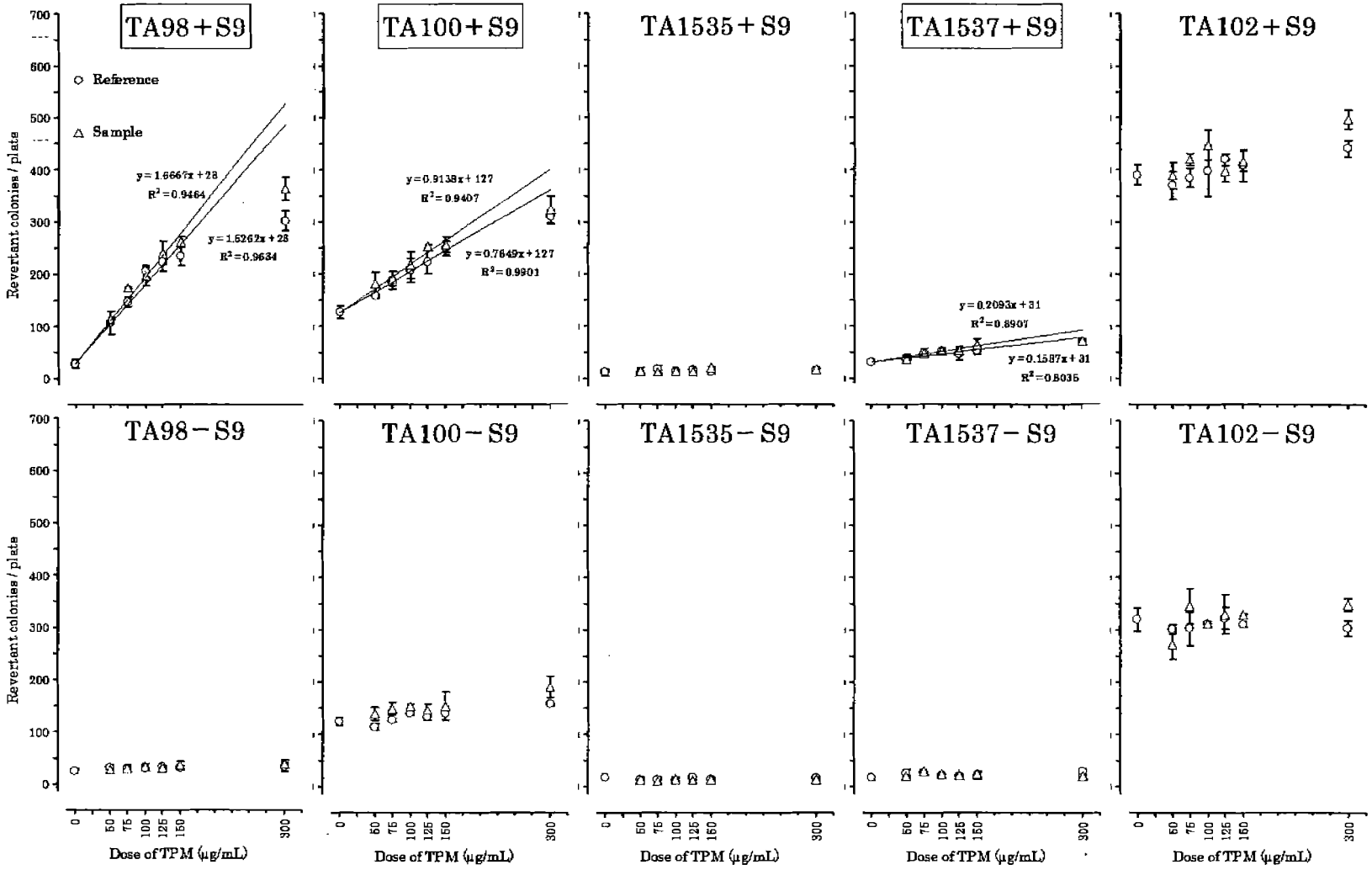
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 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



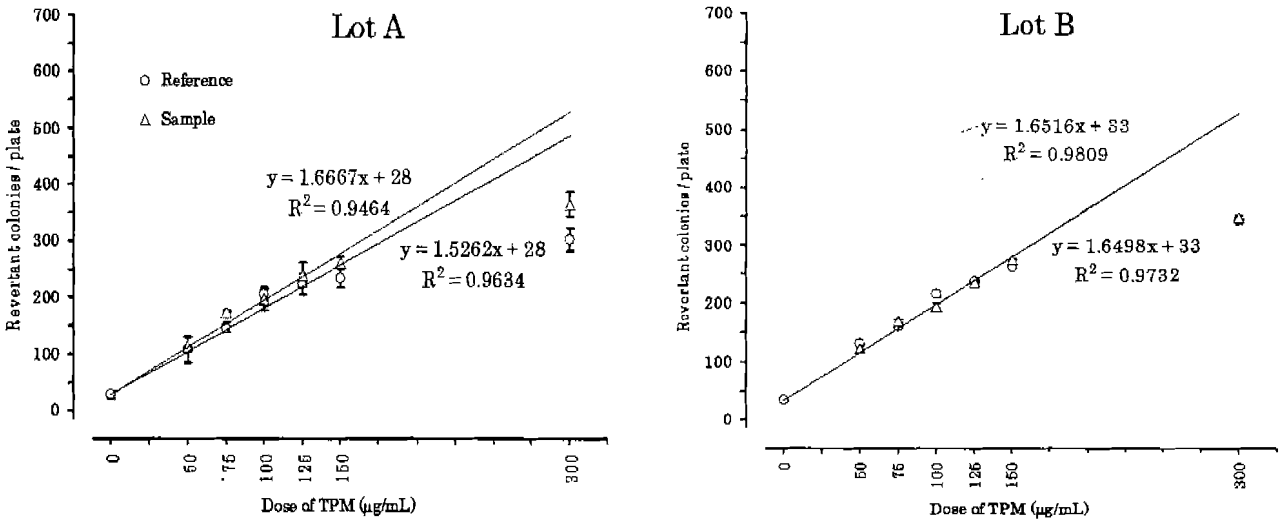
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

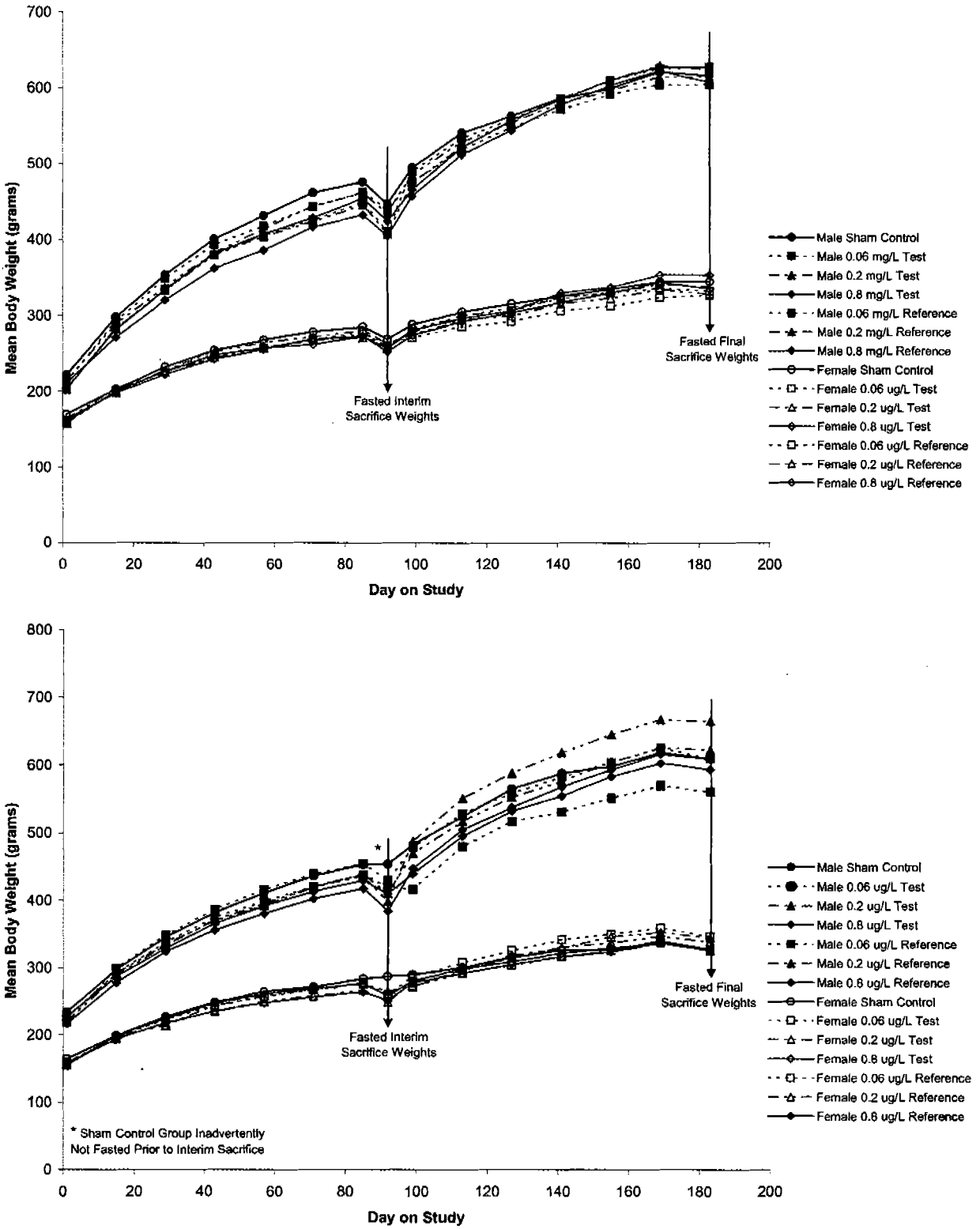


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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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MALTOL AND RELATED SUBSTANCES

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1.0 EVALUATION

1.1 Introduction

The Committee evaluated a group of seven flavouring agents (see Table 1) comprising maltol and related substances. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1, p. 170). The Committee had evaluated two members of the group previously. Maltol (No. 1480) was evaluated at the eleventh meeting (Annex 1, reference 15), when a temporary ADI of 0–1 mg/kg bw was established because no results of long-term studies were available. At its eighteenth meeting (Annex 1, reference 35), the Committee withdrew the temporary ADI because the results of the long-term studies of toxicity that had been requested at its previous meeting had not been made available. At its twenty-second meeting (Annex 1, reference 47), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw.

Table 1. Summary of results of safety evaluations of maltol and related substances used or proposed for use as flavouring agents

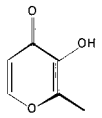
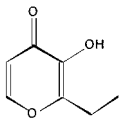
Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II							
Maltol	1480	118-71-8 	Yes Europe: 3585 USA: 2898	No	Yes. The NOEL of 100 mg/kg bw per day (Annex 1, reference 56) is > 1600 times the estimated daily intake of maltol when used as a flavouring agent.	Note 1	At its 25th meeting, JECFA established an ADI of 0–1 mg/kg bw (Annex 1, reference 56).
Ethyl maltol	1481	4940-11-8 	Yes Europe: 1851 USA: 6692	No	Yes. The NOEL of 200 mg/kg bw per day for ethyl maltol in rats (Annex 1, reference 35) is > 1800 times the estimated daily intake of ethyl maltol when used as a flavouring agent.	Note 1	At its 18th meeting, JECFA established an ADI of 0–2 mg/kg bw (Annex 1, reference 35).

Table 1 (contd)

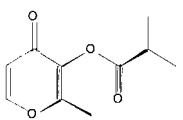
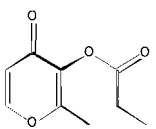
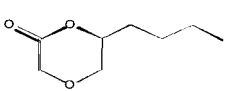
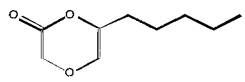
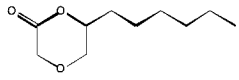
Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Maltyl isobutyrate	1482	65416-14-0 	No Europe: 23 USA: 38	NR	NR	Note 2	No safety concern
2-Methyl-3-(1-oxo-propoxy)-4*H*-pyran-4-one	1483	68555-63-5 	No Europe: ND USA: 26b	NR	NR	Note 2	No safety concern (conditional)
Structural class III							
2-Amyl-5 or 6-keto-1,4-dioxane	1485	65504-96-3 	No Europe: ND USA: 0.2	NR	NR	Note 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class III							
2-Butyl-5- or -6-keto-1,4-dioxane	1484	65504-95-2 	No Europe: ND USA: 0.5	NR	NR	Note 3	No safety concern
2-Hexyl-5 or 6-keto-1,4-dioxane	1486	65504-97-4 	No Europe: ND USA: 0.5	NR	NR	Note 3	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation because intake of the substance was determined to be of no safety concern at Step A3 of the procedure.

Step 2: All the agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure.

[a] The thresholds for human intake for structural classes II and III are 540 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intakes of flavouring agents in structural class II are 5459 μg/day in Europe and 9655 μg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 1.2 μg/day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Notes:

1. Conjugation with glucuronic acid or sulfate followed by excretion in urine
2. Hydrolysis to maltol and the corresponding carboxylic acid, followed by conjugation with glucuronic acid or sulfate and excretion in urine
3. Hydrolysis to a hydroxycarboxylic acid, followed by excretion as the glucuronic acid conjugate

At its twenty-fifth meeting (Annex 1, reference 56), the Committee evaluated additional data and assigned an ADI of 0–1 mg/kg bw. Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting (Annex 1, reference 22), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting (Annex 1, reference 35), the Committee re-evaluated ethyl maltol and confirmed the previous ADI of 0–2 mg/kg bw.

One of the seven substances, maltol (No. 1480), has been reported to occur naturally in a wide variety of foods, including wheaten and rye bread, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soya proteins, beans, and clams. Under conditions of baking (e.g. bread, beans) and roasting (cocoa, coffee, peanuts), simple sugars are partly converted to maltol (Nijssen et al., 2003).

1.2 Estimated daily per capita exposure

Annual volumes of production have been reported for six of the seven flavouring agents in the group (Nos 1480, 1481, 1482, 1484, 1485 and 1486). With respect to the remaining substance (No. 1483), anticipated annual volumes of production have been given for its proposed use as a flavouring agent. The total reported and anticipated annual volume of production of the seven flavouring agents in this group is about 38 000 kg in Europe (International Organization of the Flavor Industry, 1995) and 73 000 kg in the USA (National Academy of Sciences, 1970, 1982; Lucas et al., 1999). More than 99% of the total reported and anticipated annual volumes of production in Europe and the USA is accounted for by maltol and ethyl maltol. The per capita intakes of maltol in Europe and the USA are about 3600 and 2900 μg/day, respectively. The per capita intakes of ethyl maltol in Europe and the USA are about 1800 and 6700 μg/day, respectively. The per capita exposure to the remainder of the flavouring agents in the group is 0–23 μg/day in Europe and 0.2–38 μg/day in the USA, most of the values being at the lower end of these ranges. The per capita exposure to each agent is reported in Table 2.

1.3 Absorption, distribution, metabolism and elimination

Chemically, maltol is classified as a γ-pyrone. It is a hydroxyl-substituted 4*H*-pyran-4-one and is expected be metabolized similarly to phenol, primarily undergoing phase II conjugation of the free hydroxy substituent. Maltol (2-methyl-3-hydroxy-4*H*-pyran-4-one) and ethyl maltol (2-ethyl-3-hydroxy-4*H*-pyran-4-one) are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine (Rennhard, 1971). Maltol esters (Nos 1482 and 1483) are predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (propionic acid or isobutyric acid) (Bennett, 1998) and to undergo further metabolism similar to that of maltol and ethyl maltol.

The remaining three substances (Nos 1484, 1485, and 1486) in the group are α-pyrone derivatives and contain a saturated 3*H*-pyranone nucleus. These three substances are lactones and are readily hydrolysed to yield the corresponding ring-opened hydroxy acid derivatives. In nature, lactones are formed by acid-catalysed intramolecular cyclization of four- or five-carbon hydroxycarboxylic acids to yield five- (γ-) or six- (δ-) membered lactone rings, respectively. The stability of the lactone ring in an aqueous environment is pH-dependent. In basic media such as blood, lactones hydrolyse rapidly to the open-chain hydroxycarboxylic acid (Fishbein & Bessman, 1966; Roth & Giarman, 1966; Guidotti & Ballotti, 1970). Studies of

Table 2. Annual volumes of production of maltol and related substances used or proposed for use as flavouring agents in Europe and the USA

Agent (No.)	Reported[a] / anticipated annual volume (kg)	Exposure[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
		µg/day	µg/kg bw per day		
Maltol (1480)					
Europe	25 123	3 585	60		
USA	21 999	2 898	48	38 694	2
Ethyl maltol (1481)					
Europe	12 969	1 851	31		
USA	50 802	6 692	112	–	NA
Maltyl isobutyrate (1482)					
Europe	163	23	0.4		
USA	286	38	0.6	–	NA
2-Methyl-3-(1-oxopropoxy)-4*H*-pyran-4-one (1483)					
Europe	ND	ND	ND		
USA[e]	150	26	0.4	–	NA
2-Butyl-5- or -6-keto-1,4-dioxane (1484)					
Europe	ND	ND	ND		
USA[f]	3	0.5	0.009	–	NA
2-Amyl-5- or -6-keto-1,4-dioxane (1485)					
Europe	ND	ND	ND		
USA[f]	1	0.2	0.003	–	NA
2-Hexyl-5- or -6-keto-1,4-dioxane (1486)					
Europe	ND	ND	ND		
USA[f]	3	0.5	0.009	–	NA
Total					
Europe	38 255				
USA	73 244				

NA, not available; ND, no intake data reported; –, not reported to occur naturally in foods

[a] From International Organization of the Flavour Industry (1995) and Lucas et al. (1999) or National Academy of Sciences (1970, 1982)

[b] Exposure (µg/person per day) calculated as follows: [(annual volume, kg) x (1×10^9 µg/kg)/(population x survey correction factor x 365 days)], where population (10%, 'eaters only') = 32×10^6 for Europe and 26×10^6 for the USA; where survey correction factor = 0.6 for Europe and 0.8 for the USA, representing the assumption that only 60% and 80% of the annual flavour volume, respectively, was reported in poundage surveys (International Organization of the Flavor Industry, 1995; Lucas et al., 1999; National Academy of Sciences, 1982) or in the anticipated annual volume.
Exposure (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] Quantitative data for the USA reported by Stofberg and Grundschober (1987)

[d] The consumption ratio is calculated as follows: (annual consumption from food, kg)/(most recent reported volume as a flavouring substance, kg)

[e] The volume cited is the anticipated annual volume, which was the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use. National surveys (National Academy of Sciences, 1970, 1982, 1987; Lucas et al., 1999), if applicable, revealed no reported use as a flavour agent.

[f] Annual volume reported in previous surveys in the USA (National Academy of Sciences, 1970, 1982)

structurally related lactones (Billecke et al., 2000) indicate that the aliphatic lactones would be hydrolysed to yield the corresponding hydroxycarboxylic acid. These acids can undergo further oxidation to yield polar, excretable metabolites or enter the fatty acid pathway and undergo β-oxidative cleavage to yield polar metabolites of lower relative molecular mass, which are also excreted either unchanged or conjugated in the urine (Nelson & Cox, 2000).

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

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the intake estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure to this group of flavouring agents, the Committee assigned four of the seven agents (Nos 1480, 1481, 1482 and 1483) to structural class II and the remaining three agents (Nos 1484, 1485 and 1486) to structural class III (Cramer et al., 1978).

Step 2. All 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 per capita exposure to two of the four agents in structural class II (Nos 1482 and 1483) and of all three agents in structural class III is below the threshold of concern for their respective class (i.e. class II, 540 μg/day; class III, 90 μg/day). Four of these five substances (Nos 1482, 1484, 1485 and 1486) are reported to be used as flavouring agents. According to the Procedure, use of these four agents would not raise concern about safety at the estimated daily exposure. The other substance (No. 1483) is proposed for use as a flavouring agent. Although the Procedure indicates no safety concern with use of this flavouring agent at the estimated daily exposure derived from the anticipated annual volume of production, less uncertain exposure estimates are needed. Estimated daily exposure to the remaining two agents in structural class II, maltol (No. 1480) and ethyl maltol (No. 1481), exceed the threshold of concern for structural class II. The per capita exposure to maltol is about 3600 μg/day in Europe and 3000 μg/day in the USA, and the exposure to ethyl maltol is about 1800 μg/day in Europe and 6700 μg/day in the USA. Accordingly, the evaluation of these two agents proceeded to step A4.

Step A4. Maltol (No. 1480) and ethyl maltol (No. 1481) are not endogenous. Therefore, their evaluation proceeded to step A5.

Step A5. At its twenty-fifth meeting, the Committee established an ADI of 0–1 mg/kg bw for maltol (No. 1480) on the basis of a NOEL of 100 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference 56). This NOEL is more than 1800 times the estimated daily exposure to this agent from its use as a flavouring agent in Europe or the USA. At its eighteenth meeting, the Committee established an ADI of 0–2 mg/kg bw for ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference 35). This NOEL is more than 1800 times the estimated daily iexposure to this substance from its use as a flavouring agent in Europe or the USA. The Committee therefore concluded that the exposure to flavours in this group would not raise concerns about safety.

The exposure considerations and other information used to evaluate maltol and six related derivatives according to the Procedure are summarized in Table 1.

1.5 Consideration of combined exposure from use as flavouring agents

In the unlikely event that all four agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the human exposure threshold for class II (540 μg per person per day). All four agents in this group are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Their safety is also indicated by the results of studies on the toxicity of maltol and ethyl maltol. An evaluation of all the data indicates that combined intake would not raise concern about safety.

In the unlikely event that all three agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class III (90 μg per person per day). Their safety is also indicated by the results of studies of toxicity. An evaluation of all the data indicates that combined intake would not raise concern about safety.

1.6 Conclusions

The Committee maintained the previously established ADIs of 0–1 mg/kg bw for maltol and 0–2 mg/kg bw for ethyl maltol. The Committee concluded that use of the flavouring agents in this group of maltol and related substances would not present a safety concern at the estimated daily intakes. For one agent (No. 1483), the evaluation was conditional, because the estimated daily exposure was based on the anticipated annual volume of production. The conclusion about the safety of this substance will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of the maltol derivatives were consistent with the results of the safety evaluation made with the Procedure.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

The relevant background information summarizes the key scientific data applicable to the safety evaluation of seven flavouring agents that include maltol and related substances.

2.2 Additional considerations on exposure

Maltol (No. 1480) is the only substance in this group of flavouring agents that is reported to occur in traditional foods. Quantitative data on natural occurrence and a consumption ratio reported for maltol indicate that exposure is predominantly from consumption of traditional foods (i.e. a consumption ratio > 1) (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987). The production volumes and exposure values for each flavouring agent in this group are shown in Table 2.

2.3 Biological data

2.3.1 Biochemical data

(a) Hydrolysis

In general, aromatic esters are hydrolysed in vivo by the catalytic activity of carboxylesterases (Heymann, 1980), the most important of which are the A-esterases. Carboxylesterases are present in the endoplasmic reticulum of most mammalian tissues (Hosokawa et al., 2001), predominantly in hepatocytes (Graffner-Nordberg et al., 1998; Hosokawa et al., 2001).

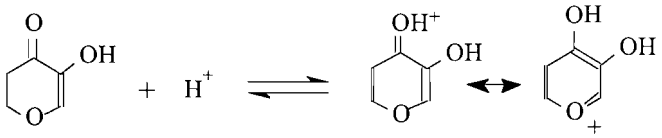
Incubation of 2-methyl-4-pyron-3-yl 2-methylpropanoate (maltol isobutyrate, No. 1482) with simulated gastric and intestinal fluid was reported to result in complete hydrolysis to the corresponding acid (isobutyric acid) and alcohol (maltol) within 10 and 15 h, respectively (Flavor and Extract Manufacturers Association of the United States, 1973). Incubation of maltol propionate (No. 1483) with simulated intestinal fluid containing pancreatin at 37 ºC resulted in essentially complete hydrolysis within 5 h (Bennett, 1998).

(b) Absorption, distribution, metabolism and excretion

Maltol (No. 1480) and derivatives (Nos 1481 to 1483) contain a γ-pyrone ring system. γ-Pyrones are relatively basic, and the behaviour as a base is partly due to the aromatic character and relative stability of the conjugate acid (see Figure 1). As that the γ-pyrone ring also contains a 3-hydroxy substituent, it is expected that maltol and its derivatives will be readily conjugated with glucuronic acid or sulfate. In addition, maltol may form a complex with metal ions (e.g. Fe^{++}), like phenols. It also has antioxidant properties in vitro and in vivo (see *Other biochemical properties*, below).

Groups of two beagle dogs of each sex were given a single intravenous injection of 10 mg/kg bw maltol (No. 1480), and urine samples were collected for 72 h. An average of 58.5% of the administered dose was excreted as a mixture of sulfate and glucuronic acid conjugates of maltol. About 98% of the total urinary excretion of conjugates occurred within the first 24 h, males and females excreting

Figure 1. Maltol acid–base reaction



an average of 42% and 73% of the administered dose, respectively. In a parallel study, groups of two beagle dogs of each sex were given a single intravenous injection of 10 mg/kg bw ethyl maltol (No. 1481). Analysis of urine samples collected over 72 h showed that an average of 66.3% of the administered dose had been excreted as a mixture of sulfate and glucuronic acid conjugates of ethyl maltol. About 97% of the total excretion occurred within the first 24 h. During that time, males and females excreted an average of 38% and 91% of the administered dose, respectively (Rennhard, 1971).

One male and one female beagle dog were given oral doses of ethyl maltol at 200 mg/kg per day on 2 consecutive days, and excreta were collected for 24 h after each dose. An average of 64% of each dose was excreted in the urine as either the sulfate (male, 12.9%; female, 11%) or the glucuronic acid (male, 46.6%; female, 57.6%) conjugate within 24 h after each dose. Small amounts of free ethyl maltol (male, 0.12%; female, 0.13%) were also detected in urine, and small amounts (male, 2.23%; female, 0.25%) of free and conjugated maltol were detected in faeces (Rennhard, 1971).

The three dioxane compounds, 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485), and 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) are expected to be metabolized similarly to lactones. Lactones undergo hydrolysis to yield the corresponding ring-opened d-hydroxycarboxylic acid. The three dioxanes in this group hydrolyse to 5-hydroxycarboxylic acid derivatives in which position 3 of the chain is occupied by an oxygen atom. This prohibits participation in the fatty acid metabolism pathway. As these hydrolysis products contain polar oxygenated functional groups, however, they are anticipated to be rapidly absorbed and excreted, either free or in conjugated form. The metabolism of aliphatic and alicyclic lactones that do not undergo γ-oxidation in the fatty acid pathway has been reviewed previously by the Committee (Annex 1, reference 166).

In conclusion, the flavouring agents in this group are anticipated to be rapidly absorbed and metabolized, either by conjugation with glucuronic acid or sulfate, like the maltol derivatives, or by hydrolysis, like the lactone derivatives.

(c) Other biochemical properties

Maltol has antioxidant properties, presumably through its ability to complex metal ions such as Fe^{++} and to promote the formation of reduced glutathione (GSH) (Murakami et al., 2001). Maltol at a concentration of 130 μmol/l inhibited iron-mediated lipid peroxidation and increased scavenging of reactive oxygen species by enhancing the supply of NADPH required for regeneration of GSH. Maltol inhibited the formation of thiobarbituric acid-reactive substances when incubated with rat liver microsomes in the presence of Fe^{++} and ascorbate. Maltol at concentrations of 130–140 μmol/l also effectively inhibited the inactivation of NADP-isocitrate dehydrogenase, the principal NADPH-generating enzyme, by Fe^{++}. Maltol significantly increased the oxidation of Fe^{++}, while dimethylpyrone had no effect. The latter results suggest that the 3-hydroxy substituent in maltol is necessary to promote Fe^{++} oxidation.

Kainic acid has been shown to induce oxidative stress (increased lipid peroxidation and decreased GSH levels) in the brain tissue of rodents, causing neurobehavioural effects (Gupta et al., 2002). In a further study, male ICR mice were given maltol at 0, 50 or 100 mg/kg bw on 5 consecutive days; 30 min after the final administration, the animals were given kainic acid in a single subcutaneous

injection of 50 mg/kg bw. Administration of kainic acid alone resulted in epileptic-like seizures, causing 50% mortality, damage to pyramidal cells of the hippocampus, marked decreases in GSH content and GSH peroxidase activity, and increases in the level of thiobarbituric acid-reactive substances in brain tissue. Administration of maltol at 100 mg/kg bw, but not 50 mg/kg bw, attenuated the neurobehavioural effects, and the loss of neurons in the hippocampus and mortality (12.5%) were significantly reduced. Maltol also restored brain GSH and GSH peroxidase activity to control levels (Kim et al., 2004).

2.3.2 Toxicological studies

(a) Acute toxicity

LD_{50} values after oral administration have been reported for three of the seven substances in this group (see Table 3), ranging from 1150 to 2800 mg/kg bw for rats and from 550 to 2100 mg/kg bw for mice. The value for guinea-pigs was 1410 mg/kg bw. These values indicate that the acute toxicity of maltol and related substances after oral intake is low (Dow Chemical Company, 1967; Pellmont, 1968a,b; Gralla et al., 1969; Moreno, 1974a,b).

(b) Short-term studies of toxicity

The results of short-term studies with maltol and related substances are summarized in Table 4.

Maltol (No. 1480)

Mice

Eight female Swiss mice were fed a diet containing 3-hydroxy-2-methyl-4-pyrone (maltol) at a level of 0.5% (w/w) for 21 weeks, calculated to provide an average daily intake of 750 mg/kg bw (Food & Drug Administration, 1993). A

Table 3. Results of studies for acute toxicity of maltol and related substances administered orally

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1480	Maltol	Mouse; F	550	Dow Chemical Co. (1967)
1480	Maltol	Mouse; M	848	Gralla et al. (1969)
1480	Maltol	Rat; F	1410	Dow Chemical Co. (1967)
1480	Maltol	Rat; M	1440	Gralla et al. (1969)
1480	Maltol	Rat; NR	2330	Moreno (1974a)
1480	Maltol	Guinea-pig; M	1410	Dow Chemical Co. (1967)
1481	Ethyl maltol	Mouse; M	780	Gralla et al. (1969)
1481	Ethyl maltol	Rat; M, F	M: 1150 F: 1200	Gralla et al. (1969)
1481	Ethyl maltol	Rat; NR	1220	Moreno (1974b)
1482	Maltyl isobutyrate	Mouse; NR	2100	Pellmont (1968a)
1482	Maltyl isobutyrate	Rat; NR	2800	Pellmont (1968b)

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

Table 4. Results of short-term studies of toxicity and long-term studies of toxicity and carcinogenicity with maltol and related substances

No.	Substance	Species; sex	No. test groups[a]/ no. per group[b]	Duration (days)	NOEL (mg/kg bw per day)	Reference
Short-term studies						
1480	Maltol	Mouse; F	1/8	147	750[c]	Bhathal et al. (1984)
1480	Maltol	Rat; M, F	1/20	90	< 1000	Gralla et al. (1969)
1480	Maltol	Rat; M, F	1/30	186	500[c]	Dow Chemical Co. (1967)
1480	Maltol	Dog; M, F	3/4	90	< 125	Gralla et al. (1969)
1481	Ethyl maltol	Rat; M, F	3/20	90	< 250	Gralla et al. (1969)
1481	Ethyl maltol	Dog; M, F	3/4	90	< 125	Gralla et al. (1969)
1484	2-Butyl-5- or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	6.59 (M) 7.35 (F)[c]	Posternak (1969a)
1485	2-Amyl-5 or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	6.65 (M) 7.33 (F)[c]	Posternak (1969b)
1486	2-Hexyl-5 or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	< 5.96 (M) < 6.76 (F)	Posternak (1969c)
Long-term studies						
1481	Ethyl maltol	Rat; M, F	3/50	730	200[c]	Gralla et al. (1969)
1481	Ethyl maltol	Dog; M, F	3/8	730	200[c]	Gralla et al. (1969)

M, male; F, female

[a] Total number of test groups does not include control animals.

[b] Total number per test group includes both male and female animals.

[c] Study performed with either a single dose or multiple doses that had no adverse effect. The value is therefore not a true NOEL but is the highest dose tested that had no adverse effects. The actual NOEL might be higher.

concurrent control group was maintained. Food and water were provided ad libitum, and body weights were recorded weekly. At termination, no differences in general health, behaviour, body-weight gain or relative liver weights were reported. Gross and microscopic examination revealed no histological abnormalities in the livers of the treated mice when compared with the controls (Bhathal et al., 1984).

Rats

Groups of 10 Charles River weanling albino rats of each sex were maintained on a diet containing maltol at a level calculated to provide an average daily intake of 1000 mg/kg bw for 90 days. Concurrent control groups of an unspecified number of male and female rats were maintained on basal diet. Body weight and food consumption were recorded weekly. Blood and urine samples were collected from five male and five female rats in each group 45 and 90 days after the beginning of the study. Blood samples were analysed for haemoglobin, erythrocyte volume fraction, red blood cell count, total white blood cell count and differential count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was examined microscopically after centrifugation. At the end of the study, all rats were necropsied; organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries), and gross and microscopic examinations were made of brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland. Decreased body-weight gain was reported in males and females after weeks 3 and 9, respectively, the male rats being more severely affected. A decrease in haemoglobin and slightly amber-coloured serum were observed in one male and one female at study termination. A high incidence of albuminuria was observed in all treated rats. No significant gross pathological changes were detected, and no differences between test and control animals in organ weights were recorded. Microscopic examination revealed kidney lesions identified as dilated acellular glomerular tufts with protein extravasation into Bowman capsules and cast formation within the lumina of dilated corticomedullary tubules. The deaths of two of the treated rats were attributed by the authors to renal failure (Gralla et al., 1969).

Groups of 15 male and 15 female 4- to 5-week-old rats were fed a diet containing 1% maltol for 6 months, calculated to provide an average daily intake of 500 mg/kg bw (Food & Drug Administration, 1993). Concurrent control groups of 15 rats of each sex were maintained on a basal diet. Body weights were recorded twice weekly. The six male and nine female controls and the four male and nine female treated rats that died during the experiment were examined for gross pathological lesions. At study termination, haematological parameters were evaluated in eight treated and control males, and all remaining animals were necropsied. Major organs were examined grossly and weighed, and selected tissues were fixed and stained for microscopic examination. No significant differences in appearance, behaviour, body weights or organ weights were observed between the treated and control animals during the study. The haematological values of treated males were normal

after 6 months. Histopathological examination of the liver, kidney, spleen, adrenals, pancreas and testes of males and females revealed no evidence of lesions that could be associated with treatment. The NOEL was 500 mg/kg bw per day (Dow Chemical Co., 1967).

Dogs

Groups of four male and four female beagle dogs were given capsules containing maltol at a dose of 125, 250 or 500 mg/kg bw per day for 90 days. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, renal function (measured by the bromosulfphthalein excretion test) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were evaluated at the beginning of the study and on days 14, 30, 60 and 90. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid, brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus and ovary) were removed and weighed. Selected tissues (brain, cervical spinal cord, sciatic nerve, hypophysis, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were evaluated microscopically. Three of four animals (sex not specified) at 500 mg/kg bw per day died within 21–41 days, and the fourth was killed when it became moribund. The symptoms before death included weight loss, episcleritis, icteric mucous membranes, emesis, ataxia and prostration. Two dogs (sex not specified) had decreased haemoglobin concentrations, erythrocyte volume fractions and red blood cell counts and increased blood urea nitrogen. Three dogs (sex not specified) had elevated aspartate and alanine aminotransferase activities, and all four (sex not specified) had increased bilirubin levels. Pathological examination of the tissues revealed pulmonary oedema, hepatic and adrenal cortical and medullary necrosis, fatty degeneration of the myocardium and testicular degeneration. Except for slight decreases in haemoglobin, erythrocyte volume fraction, red blood cell count and bilirubin values at 250 mg/kg bw per day, no effects were reported at 125 or 250 mg/kg bw per day. The NOEL was 250 mg/kg per day (Gralla et al., 1969).

Ethyl maltol (No. 1481)

Rats

Groups of 10 Charles River weanling albino rats of each sex were maintained on a diet containing ethyl maltol at levels calculated to provide an average daily intake of 250, 500 or 1000 mg/kg bw for 90 days. Concurrent control groups of unspecified numbers of male and female rats were maintained on a basal diet. Body weight and food consumption were recorded weekly. Blood and urine samples were collected from five male and five female rats from each group 45 and 90 days after the start of the study. Blood samples were analysed for haemoglobin, erythrocyte

volume fraction, red blood cell count, total white blood cell count and differential count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was analysed microscopically after centrifugation. At study termination, all rats were necropsied and organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries); gross and microscopic examinations were made of major tissues (brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland). There were no significant effects on body-weight gain. Two females and three males at the lowest dose had decreased haemoglobin concentration and slightly amber-coloured serum, but these changes were not seen at higher doses. No significant gross pathological changes or changes in organ weights were reported. Microscopic examination revealed a low incidence of kidney lesions, characterized as dilated acellular glomerular tufts with protein extravasation into Bowman capsule and cast formation within the lumina of dilated corticomedullary tubules, in rats at 1000 mg/kg bw per day; however, the incidence was less than that in rats given the same dose of maltol (Gralla et al., 1969).

Dogs

Groups of four male and four female beagle dogs were given capsules containing ethyl maltol at a dose of 125, 250 or 500 mg/kg bw per day for 90 days. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, renal function (bromosulfphthalein excretion test) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were evaluated at the start of the study and on days 14, 30, 60 and 90. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid, brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus, and ovary) were weighed. Selected tissues (brain, cervical spinal cord, sciatic nerve, hypophysis, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were examined microscopically. On day 30, all dogs receiving 500 mg/kg bw per day and half of those receiving 250 mg/kg bw per day showed elevated bilirubin levels, which returned to normal in the dogs at 250 mg/kg bw per day. Microscopic examination of the liver revealed that dogs at 250 mg/kg bw per day had a few or a moderate number of Kupffer cells containing both haemosiderin and small amounts of intracellular bilirubin. In dogs at 125 mg/kg bw per day, a few Kupffer cells contained haemosiderin, but no bilirubin was detected. No other effects were reported (Gralla et al., 1969).

2-Butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) and 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486)

Rats

In three studies, groups of 14 Charles River rats of each sex were fed diets containing 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) or 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) as a 16.7% emulsion in gum arabic for 90 days. The gum mixture was added to the diet at a concentration of 51 mg/kg for the first 4 weeks, 85 mg/kg for weeks 5–10 and 102 mg/kg for weeks 11–13. The doses provided by these concentrations in males and females, respectively, were: 6.59 and 7.35 mg/kg bw per day of 2-butyl-5- or -6-keto-1,4-dioxane, 6.65 and 7.33 mg/kg bw per day of 2-amyl-5- or -6-keto-1,4-dioxane or 5.96 and 6.76 mg/kg bw per day of 2-hexyl-5- or -6-keto-1,4-dioxane. Concurrent control groups (10–14 rats of each sex) were maintained on a basal diet. Body weights and food consumption were recorded weekly. Haematological examinations (haemoglobin concentration, erythrocyte count, erythrocyte volume fraction and total and differential leucocyte counts) and blood urea determinations were performed on 50% of the rats at week 7 and on all rats at week 13. At termination, the livers and kidneys were weighed, and gross and histological examinations were conducted on major organs. Liver, spleen, pancreas, stomach, large and small intestines, epididymis and testicles or ovaries and uterus, kidneys, bladder, heart, lungs, thyroid, adrenal glands, pituitary gland, submaxillary gland, sternal marrow, spinal cord and brain were examined microscopically (Posternak, 1969a,b,c).

In the study with 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), no significant differences were found in body weights between treated and control animals. There was no significant difference in absolute liver weights between the two groups, but a significant increase in relative liver weight was reported in treated males and females. Histopathological examination revealed no evidence of alteration in any organ or tissue (Posternak, 1969a).

In the study with 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485), slight, transient increases in haemoglobin concentration in males and in blood urea in females were reported in week 7 but not at the end of the study. A slight decrease in blood urea in males at week 13 was also reported. Body weights were similar in the two groups. Absolute liver weights were similar in treated and control groups, but an increased relative liver weight was reported in treated males. Histopathological examination revealed no evidence of alteration in any organ or tissue (Posternak, 1969b).

In the study with 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486), increased mean corpuscular haemoglobin was reported in animals of each sex at week 7 and in females also at week 13. In addition, haemoglobin concentrations were increased in treated females at week 7 only. A slight decrease (12.4%) in body weights occurred in treated males in comparison with control males. Absolute kidney and liver weights did not differ significantly between test and control animals; however, mainly because of depressed body weights in males, the relative kidney:body weight ratio was increased in treated males. Histopathological examination of the kidneys revealed pathological lesions in all treated males and females, while control animals had no such lesions. The lesions were less pronounced in females and were characterized by enlargement of the Bowman space and vacuolization of the proximal and distal convoluted tubules. There were no changes in any other organ or tissue examined (Posternak, 1969c).

The effects on relative liver and kidney weights and on clinical chemistry and haematological parameters after consumption of 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) or 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) were considered to be minimal when the test values were compared with composite rather than individual control groups. The effects were therefore deemed not to be toxicologically significant (Posternak et al., 1969).

(c) Long-term studies of toxicity and carcinogenicity

The results of long-term studies with maltol-related substances are summarized in Table 4.

Ethyl maltol (No. 1481)

Rats

Groups of 25 Charles River weanling albino rats of each sex were maintained on diets containing ethyl maltol at levels calculated to provide an average daily intake of 50, 100 or 200 mg/kg bw per day for 2 years. Body weight and food consumption were recorded weekly. Blood and urine samples were collected at 3, 6, 9, 12, 18 and 24 months. Blood samples were analysed for haemoglobin, erythrocyte volume fraction, red blood cell count and total and differential white blood cell count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was examined microscopically after centrifugation. At the end of the study, all rats were necropsied; organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries), and gross and microscopic examinations were made of major tissues (brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland). No difference in general health or behaviour was observed between treated and control rats. All rats, including controls, showed a tendency toward albuminuria. Measurements of body weight, haematology, clinical chemistry and histopathology revealed no significant differences between treated and control animals. Neoplasms occurred randomly in test and control animals with no apparent relation between the number, location or type of tumour and treatment with ethyl maltol (Gralla et al., 1969).

Dogs

Groups of eight male and eight female beagle dogs were given capsules containing ethyl maltol at a dose of 50, 100 or 200 mg/kg bw per day for 2 years. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, a renal function test (bromosulfphthalein excretion) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were conducted at 0, 3, 6, 8, 12, 18 and 24 months. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid,

brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus and ovary) were weighed. Selected tissues (brain, cervical spinal cord, hypophysis, sciatic nerve, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were examined microscopically. Two dogs per group were killed after 1 year of treatment and the remaining animals at the end of the study. Four dogs (sex not specified) receiving 200 mg/kg per day had slightly elevated serum alanine aminotransferase activity; however, all other measures of liver function were normal, as was liver morphology. At necropsy, pathological and microscopic examination revealed no dose-related effects (Gralla et al., 1969).

(d) Genotoxicity

Two representative flavouring agents in this group have been tested for genotoxicity. The results are summarized in Table 5.

In vitro

Maltol (No. 1480) and ethyl maltol (No. 1481) were weakly mutagenicity (two- to threefold increases in number of revertants) in *Salmonella typhimurium* TA100 at concentrations of 1–3 mg/plate either alone or with an exogenous liver-derived bioactivation system. Activity against TA98 was not detected (Bjeldanes & Chew, 1979). Maltol tested at concentrations of 0.1–10.0 mg/plate increased the number of revertants in strain TA97 at 1 mg/plate by about twofold. No increase was found in the presence of an activation system, or in TA102 alone or with activation (Fujita et al., 1992). In other studies with in *S. typhimurium*, neither maltol (Hayashi et al., 1988; Gava et al., 1989) nor ethyl maltol (Wild et al., 1983) was consistently mutagenic when tested at concentrations up to 10 000 μg/plate alone or in the presence of an activation system.

No evidence of DNA damage was reported when maltol was incubated with *Escherichia coli* strain PQ37 at a concentration of 5 mmol/l (631 μg/ml) for 2 h at 37 °C (Ohshima et al., 1989).

Maltol at concentrations ranging from 0.1 to 1.5 μmol/ml induced sister chromatid exchanges in Chinese hamster ovary cells (Gava et al., 1989) and in human lymphocytes (Jannson et al., 1986; Gava et al., 1989). Gava et al. (1989) suggested that these results were due to an indirect action of maltol and not to its direct reactivity with DNA.

In vivo

When groups of 8-week-old male ddY mice were given a single intraperitoneal injection of 125, 250 or 500 mg/kg bw of maltol and their bone marrow was sampled at 24 h, a dose-dependent increase in the incidence of micronucleated polychromatic erythrocytes was observed at the two highest doses (Hayashi et al., 1988). No evidence of micronucleus formation was reported when ethyl maltol was administered by intraperitoneal injection to groups of 10–14-week-old male and female NMRI mice at a concentration of 420, 700 or 980 mg/kg bw with sampling 30 h later or in

Table 5 .Studies of genotoxicity with maltol and related substances

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
1480	Maltol	Reverse mutation	*S. typhimurium* TA100	4.44 µmol/plate[a] (560 µg/plate)	Negative[b,c]	Kim et al. (1987)
1480	Maltol	Reverse mutation	*S. typhimurium* TA98, TA100	≤ 3 mg/plate (3000 µg/plate)	Positive[d,e]	Bjeldanes & Chew (1979)
1480	Maltol	Reverse mutation	*S. typhimurium* TA92, TA98, TA100, TA104	1.5–11 µmol/plate[a] (189–1387 µg/plate)	Negative	Gava et al. (1989)
1480	Maltol	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	33–10 000 µg/plate	Negative[b,f,g]	Mortelmans et al. (1986)
1480	Maltol	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	33–3333 µg/plate	Negative[b,c,g]	Mortelmans et al. (1986)
1480	Maltol	Reverse mutation	*S. typhimurium* TA97, TA102	0.1, 0.5, 1, 5 or 10 mg/plate (100, 500, 1000, 5000 or 10 000 µg/plate)	Weakly positive[b,d,h,i]	Fujita et al. (1992)
1480	Maltol	DNA damage (SOS Chromotest)	*E. coli* PQ37	5 mmol/l[a] (631 µg/ml)	Negative[j]	Ohshima et al. (1989)
1480	Maltol	Sister chromatid exchange	Chinese hamster ovary cells	≤ 1.5 mmol/ml[a] (12.6–189 µg/ml)	Positive[c,k]	Gava et al. (1989)
1480	Maltol	Sister chromatid exchange	Human lymphocytes	≤ 1.0 mmol/l[a] (126.11 µg/ml)	Positive	Jansson et al. (1986)
1481	Ethyl maltol	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	≤ 3.6 mg/plate (3600 µg/plate)	Negative[b,d,l]	Wild et al. (1983)
1481	Ethyl maltol	Reverse mutation	*S. typhimurium* TA98, TA100	≤ 2 mg/plate (2000 µg/plate)	Positive[d,e]	Bjeldanes & Chew (1979)
In vivo						
1480	Maltol	Micronucleus formation	ddY mouse bone marrow	125, 250 or 500 mg/kg	Positive[m]	Hayashi et al. (1988)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	6000 ppm (6000 µg/ml)	Equivocal[n]	Zimmering et al. (1989)
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 000 ppm (10 000 µg/ml)	Negative[m]	Mason et al. (1992)
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 000 ppm (10 000 µg/ml)	Negative[n]	Mason et al. (1992)
1481	Ethyl maltol	Micronucleus formation	NMRI mouse bone marrow	420, 700 or 980 mg/kg	Negative[m]	Wild et al. (1983)
1481	Ethyl maltol	Micronucleus formation	NMRI mouse bone marrow	980 mg/kg	Negative[m,o]	Wild et al. (1983)
1481	Ethyl maltol	Sex-linked recessive lethal mutation (Basc test)	*Drosophila melanogaster*	14 or 50 mmol/l[p] (1962 or 7007 mg)	Negative[n]	Wild et al. (1983)

[a] Calculated from relative molecular mass of maltol = 126.11
[b] Assay performed with pre-incubation method
[c] Without metabolic activation
[d] With and without metabolic activation
[e] Dose-related mutagenic activity reported only in TA100
[f] With metabolic activation
[g] Dose-dependent increase in number of revertants observed, but the number of revertants was less than twofold higher than that in negative controls.
[h] Weak mutagenicity reported in TA97 only without metabolic activation
[i] Maltol was nitrosated (incubated for 60 min at 37 °C with 25 mmol/l sodium nitrite) before the SOS Chromotest was performed.
[j] Cytotoxicity observed at highest dose
[k] Weak mutagenicity in TA100 at concentrations > 1000 µg/plate, but significant increases not reproducible
[l] Cytoxocoty observed at highest dose
[m] Administered by intraperitoneal injection
[n] Administered orally
[o] Modified test with expression times of 24, 48 and 72 h after treatment
[p] Calculated from relative molecular mass of ethyl maltol = 140.14

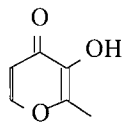
a modified test with sampling 24, 48 or 72 h after treatment with 980 mg/kg bw (Wild et al., 1983).

Equivocal results were obtained for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* larvae fed a concentration of 6000 mg/kg (Zimmering et al., 1989). On the basis of new data and a re-examination of the criteria used to determine mutagenicity in the published data, Mason et al. (1992) reported that maltol did not induce sex-linked recessive lethal mutations in *Drosophila* at concentrations up to 10 000 mg/kg. Ethyl maltol did not induce sex-linked recessive lethal mutations when fed to *D. melanogaster* larvae at concentrations of 14–50 mmol/l (Wild et al., 1983).

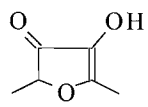
Conclusion

Equivocal or weakly positive results were obtained with maltol and ethyl maltol in some tests for genotoxicity in vitro, and positive or equivocal results were found in vivo with maltol but not with ethyl maltol. The mechanism of the effects is not known, but there is a structural similarity between maltol, ascorbic acid and 4-hydroxy-3-(2*H*)furanones (see Figure 2), which also contain an oxidizable enol functionality. In the presence of metals (e.g. Fe^{3+}) and dissolved oxygen, the enolic OH of ascorbate is oxidized by single-electron transfer to yield the corresponding carbon-centred radical and a reduced metal ion (e.g. Fe^{2+}). The carbon-centred radical can couple to molecular oxygen to produce a peroxyl radical, which might damage DNA. Alternatively, the reduced metal ion could autoxidize to form superoxide radical anion. Superoxide radical would then dismutate into hydrogen peroxide (H_2O_2). It is well recognized that reduced metals react with H_2O_2 to form the hydroxyl radical, which is a powerful oxidizing agent that might cause DNA strand breaks (see Figure 3).

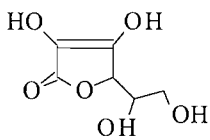
Figure 2. α-Hydroxyenol derivatives



Maltol



2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone



Ascorbic acid

Figure 3. Mechanism of oxidation of α-hydroxyenol derivatives in vitro

$O_2^{-\cdot}$ → Superoxide dismutase → H_2O_2 → $OH\cdot$ Hydroxy radical, DNA strand breaking

Hydrogen peroxide also oxidizes glutathione, leading to decreased GSH:GSSG and increased cellular oxidative stress.

Ascorbic acid is genotoxic in test systems similar to those in which maltol gave positive results. It induces reverse mutations in *S. typhimurium* strains TA104, TA102, TA100 and TA98 at concentrations of 352–1761 μg/plate (Ichinotsubo et al., 1981; D'Agostini et al., 2000). Increased levels of micronuclei were observed when ascorbic acid (400, 500 or 600 μg/ml) was incubated with Chinese hamster cells (Miller et al., 1995), and increased sister chromatid exchange was observed in Chinese hamster ovary cells in the presence of 500 μg/ml of ascorbic acid without metabolic activation (Tennant et al., 1987). In a standard assay for micronucleus formation in mouse bone marrow, 1500 mg/kg bw ascorbic acid induced a significant increase in B6C3F_1 mice (Shelby et al., 1993). The structural similarity of maltol to ascorbate suggests that a similar mechanism might be responsible for its mutagenicity, although this has not been tested experimentally.

In animals, absorbed maltol is rapidly conjugated with glucuronic acid and sulfate in the liver (see section 1.3). Only minute amounts of free maltol and ethyl maltol are detected in the urine of rats or dogs given high doses; most of an administered dose of maltol or ethyl maltol is rapidly excreted as glucuronic acid and sulfate conjugates in urine. Therefore, despite the structural similarity of maltol and ascorbate, it seems unlikely that the mutagenic activity of maltol would be expressed under the conditions of oral human intake.

(e) Other relevant studies

Maltol (No. 1480)

A three-generation study of reproductive toxicity was conducted, in which groups of 20 male and 20 female rats were given diets containing maltol at concentrations resulting in 100, 200 or 400 mg/kg bw per day. On day 134, animals of the F_1 generation showed signs of sialodacryodenitis due to a contagious virus. No deaths occurred, and the signs diminished within 10 days. Maltol had no effect on copulation rate, mating viability index, lactation, offspring sex ratio or 21-day pup survival index. Discrepancies in F_1 growth rates appeared to be related to the sialodacryodenitis outbreak in the colony. No maltol-related abnormalities or lesions were reported in pups. F_1 pups were weaned and then maintained on the same maltol-containing diets, and F_1 rats were mated on days 189 and 245 to provide the F_{2a} and F_{2b} generations. On day 418, all animals showed signs of sialodacryodenitis. No deaths occurred, and the signs diminished within 10 days. Pup survival was comparable to that of controls. The results of ophthalmic examinations performed during months 12, 18 and 24 were unremarkable. At autopsy, haematological comparisons of test and control animals revealed significant increases in K^+, Cl^-, urea and bilirubin concentrations in males and females at the highest dose. Increased urea and K^+ were found in males at the intermediate dose. No compound-related lesions were found in organs or tissues of the F_{2a} and F_{2b} generations. There was no indication that maltol affected tumour incidence (Annex 1, reference 57).

In a study conducted to develop a long-term animal model of the toxicity of aluminium administered intravenously, groups of 15–16 young adult New Zealand white male rabbits were given 0.075 mmol aluminium maltol or 0.675 mmol maltol (85 mg or approximately 21.3 mg/kg bw) three times per week for 8–30 weeks. A

concurrent control group of 15 animals was maintained. The rabbits were fed standard rabbit chow and had access to food and water ad libitum. All animals were weighed weekly and observed daily for changes in general appearance, food and water consumption and urine and faecal production. The rabbits were also monitored for signs of weakness or loss of neurological function. A statistically insignificant weight gain was reported in the maltol-treated animals. No treatment-related changes in blood chemistry, histology or neurological function were reported in the maltol-treated animals during the study (Berthole et al., 1989).

Ethyl maltol (No. 1481)

Groups of 25 Charles River weanling albino rats of each sex were maintained on a diet containing ethyl maltol at levels calculated to result in an average daily intake of 50, 100 or 200 mg/kg bw for 2 years. Ten pairs of rats at each dose were mated between weeks 15–21 and weeks 30–36. The offspring were killed at weaning. In the parental group, five of each sex were killed after 1 year and the remaining five of each sex at the end of the study. Gross and microscopic examination of parents and offspring indicated no significant effects on fertility, gestation, parturition, lactation or fetal development. Treated and control animals observed for 2 years showed no differences in general health or behaviour, body weight, haematology, clinical chemistry, histopathology results or the incidence of neoplasms (Gralla et al., 1969).

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 83, Revision 1 (FGE.83Rev1):

Consideration of ethyl maltol and two 6-keto-1,4-dioxane derivatives substances evaluated by JECFA (65th meeting)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The JECFA has evaluated a group of seven flavouring substances consisting of four maltol related substances and three 6-keto-1,4-dioxane derivatives, in the JECFA group "Maltol and related substances". Two of the JECFA evaluated substances are not in the Register (2-hexyl-5 or 6-keto-1,4-dioxane (JECFA-no: 1486) and 2-methyl-3-(1-oxypropoxy)-4H-pyran-4-one (JECFA-no: 1483)) and three substances [FL-no: 07.014, 07.047 and 09.525] are alpha,beta-unsaturated ketones.

The genotoxicity of the three substances being alpha,beta-unsaturated ketones [FL-no: 07.014, 07.047 and 09.525] has been considered in FGE.213 (EFSA, 2009x). The Panel concluded that for two substances [FL-no: 07.014 and 09.525] a final conclusion as to their genotoxic properties could not be

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reached and additional data were requested. Accordingly, these two substances will not be considered in this FGE.

For ethyl maltol [FL-no: 07.047], also considered in FGE.213, the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that the substance can be evaluated through the Procedure. The present FGE.83Rev1 therefore in total deals with three substances [FL-no: 09.047, 13.027 and 13.028]. The Panel concluded that no corresponding FGE is available.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for the three substances considered in this FGE. However, the JECFA evaluation of two of the three substances is only based on Maximised Survey-derived Daily Intake (MSDI) values derived from USA production figures. EU production figures are needed in order to finalise the evaluation of these two substances [FL-no: 13.027 and 13.028].

For all three substances evaluated through the Procedure use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the three JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. For the two JECFA evaluated substances [FL-no: 13.027 and 13.028] information on the stereoisomeric composition has not been provided.

Thus, for two substances [FL-no: 13.027 and 13.028] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and missing information on stereoisomerism). For the remaining substance [FL-no: 07.047] the Panel agrees with the JECFA conclusion “No safety concern at estimated level of intake as flavouring substance” based on the MSDI approach.

KEY WORDS

Ethyl maltol, 6-keto-1,4-dioxane derivatives, flavourings, JECFA, 65th meeting.

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BACKGROUND

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

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available,

meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

At its 65th meeting the JECFA evaluated a group of seven flavouring substances consisting of four maltol related substances and three 6-keto-1,4-dioxane derivatives, in the JECFA group " Maltol and related substances". Two of the JECFA evaluated substances are not in the Register (2-hexyl-5 or 6-keto-1,4-dioxane (JECFA-no: 1486) and 2-methyl-3-(1-oxypropoxy)-4H-pyran-4-one (JECFA-no: 1483)) and three substances [FL-no: 07.014, 07.047 and 09.525] are alpha,beta-unsaturated ketones. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), they have been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The remaining 2 flavouring substances have originally been considered by EFSA in the FGE.83 (EFSA, 2008ay).

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship ((Q)SAR) prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220).

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.83	December 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902221126.htm	2
FGE.83Rev1	November 2009		3

The present Revision of FGE.83, FGE.83Rev1, includes the assessment of one additional substance, ethyl maltol, [FL-no: 07.047] originally considered in FGE.213 and for which the Panel concluded that the genotoxicity data available do not preclude its evaluation through the Procedure.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of seven flavouring substances consisting of four maltol related substances and three 6-keto-1,4-dioxane derivatives, in the JECFA group "Maltol and related substances" (JECFA, 2006d).

1.1.2. EFSA Considerations

Two of the seven JECFA evaluated substances are not in the Register (2-hexyl-5 or 6-keto-1,4-dioxane (JECFA-no: 1486) and 2-methyl-3-(1-oxypropoxy)-4H-pyran-4-one (JECFA-no: 1483)) and three substances [FL-no: 07.014, 07.047 and 09.525] are alpha,beta-unsaturated ketones. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these three substances were given special considerations.

The genotoxicity of the three substances being alpha,beta-unsaturated ketones [FL-no: 07.014, 07.047 and 09.525] has been considered in FGE.213 (EFSA, 2009x). The Panel concluded that for two substances a final conclusion as to their genotoxic properties could not be reached and additional data were requested. Accordingly, these two substances will not be considered in this FGE. The Panel further concluded that the data available on ethyl maltol [FL-no: 07.047] did rule out the concern for genotoxicity and thus concluded that the substance can be evaluated through the Procedure.

The remaining two flavouring substances [FL-no: 13.027 and 13.028] have originally been considered by EFSA in the FGE.83 (EFSA, 2008ay).

The present FGE.83Rev1 therefore in total deals with three substances [FL-no: 07.047, 13.027 and 13.028].

The Panel has concluded that no corresponding FGE is available for the three JECFA evaluated substances.

1.2. Isomers

1.2.1. JECFA Status

Two substances [FL-no: 13.027 and 13.028] in the group of the JECFA evaluated ethyl maltol and 5- or 6-keto-1,4-dioxane derivatives have a chiral centre.

1.2.2. EFSA Considerations

Information has not been provided about the stereoisomerism for the two substances [FL-no: 13.027 and 13.028].

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for the three substances (JECFA, 2005d; JECFA, 2008c). See Table 1.

1.3.2. EFSA Considerations

For two substances [FL-no: 13.027 and 13.028] information on stereoisomerism has not been provided (see Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For one substance evaluated through the JECFA Procedure intake data are available for the EU (see Table 3.1). For two substances production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for the two substances [FL-no: 13.027 and 13.028], MSDI values for the EU cannot be calculated.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[4] from the JECFA (JECFA, 2006d)

In vitro

Ethyl maltol [FL-no: 07.047] was weakly mutagenicity (two-to threefold increases in number of revertants) in *Salmonella typhymurium* TA100 at concentrations of 1 – 3 mg/plate either alone or with an exogenous liver-derived bioactivation system. Activity against TA98 was not detected (Bjeldanes & Chew, 1979). In other studies with *S. typhymurium* ethyl maltol (Wild et al., 1983) was consistently mutagenic when tested at concentrations up to 10000 microgram/plate alone or in the presence of an activation system.

In vivo

No evidence of micronucleus formation was reported when ethyl maltol was administerred by intraperitoneal injection to groups of 10-14 weeks-old male and female NMRI mice at a concentration of 420, 700 or 980 mg/kg bw with sampling 30 h later or in a modified test with sampling 24, 48 or 72 h after treatment with 980 mg/kg bw (Wild et al., 1983). Ethyl maltol did not induce sex-linked recessive lethal mutations when fed to *D. Melanogaster* larvae at concentrations of 14-50 mmol/L (Wild et al., 1983).

Conclusion

Equivocal or weakly positive results were obtained with ethyl maltol (and maltol) in some tests for genotoxicity *in vitro*. The JECFA considered that the mechanism of action would be similar to that of ascorbate which shows genotoxicity in similar test systems.

Only minute amounts of free ethyl maltol are detected in the urine of rats or dogs given high doses; most of an administered dose of ethyl maltol is rapidly excreted as glucoronic acid an sulphate conjugates in urine.

For a summary of *in vitro/in vivo* genotoxicity data evaluated by the JECFA see Table 2.1.

[4] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed*.

3.2. Genotoxicity and Carcinogenicity – Text Taken[5] from FGE.213 (EFSA, 2009x)

In vitro / in vivo

For ethyl maltol [FL-no: 07.047] two *in vitro* and one *in vivo* study were evaluated. Ethyl maltol induced gene mutations in bacteria (Bjeldanes & Chew, 1979). The validity of the other studies was considered limited.

Groups of 25 male and female rats were fed for two years on diets containing ethyl maltol [FL-no: 07.047] calculated to deliver 0, 50, 100 and 200 mg ethyl maltol/kg bw/day. No abnormalities were seen as regards survival, clinical appearance, growth rate or food consumption, clinical chemistry, haematology and urinalysis. No histopathological changes and no increases in neoplasms were seen after the treatment with ethyl maltol (Gralla et al., 1969).

Conclusion on Genotoxicity and Carcinogenicity

There is a carcinogenicity study on ethyl maltol [FL-no: 07.047] in rats. Although the number of animals per group were lower than suggested in OECD guidelines they were in accordance with the standards at the time the study was performed and the Panel concluded that the result could overrule the mutagenicity observed with ethyl maltol in bacteria. The data available do not indicate a genotoxic or carcinogenic potential for ethyl maltol.

For a summary of *in vitro/in vivo* genotoxicity data evaluated in FGE.213, see Table 2.2 and 2.3.

3.3. EFSA Considerations

Ethyl maltol [FL-no: 07.047] induced gene mutations in bacteria but was negative in an *in vivo* in the micronucleus assay and furthermore not considered carcinogenetic in rats. Although the data were of limited validity, the Panel concluded that the data available did not indicate a genotoxic or carcinogenic potential of ethyl maltol and therefore this compound could be evaluated through the Procedure.

The two substances, 2-pentyl-5 or 6-keto-1,4-dioxane [FL-no: 13.027] and 2-butyl-5 or 6-keto-1,4-dioxane [FL-no: 13.028], are expected to be rapidly hydrolysed similarly to other lactones to yield the corresponding 5-hydroxycarboxylic acid derivatives. The Panel concluded that data available do not preclude evaluation of the two flavourings through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to Ethyl Maltol and 6-Keto-1,4-dioxane Derivatives by the JECFA (JECFA, 2006d)

According to the JECFA ethyl maltol belongs to structural class II and the two other substances belong to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded the two 5- or 6-keto-1,4-dioxane derivatives at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for the substances are below the thresholds for the structural classes III (step A3).

Ethyl maltol, [FL-no: 07.047] was concluded at step A5 – i.e. the estimated daily intake is above the threshold for the structural class, the substance is not endogenous, but a No Observed Adverse Effect Level (NOAEL) of 200 mg/kg body weight (bw) is available (Gralla et al., 1969) that can provide an

[5] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

adequate margin of safety to the MSDI value for the substance. For this substance the estimated daily intake used in the JECFA evaluation was based on the calculated MSDI for USA (worst case) which was 6692 microgram/capita/day. The calculated MSDI for Europe was 1580 microgram/capita/day.

The JECFA concluded the substances to be of no safety concern at the estimated levels of intake as flavouring substances, based on the MSDI approach.

The evaluations of the three substances are summarised in Table 3: Summary of Safety Evaluation of Ethyl Maltol and 5- or 6-Keto-1,4-Dioxane Derivatives (JECFA, 2006d).

4.2. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for the three substances in the group of ethyl maltol and 5- or 6-keto-1,4-dioxane derivatives.

However, for the two substances [FL-no: 13.027 and 13.028] no European production figures were available and consequently no European exposure estimates could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for the substances.

5. Conclusion

The JECFA has evaluated a group of seven flavouring substances consisting of four maltol related substances and three 6-keto-1,4-dioxane derivatives, in the JECFA group "Maltol and related substances". Two of the JECFA evaluated substances are not in the Register (2-hexyl-5 or 6-keto-1,4-dioxane (JECFA-no: 1486) and 2-methyl-3-(1-oxypropoxy)-4H-pyran-4-one (JECFA-no: 1483)) and three substances [FL-no: 07.014, 07.047 and 09.525] are alpha,beta-unsaturated ketones.

The genotoxicity of the three substances being alpha,beta-unsaturated ketones [FL-no: 07.014, 07.047 and 09.525] has been considered in FGE.213 (EFSA, 2009x). The Panel concluded that for the two substances [FL-no: 07.014 and 09.525] a final conclusion as to their genotoxic properties could not be reached and additional data were requested. Accordingly, these two substances will not be considered in this FGE.

For ethyl maltol [FL-no: 07.047], also considered in FGE.213, the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that the substance can be evaluated through the Procedure. The present FGE.83Rev1 therefore in total deals with three substances [FL-no: 09.047, 13.027 and 13.028]. The Panel concluded that no corresponding FGE is available.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for the three substances considered in this FGE. However, the JECFA evaluation of two of the three substances is only based on MSDI values derived from USA production figures. EU production figures are needed in order to finalise the evaluation of these substances two [FL-no: 13.027 and 13.028].

For all three substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

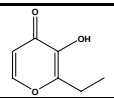
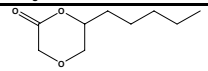
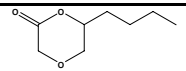
In order to determine whether the conclusion for the three JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. For the two JECFA evaluated substances [FL-no: 13.027 and 13.028] information on the stereoisomeric composition has not been provided.

Thus, for two substances [FL-no: 13.027 and 13.028] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and missing information on stereoisomerism). For the remaining substance [FL-no: 07.047] the Panel agrees with the JECFA conclusion "No safety concern at estimated level of intake as flavouring substance" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: specifications summary for the JECFA evaluated substances in the present group (JECFA, 2005d; JECFA, 2008c)

Table 1: Specification Summary of Ethyl Maltol and 6-Keto-1,4-dioxane Derivatives evaluated by JECFA (JECFA, 2005d; JECFA, 2008c)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
07.047 1481	Ethyl maltol		3487 692 4940-11-8	Solid $C_7H_8O_3$ 140.14	Soluble Soluble	 89-93 NMR 99 %	n.a. n.a.	
13.027 1485	2-Pentyl-5 or 6-keto-1,4-dioxane 6)		2076 2205 65504-96-3	Liquid $C_9H_{16}O_3$ 172.22	Slightly soluble Soluble	101-103 (20hPa) NMR 97 %	1.480-1.486 1.288-1.294	CASrn in Register does not specify stereoisomers.
13.028 1484	2-Butyl-5 or 6-keto-1,4-dioxane 6)		2204 2206 65504-45-2	Liquid $C_8H_{14}O_3$ 158.20	Slightly soluble Soluble	98-99 (17 hPa) NMR 97 %	1.472-1.478 1.292-1.296	JECFA CASrn: 65504-95-2 does not specify stereoisomers. CASrn in Register to be changed.

1) *Solubility in water, if not otherwise stated.*

2) *Solubility in 95 % ethanol, if not otherwise stated.*

3) *At 1013.25 hPa, if not otherwise stated.*

4) *At 20°C, if not otherwise stated.*

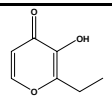
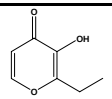
5) *At 25°C, if not otherwise stated.*

6) *Stereoisomeric composition not specified.*

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (in vitro / in vivo) (JECFA, 2006d)

Table 2.1: Summary of genotoxicity data of the group of ethyl maltol and two 6-keto-1,4-dioxane derivatives evaluted by JECFA (JECFA, 2006d)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
07.047 1481	Ethyl maltol		Reverse Mutation	*S. typhimurium* TA 1535, TA1537, TA1538, TA98 and TA100	Up to 3.6 mg/plate (3600 µg/plate)	Negative[a,b,c]	(Wild et al., 1983)
			Reverse Mutation	*S. typhimurium* TA98 and TA100	Up to 2 mg/plate (2,000 µg/plate)	Positive[a,d]	(Bjeldanes & Chew, 1979)
In vivo							
07.047 1481	Ethyl maltol		Micronucleus formation	NMRI Mouse bone marrow cells	420, 700 or 980 mg/kg	Negative[e]	(Wild et al., 1983)
			Micronucleus formation	NMRI Mouse bone marrow cells	980 mg/kg	Negative[e,g]	(Wild et al., 1983)
			Sex-linked Recessive Lethal Mutation (Basc test)	*Drosophila melanogaster*	14 or 50 mmol/l (1962 or 7007 mg)	Negative[f]	(Wild et al., 1983)

[a:] *With and without metabolic activation.*

[b] *Assay performed with pre-incubation.*

[c] *Cytotoxicity observed at highest dose.*

[d] *Dose-related mutagenic activity reported only in TA100.*

[e] *Administered by intraperitoneal injection.*

[f] *Administered orally.*

[g] *Modified test with expression times of 24, 48 and 72 h after treatment.*

Table 2.2: Genotoxicity (in vitro) FGE.213 (EFSA, 2009x)

Table 2.2: GENOTOXICITY (*in vitro*) FGE.213 (EFSA, 2009x)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [d]
beta-Ionone [07.008]	Gene mutation(preincubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	1-180 µg/plate	Negative[a]	(Mortelmans et al., 1986)	Valid.
	Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 mmol/plate	Negative[a]	(Florin et al., 1980)	Insufficient validity (spot test, not according to OECD guideline, methods and results insufficiently reported).
3-Methylcyclopentan-1,2-dione [07.056]	Reverse mutation	S. typhimurium TA1535,	10 000 µg/plate	Negative[b]	(Heck et al., 1989)	Validity cannot be evaluated (result not reported in detail).
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg/plate	Negative[b]	(Heck et al., 1989)	Validity cannot be evaluated (result not reported in detail).
Maltol [07.014]	Reverse Mutation	*Salmonella typhimurium* TA100	4.44 µmol/plate (560 µg/plate) [d]	Negative[c]	(Kim et al., 1987b)	Insufficient validity (only one concentration was tested with only one bacterial strain without metabolic activation). The main purpose of the study was to investigate antimutagenic effects.
	Reverse Mutation	*Salmonella typhimurium* TA98 and TA100	Up to 3 mg/plate (3,000 µg/plate) [d]	Positive[a]	(Bjeldanes & Chew, 1979)	Valid.
	Reverse Mutation	*Salmonella typhimurium* TA92, TA98, TA100 and TA104	1.5 to 11 µmol/plate (189 to 1,387 µg/plate)	Negative	(Gava et al., 1989)	Limited validity (data not reported in detail).
	Reverse Mutation	*Salmonella typhimurium* TA1535, TA98, TA100 and TA1537	33 to 10,000 µg/plate	Positive[b]	(Mortelmans et al., 1986)	Valid .
	Reverse Mutation	*Salmonella typhimurium* TA97 and TA102	0.1, 0.5, 1, 5, or 10 mg/plate (100, 500, 1,000, 5,000, or 10,000 µg/plate)	Weak Positive[a]	(Fujita et al., 1992)	Result is considered equivocal. Limited validity (the use of only two strains is not according to OECD guideline).
	DNA Damage (SOS Chromotest)	*Escherichia coli* PQ37	5mM (631 µg/ml) [d]	Negative	(Ohshima et al., 1989)	The test system used is considered inappropriate, due to insufficient validity.
	Sister Chromatid Exchange	Chinese hamster ovary cells	Up to 1.5 µmol/ml (12.6 to 189 µg/ml) [d]	Positive[c]	(Gava et al., 1989)	Validity cannot be evaluated (insufficiently reported: number of cells analysed not reported. Statistical test used not reported). SCEs were reported as SCE per chromosome. Effect was less than twofold compared to control.
	Sister Chromatid Exchange	Human lymphocytes	Up to 1.0 mM (126.11 µg/ml)	Positive	(Jansson et al., 1986)	Validity cannot be evaluated. Relevance of test system for the evaluation of genotoxicity uncertain.

Table 2.2: GENOTOXICITY (*in vitro*) FGE.213 (EFSA, 2009x)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [d]
Ethyl maltol [07.047]	Reverse Mutation	*Salmonella typhimurium* TA 1535, TA1537, TA1538, TA98 and TA100	5 concentrations up to cytotoxicity, or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (result not reported in details, no TA 102 or E. Coli).
	Reverse Mutation	*Salmonella typhimurium* TA98 and TA100	Up to 2 mg/plate (2,000 µg/plate)	Positive[a]	(Bjeldanes & Chew, 1979)	Valid.

a: With and without metabolic activation.

b: With metabolic activation.

c: Without metabolic activation.

d: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

Table 2.3: Genotoxicity (in vitro) FGE.213 (EFSA, 2009x)

Table 2.3: GENOTOXICITY (*in vivo*) FGE.213 (EFSA, 2009x)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments [a]
Maltol [07.014]	Micronucleus formation	ddY Mouse bone marrow cells	intraperitoneal injection (i.p.)	125, 250, or 500 mg/kg	Positive	(Hayashi et al., 1988)	Valid. The induction of micronuclei was up to about 10-fold compared to control.
	Sex-linked Recessive Lethal Mutation	*Drosophila melanogaster*	feeding	6,000 ppm (6,000 µg/ml)	Equivocal	(Zimmering et al., 1989)	Limited validity (only one exposure level tested). Test system considered of limited relevance.
	Sex-linked Recessive Lethal Mutation	*Drosophila melanogaster*	feeding	10,000 ppm (10,000 µg/ml)	Negative	(Mason et al., 1992)	Valid, however, test system considered of limited relevance.
	Sex-linked Recessive Lethal Mutation	*Drosophila melanogaster*	injection	0.2 – 0.3 µl, 10,000 ppm (10,000 µg/ml)	Negative	(Mason et al., 1992)	Valid, however, test system considered of limited relevance.
Ethyl maltol [07.047]	Micronucleus formation	NMRI Mouse bone marrow cells	intraperitoneal injection (i.p.).	420, 700, or 980 mg/kg	Negative	(Wild et al., 1983)	Limited validity (injected twice; only analysis at one time point; no PCE/NCE ratio reported).
	Micronucleus formation	NMRI Mouse bone marrow cells	intraperitoneal injection (i.p.)	980 mg/kg	Negative	(Wild et al., 1983)	Limited validity (single injection, analysis at three time points, no PCE/NCE ratio reported).
	Sex-linked Recessive Lethal Mutation (Basc test)	*Drosophila melanogaster*	feeding	14, 25 or 50 mM	Negative	(Wild et al., 1983)	Limited validity (limited reporting, test system considered of limited relevance).

a: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

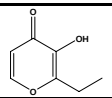
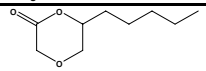
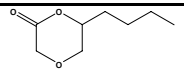
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3: Summary of safety evaluations of the JECFA evaluated substances in the present group (JECFA, 2006d)

Table 3: Summary of Safety Evaluation of Ethyl Maltol and 5- or 6-Keto-1,4-dioxane Derivatives (JECFA, 2006d)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.047 1481	Ethyl maltol		1580 6692	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.
13.027 1485	2-Pentyl-5 or 6-keto-1,4-dioxane		ND 0.2	Class III A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	Stereoisomeric composition to be specified. No European production volumes available, preventing them to be evaluated using the Procedure.
13.028 1484	2-Butyl-5 or 6-keto-1,4-dioxane		ND 0.5	Class III A3: Intake below threshold	4)	No European production volumes available, preventing them to be evaluated using the Procedure.	CASrn in Register to be changed to 65504-95-2. Stereoisomeric composition to be specified. No European production volumes available, preventing them to be evaluated using the Procedure.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
ND: not determined.

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ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
Ip	Intraperitoneal
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte

SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

SCIENTIFIC OPINION

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Scientific Opinion on Flavouring Group Evaluation 213, Revision 2 (FGE.213Rev2): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

Abstract

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) of the European Food Safety Authority (EFSA) was requested to evaluate the genotoxic potential of 26 flavouring substances from subgroup 2.7 of FGE.19 in Flavouring Group Evaluation (FGE) 213. In the first version of FGE.213 the Panel concluded, based on available genotoxicity data, that a concern regarding genotoxicity could be ruled out for 11 substances [FL-nos: 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.117, 07.118, 07.119, 07.120 and 07.168], but for the remaining 15 substances in subgroup 2.7 further genotoxicity data were required. Based on new submitted genotoxicity data, the Panel concluded in FGE.213Rev1 that the concern regarding genotoxicity could be ruled out for 13 substances in subgroup 2.7 [FL-nos: 02.106, 07.008, 07.010, 07.041, 07.083, 07.089, 07.108, 07.109, 07.127, 07.136, 07.200, 07.224 and 09.305] but not for maltol [FL-no: 07.014] and maltyl isobutyrate [FL-no: 09.525]. In FGE.213Rev2, new data on maltol were considered and the Panel concluded that for maltol [FL-no: 07.014] and maltyl isobutyrate [FL-no: 09.525] in food the concern for genotoxicity could be ruled out. Moreover, the Panel reconsidered the available data on *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127], based on new data on the structurally related substance pulegone, and concluded that additional genotoxicity data are needed to rule out the concern for genotoxicity of *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127].

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Keywords: FGE.213, α,β-unsaturated alicyclic ketones, flavouring substances, safety evaluation, subgroup 2.7, FGE.19

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Summary

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel was asked to evaluate flavouring substances using the procedure referred to in Commission Regulation EC No 1565/2000 (hereafter 'the Procedure').

The Flavouring Group Evaluation (FGE) 213 concerns 26 substances, corresponding to subgroup 2.7 of FGE.19. Twenty-three of the substances are α,β-unsaturated alicyclic ketones [Flavour Information System (FL)-nos: 07.008, 07.010, 07.014, 07.041, 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.083, 07.089, 07.108, 07.109, 07.117, 07.118, 07.119, 07.120, 07.127, 07.136, 07.168, 07.200 and 07.224] and three are precursors for such ketones [FL-nos: 02.106, 09.305 and 09.525].

In the first version of FGE.213 the Panel concluded that the genotoxicity concern for ethyl maltol [FL-no: 07.047], 3-ethylcyclopentan-1,2-dione [FL-no: 07.057] and the nine structurally related substances [FL-nos: 07.117, 07.118, 07.119, 07.120, 07.056, 07.168, 07.075, 07.076 and 07.080] could be ruled out and the 11 substances could accordingly be evaluated through the Procedure.

For maltol [FL-no: 07.014], a micronucleus assay after oral application was required in addition to an *in vivo* comet assay in order to clarify the genotoxic potential. The outcome would also be applicable to maltyl isobutyrate [FL-no: 09.525].

The remaining 13 substances (including two precursors of a ketone) [FL-nos: 02.106, 07.008, 07.010, 07.041, 07.083, 07.089, 07.108, 07.109, 07.127, 07.136, 07.200, 07.224 and 09.305] could not be evaluated through the Procedure. Accordingly, additional data on genotoxicity were required for representatives of these 13 substances.

The Flavour Industry informed that it no longer supports the representative flavouring substance, piperitenone oxide [FL-no: 16.044], for which the Panel requested additional data. In FGE.213Rev1, one additional substance has been included in subgroup 2.7, *tr*-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.224], which is structurally related to the other substances for which the genotoxic potential could not be ruled out.

In FGE.213Rev1, the Panel evaluated the new data submitted by the Flavour Industry in response to the data request presented in FGE.213. Based on these new data, the Panel concluded that the genotoxicity concern could be ruled out for the representative substances β-ionone [FL-no: 07.008], β-damascone [FL-no: 07.083], nootkatone [FL-no: 07.089], 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109] and the nine substances that they represent [FL-nos: 02.106, 07.010, 07.041, 07.108, 07.127, 07.136, 07.200, 07.224 and 09.305].

In the case of maltol, positive results were observed in an *in vitro* micronucleus assay in human peripheral blood lymphocytes and in an *in vivo* micronucleus assay in mouse bone marrow after intraperitoneal application. Maltol was also tested in rats (administered by gavage) in a combined bone marrow micronucleus assay and comet assay in liver. Both tests showed negative results, but no clinical signs and no bone marrow toxicity were observed. To investigate the systemic exposure, plasma bioanalysis was performed, but results were inconsistent. Owing to the intended use of maltol as a food-flavouring agent, the *in vivo* study performed with administration of maltol by gavage is considered more relevant than the study performed by intraperitoneal application. Therefore, the Panel concluded in Revision 1 of this FGE that for maltol [FL-no: 07.014] and maltyl isobutyrate [FL-no: 09.525] the concern for genotoxicity could not be ruled out.

The Flavour Industry has submitted a new plasma bioanalysis for maltol, which is evaluated in the present revision of FGE.213 (FGE.213Rev2). The Panel considered this new plasma bioanalysis and concluded that it seems justifiable to assume that animals were systemically exposed to maltol and that the bone marrow was exposed in the *in vivo* micronucleus assay. Therefore, the negative result of the *in vivo* micronucleus assay can be considered reliable and, accordingly, the concern for genotoxicity for maltol [FL-no: 07.014] and for maltyl isobutyrate [FL-no: 09.525] in food is ruled out; both substances were evaluated by JECFA before 2000 and no EFSA consideration is required.

Moreover, the Panel reconsidered the available data on *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127], based on new data on the structurally related substance pulegone and concluded that additional genotoxicity data are needed to rule out the concern for genotoxicity on *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127].

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The use of flavourings is regulated under Regulation (EC) No 1334/2008[1] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) N° 1565/2000.

On 10 April 2014 the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 213, Revision 1 (FGE.213Rev1): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19.[3]

The Panel concluded that, for maltol [FL-no: 07.014] and for maltyl isobutyrate [FL-no: 09.525] the Panel's concern with respect to genotoxicity could not be ruled out and subsequently additional data are requested. In particular it was pointed out that the data provided to prove systemic availability were considered inconclusive due to the inconsistency of the data.

On 6 January 2015 the applicant has submitted additional data on the representative substance maltol [FL-no: 07.014] in response to this EFSA evaluation. This additional data regards a study intended to look at systemic exposure of rats following oral administration of this substance, using the same dosing regimen employed in the combined micronucleus and comet test previously submitted.

Terms of Reference as provided by the European Commission

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and, depending on the outcome, proceed to the full evaluation on this flavouring substance in accordance with Commission Regulation (EC) N° 1565/2000[4].

[1] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34-50

[2] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161

[3] EFSA Journal 2014;12(2):3587.

[4] 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. OJ L 180, 19.7.2000, p. 8-16

2. Data and Methodologies

2.1. History of the evaluation of FGE.19 substances

Flavouring Group Evaluation (FGE) 19 (FGE.19) contains 360 flavouring substances from the European Union (EU) Register being α,β-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and/or oxidation (EFSA, 2008a).

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

The α,β-unsaturated carbonyls were subdivided into subgroups on the basis of structural similarity (EFSA, 2008a). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure–activity relationship ((Q)SAR) prediction of the genotoxicity of these substances was undertaken considering a number of models (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models, (Gry et al., 2007)).

The Panel noted that for most of these models internal and external validation has been performed, but considered that the outcome of these validations was not always extensive enough to appreciate the validity of the predictions of these models for these α,β-unsaturated carbonyls. Therefore, the Panel considered it inappropriate to totally rely on (Q)SAR predictions at this point in time and decided not to take substances through the procedure based on negative (Q)SAR predictions only.

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a, b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. Based on these data the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008b) could not be evaluated through the Procedure because of concerns with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established: FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225.

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavour Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220 the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related α,β-unsaturated substances in the different subgroups for which additional data are requested, EFSA worked out a list of representative substances for each subgroup (EFSA, 2008c). Likewise, an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

The Flavour Industry has been requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

The Flavour Industry has now submitted additional data and the present FGE concerns the evaluation of these data requested on genotoxicity.

2.2. History of the evaluation of the substances belonging to FGE.213

In the EFSA Opinion 'List of α,β-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing' (EFSA, 2008c), representative flavouring substances have been selected for FGE.19 subgroup 2.7, corresponding to FGE.213.

In the first scientific opinion on FGE.213 (EFSA, 2009), the Panel concluded that, based on the data available, the concern with respect to genotoxicity could be ruled out for 11 substances [FL-nos: 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 7.117, 07.118, 07.119, 07.120 and 07.168]. Nine of these substances have been evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) before 2000 to be of no safety concern and, in accordance with Commission Regulation (EC) No 1565/2000, no further consideration is requested. The remaining two substances, ethyl maltol [FL-no: 07.047] and 2-hydroxypiperitone [FL-no: 07.168], were evaluated in FGE.83Rev1 (EFSA CEF Panel, 2010) and FGE.11Rev2 (EFSA CEF Panel, 2011), respectively, using the Procedure.

For maltol [FL-no: 07.014], the Panel requested a combined *in vivo* micronucleus and comet assay in order to clarify the genotoxic potential. The outcome would also be applicable to maltyl isobutyrate [FL-no: 09.525].

For the remaining 13 substances [FL-nos: 02.106, 07.008, 07.010, 07.041, 07.083, 07.089, 07.108, 07.109, 07.127, 07.136, 07.200, 07.224 and 09.305] additional data on genotoxicity were required for the representative substances, according to the Opinion of the CEF Panel on the 'Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19' (EFSA, 2008b).

FGE	Adopted by EFSA	Link	No. of Substances
FGE.213	27 November 2008	http://www.efsa.europa.eu/en/efsajournal/pub/879.htm	26
FGE.213Rev1	10 April 2014	http://www.efsa.europa.eu/it/efsajournal/pub/3661.htm	26
FGE.213Rev2	09 September 2015	http://www.efsa.europa.eu/it/efsajournal/pub/4244.htm	26

In FGE.213 Revision 1 (FGE.213Rev1) the Panel evaluated additional genotoxicity data submitted by the Flavouring Industry (IOFI, 2012, 2013) in response to a data request presented in FGE.213 (EFSA, 2009).

The new data submitted concerned five of the original six representative substances requested by the Panel (EFSA, 2008c), namely β-ionone [FL-no: 07.008], maltol [FL-no: 07.014], β-damascone [07.083], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109] (Table 1).

The Flavour Industry informed that it no longer supports the representative flavouring substance, piperitenone oxide [FL-no: 16.044], for which the Panel requested additional data. However, since piperitenone oxide was a self-representative substance, this did not affect the evaluation of the remaining substances in FGE.213Rev1.

In FGE.213Rev1, one additional substance was included in subgroup 2.7, tr-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.224], which is structurally related to the other substances for which the genotoxic potential could not be ruled out.

In FGE.213Rev1, the Panel concluded that the *in vitro* and *in vivo* genotoxicity data for the selected representative substances β-ionone [FL-no: 07.008], β-damascone [FL-no: 07.083], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109] do not indicate a genotoxic potential. Therefore, these substances, and the nine substances that they represent [FL-no: 02.106, 07.010, 07.041, 07.108, 07.127, 07.136, 07.200, 07.224 and 09.305] could be evaluated through the Procedure.

During the evaluation of *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127], using the Procedure (in FGE.57Rev1), the Panel noted that the chemical structure of *p*-mentha-1,4(8)-dien-3-one [FL-no:

07.127] is actually more closely related to the structure of pulegone (Table 2) than to the structures used for the read-across approach in FGE.213Rev1. New information (NTP, 2011) was found on genotoxicity and carcinogenicity of pulegone, from which additional data are expected to be provided by the applicant. The data available, at present, on pulegone and on the structurally related *p*-mentha-1,4(8)-dien-3-one [FL-no 07.127] do not rule out the concern for genotoxicity and carcinogenicity. Therefore, the genotoxicity of *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] will be reconsidered based on additional data.

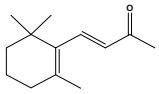
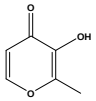
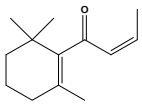
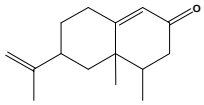
For maltol [FL-no: 07.014], a combined bone marrow micronucleus test and comet assay in rats (Beevers, 2013a) was evaluated by the Panel in FGE.213Rev1. The results of the micronucleus test showed that there were no statistically significant increases in micronucleus frequency for any dose group after oral treatment with maltol when compared with the vehicle control group. The comet assay did not reveal cytotoxicity, necrosis or apoptosis in the hepatocytes as assessed by cloud and halo analysis, and the groups treated with maltol showed mean percentage tail intensities and tail moments that were similar to vehicle controls and fell within historical control ranges.

The Panel noted that, at the dose levels selected, no clinical signs and no bone marrow toxicity were observed in any animal in the maltol-treated groups, which may reflect the possibility that the bone marrow and the liver were not exposed. Therefore, plasma analysis for proof of exposure was requested. Analysis of maltol in plasma was performed using a gas chromatography with mass selective detection (GC–MSD) method. Results showed marked inconsistencies among animals and between sampling times. The Panel concluded that negative findings observed in the combined bone marrow micronucleus test and comet assay in the liver of treated rats could not rule out the concern for genotoxicity of maltol since the data provided to prove systemic availability were considered inconclusive due to the inconsistency of the data.

Following the Panel's conclusion in FGE.213Rev1, the Flavour Industry has submitted a new plasma analysis (Beevers, 2015) performed on the same strain of rats and using the same dosing regimen of the combined micronucleus test and comet assay (Beevers, 2013a). These new data are evaluated in this revision of FGE.213, FGE.213Rev2.

The new data provided show that detectable levels of maltol were found in all plasma samples isolated at 0.5, 1 and 2 hours after dosing. Peak plasma levels of maltol were seen in the majority of animals at 0.5 hours after dose administration. The concentration of maltol detected in plasma was different between the animals of 2 separate cages and the authors of the study did not identify any technical reasons that could account for this difference. The new data submitted are described and evaluated in Section 3 of the present revision. Sections 2.4 and 2.5 report the same information that was present in FGE.213 and FGE.213Rev1, respectively.

Table 1: Representative substances for subgroup 2.7 of FGE.19

FL-no JECFA-no	EU Register name	Structural formula	Comments
07.008 **389**	β-Ionone		*In vitro* assays in bacteria and mammalian cells submitted
07.014 **1480**	Maltol		*In vitro* assays in bacteria and mammalian cells and an *in vivo* combined comet and micronucleus assay submitted
07.083 **384**	β-Damascone		*In vitro* assays in bacteria and mammalian cells and an *in vivo* combined comet and micronucleus assay submitted
07.089 **1398**	Nootkatone		*In vitro* assays in bacteria and mammalian cells submitted

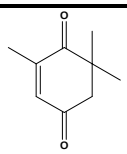
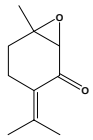
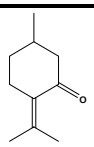
FL-no JECFA-no	EU Register name	Structural formula	Comments
07.109 1857	2,6,6-Trimethylcyclohex-2-en 1,4-dione		*In vitro* assays in bacteria and mammalian cells submitted
16.044 1574	Piperitenone oxide		No longer supported by the Flavour Industry and no data submitted

Table 2: Supporting substance for subgroup 2.7 of FGE.19

FL-no JECFA-no	Substance name	Structural formula	Comments
Not in Register 753	Pulegone		Additional *in vitro* and *in vivo* data (NTP, 2011)

2.3. Presentation of the substances in flavouring group evaluation 213

2.3.1. Description

The Flavouring Group Evaluation 213 (FGE.213) concerned 26 substances (Table 4), corresponding to subgroup 2.7 of FGE.19. Twenty-three of the substances are α,β-unsaturated alicyclic ketones [FL-nos: 07.008, 07.010, 07.014, 07.041, 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.083, 07.089, 07.108, 07.109, 07.117, 07.118, 07.119, 07.120, 07.127, 07.136, 07.168, 07.200 and 07.224] (one substance [FL-no 16.044] is no longer supported by the Flavour Industry and one new substance [FL-no 07.224] has been included in Revision 1) and three are precursors for such ketones [FL-nos: 02.106, 09.305 and 09.525]. Two of these substances [FL-nos: 02.106 and 09.305] are precursors of the ketone β-ionone [FL-no: 07.008] and one [FL-no: 09.525] is a precursor of the ketone maltol [FL-no: 07.014]. Ten of the ketones have the possibility for keto–enol tautomerism [FL-nos: 07.056, 07.057, 07.075, 07.076, 07.080, 07.117, 07.118, 07.119, 07.120 and 07.168]. Based on experimental evidence for other diketones it is anticipated that the enol is the predominant form.

Twenty-two of the substances in FGE.213 (including the new substance [FL-no 07.224], excluding [FL-no 16.044]) have formerly been evaluated by the JECFA (JECFA, 1999, 2001, 2006a, b, 2009a), a summary of their current evaluation status by the JECFA is given in Table 5.

As the α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a) the available data on genotoxic or carcinogenic activity for the 26 unsaturated alicyclic ketones and precursor in subgroup 2.7 will be considered in this FGE.

The Panel has also taken into consideration the outcome of the predictions from five selected (Q)SAR models (Benigni and Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on 22 ketones [FL-nos: 07.008, 07.010, 07.014, 07.041, 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.083, 07.089, 07.108, 07.109, 07.117, 07.118, 07.119, 07.120, 07.127, 07.136, 07.168 and 07.200]. The 22 ketones and their (Q)SAR predictions are shown in Table 6.

2.4. Data evaluated by the Panel in FGE.213[5]

2.4.1. (Q)SAR predictions

In Table 6 the outcomes of the (Q)SAR predictions for possible genotoxic activity in five *in vitro* (Q)SAR models (ISS-Local Model–Ames test, DTU–NFI-MULTICASE–Ames test, Chromosomal aberration test (using Chinese hamster ovary (CHO) cells), Chromosomal aberration test (using Chinese hamster lung (CHL) cells) and Mouse lymphoma test) are presented.

Maltol [FL-no: 07.014], ethyl maltol [FL-no: 07.047] and nootkatone [FL-no: 07.089] were predicted positive with the MultiCASE model on chromosomal aberrations in CHL cells. All other predictions were negative or the substances were out of domain.

2.4.2. Genotoxicity studies

In subgroup 2.7 there are studies available for four substances. For maltol [FL-no: 07.014] eight *in vitro* and three *in vivo* studies have been evaluated. For ethyl maltol [FL-no: 07.047] two *in vitro* and one *in vivo* study were evaluated. Two *in vitro* studies concerning β-ionone [FL-no: 07.008] and one *in vitro* study for 3-methylcyclopentan-1,2-dione [FL-no: 07.056] were evaluated.

Study validation and results are presented in Tables 7 and 8.

In studies that were considered valid, the following results were obtained:

- Maltol-induced gene mutations in bacteria (Bjeldanes and Chew, 1979) and sister chromatid exchanges (SCEs) in human lymphocytes (Jansson et al., 1986). *In vivo*, maltol-induced micronuclei in mouse bone marrow after intraperitoneal application (Hayashi et al., 1988). Negative results were obtained in a sex-linked recessive lethal mutation assay in *Drosophila* (Mason et al., 1992). However, the micronucleus assay is considered more relevant than the *Drosophila* assay.
- Ethyl maltol-induced gene mutations in bacteria (Bjeldanes and Chew, 1979).
- A negative result was obtained with β-ionone in a gene mutation assay in bacteria (Mortelmans et al., 1986).

The validity of other studies was limited or could not be evaluated.

2.4.3. Carcinogenicity studies

In a combined study of developmental toxicity and carcinogenicity, three successive generations of male and female Charles River CD-COBS rats received 3-ethyl-2-hydroxy-2-cyclopenten-1-one (owing to keto–enol tautomerism this substance can exist as two isomers; the keto-isomer is 3-ethylcyclopentan-1,2-dione [FL-no: 07.057], a synonym for the keto-isomer is ethylcyclopentenolone) in the basal diet at doses of 0 (untreated control), 0 (propylene glycol control), 30, 80 or 200 mg/kg body weight (bw) per day. The F1 generation was initially exposed *in utero*, subsequently via the dams' milk until weaning, and then treated for two years and bred twice (at days 99 and 155). In the F1 generation, there were 100 animals of each sex in the untreated control group, 50 animals of each sex in both the propylene glycol control and 3-ethyl-2-hydroxy-2-cyclopenten-1-one-treated groups. Survival, clinical symptoms, food consumption, reproductive performance, haematological and clinical chemistry parameters were not adversely affected. Gross pathological and histopathological examination revealed no significant treatment-related effects. The incidence of benign or malignant tumours in treated animals was similar to that in controls. The No Observed Effect Level (NOEL) was 200 mg/kg bw per day (King et al., 1979).

The Panel concluded that 3-ethyl-2-hydroxy-2-cyclopenten-1-one (3-ethylcyclopentan-1,2-dione [FL-no: 07.057]) was not carcinogenic in rats under the study conditions.

Groups of 25 male and female rats were fed for two years on diets containing ethyl maltol [FL-no: 07.047] calculated to deliver 0, 50, 100 and 200 mg ethyl maltol/kg bw/day. No abnormalities were

[5] The data presented in Section 2.4 are cited from the first version of the present FGE.213. These data are the basis for the conclusions in FGE.213 requesting additional genotoxicity data.

seen as regards survival, clinical appearance, growth rate or food consumption, clinical chemistry, haematology and urinalysis. No histopathological changes and no increases in neoplasms were seen after the treatment with ethyl maltol (Gralla et al., 1969).

Study validation and results are presented in Table 9.

The Panel noted that this study was performed before Organisation for Economic Co-operation and Development (OECD) test guidelines 451/453 (1981a, b) had been established and it does not meet the criteria of these OECD test guidelines with respect to the number of animals. However, the Panel concluded that ethyl maltol was not carcinogenic in rats in this study.

2.4.4. Conclusion on genotoxicity and carcinogenicity

For the substances of this group, the applicability of the (Q)SAR models is very limited since many substances were out of domain in the ISS model and the MultiCASE models.

Two substances [FL-nos: 02.106 and 09.305] are precursors of β-ionone [FL-no: 07.008] and therefore, the conclusions for these two precursors could be based on the conclusions drawn for the corresponding ketone [FL-no: 07.008]. Maltyl isobutyrate [FL-no: 09.525] is a precursor of maltol [FL-no: 07.014], and accordingly, the conclusion for maltyl isobutyrate could be based on the conclusion drawn for maltol.

Maltol and ethyl maltol were considered separately because, in contrast to the other substances in this subgroup, they contain a ring-oxygen atom.

A carcinogenicity study on ethyl maltol [FL-no: 07.047] in rats (Gralla et al., 1969) has been evaluated. Although there were fewer animals per group than that suggested in OECD guidelines 451/453 (1981a, b), the study was in accordance with the standards available at that time. The Panel concluded that the result from this study could overrule the mutagenicity observed with ethyl maltol in bacteria, but not the mutagenicity observed with maltol [FL-no: 07.014] *in vitro* and *in vivo*. Since the micronuclei induced by maltol in mice were analysed after intraperitoneal application, a micronucleus assay after oral application is required, in addition to an *in vivo* comet assay, in order to clarify the genotoxic potential of maltol. A combination of the micronucleus assay and the comet assay in a single study would also be acceptable. The results of these assays would also be applicable to maltyl isobutyrate [FL-no: 09.525], which is a precursor of maltol.

No carcinogenicity was observed with 3-ethyl-2-hydroxy-2-cyclopenten-1-one [FL-no: 07.057] in rats. This substance was considered representative for nine substances [FL-nos: 07.117, 07.118, 07.119, 07.120, 07.056, 07.168, 07.075, 07.076 and 07.080]. Therefore, the Panel concluded that the structural alert for genotoxicity is overruled for 3-ethyl-2-hydroxy-2-cyclopenten-1-one [FL-no: 07.057] as well as for the nine structurally related substances.

For the 13 remaining substances (including two precursors of a ketone) [FL-nos: 02.106, 07.008, 07.010, 07.041, 07.083, 07.089, 07.108, 07.109, 07.127, 07.136, 07.200, 09.305 and 16.044] a genotoxic potential could not be ruled out since only one valid negative bacterial genotoxicity study on [FL-no: 07.008] is available for these substances.

2.4.5. Conclusion based on the data available to the Panel in FGE.213

The Panel concluded that ethyl maltol [FL-no: 07.047], 3-ethylcyclopentan-1,2-dione [FL-no: 07.057] and the nine structurally related substances [FL-nos: 07.117, 07.118, 07.119, 07.120, 07.056, 07.168, 07.075, 07.076 and 07.080] can be evaluated through the Procedure.

For maltol [FL-no: 07.014], a micronucleus assay after oral application is required, in addition to an *in vivo* comet assay, in order to clarify the genotoxic potential. A combination of the micronucleus assay and the comet assay in a single study would also be acceptable. The outcome would also be applicable to maltyl isobutyrate [FL-no: 09.525].

At present, the remaining 13 substances (including two precursors of a ketone) [FL-nos: 02.106, 07.008, 07.010, 07.041, 07.083, 07.089, 07.108, 07.109, 07.127, 07.136, 07.200, 09.305 and 16.044] cannot be evaluated using the Procedure. Additional data on genotoxicity are requested for the representative substances of this subgroup according to the opinion of the Panel on the 'Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19' (EFSA, 2008b).

2.5. Additional genotoxicity data evaluated by the Panel in FGE.213Rev1[6]

2.5.1. Presentation of the additional data

Based on Panel requirements published in FGE.213 (EFSA, 2009), additional data have been provided by the Flavour Industry for the representative substances: β-ionone [FL-no: 07.008], maltol [FL-no: 07.014], β-damascone [FL-no: 07.083], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109].

FGE.213, Revision 1 (FGE.213Rev1), includes the assessment of these additional genotoxicity studies (Table 3).

Table 3: Studies evaluated in FGE.213Rev1

Substance/study type	Bacterial Reverse Mutation assay	*In Vitro* Micronucleus test	*In Vivo* combined Micronucleus test and Comet assay
β-Ionone [FL-no: 07.008]	Ballantyne, 2011	Stone, 2011a	
Maltol [FL-no: 07.014]	Ballantyne, 2012	Whitwell, 2012	Beevers, 2013a
β-Damascone [FL-no: 07.083]	Bowen, 2011b	Stone, 2012	Beevers, 2013b, c
Nootkatone [FL-no: 07.089]	Marzin, 1998	Stone, 2011b	
2,6,6-Trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]	Bowen, 2011a	Lloyd, 2011	

2.5.2. *In vitro* data

Bacterial reverse mutation assay

β-Ionone [FL-no: 07.008]

β-Ionone [FL-no: 07.008] was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix (Ballantyne, 2011). In the first experiment, the concentrations used were 0.32, 1.6, 8, 40, 200, 1 000 and 5 000 µg/plate of β-ionone and the plate incorporation methodology was used. Toxicity ranging from slight thinning of the background lawn to complete killing of the tester strains was observed at 1 000 and/or 5 000 µg/plate for all tester strains in the absence and presence of S9-mix. In the second experiment, the concentrations tested were 10.24, 25.6, 64, 160, 400 and 1 000 µg/plate and the treatments in the presence of S9-mix used the pre-incubation method. Toxicity ranging from thinning of the background lawn and/or reduction in revertant numbers to complete killing of the tester bacteria occurred in all strains at 1 000 µg/plate in the absence and presence of S9-mix, and was also seen down to 160 and/or 400 µg/plate for some individual strains. The study design complied with current recommendations and an acceptable highest concentration was achieved. There was clearly no evidence of any mutagenic effect induced by β-ionone in any of the strains, either in the absence or presence of S9-mix.

Maltol [FL-no: 07.014]

Maltol [FL-no: 07.014] was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix (Ballantyne, 2012). In the first experiment, the concentrations were 0.32, 1.6, 8, 40, 200, 1 000 and 5 000 µg/plate of maltol and the plate incorporation methodology was used. Toxicity in the form of reduction of the number of revertants in the tester strain TA102 was observed at concentrations of 200 µg/plate and greater in the presence of

[6] The data presented in Section 2.5 are cited from FGE.213Rev1. These data are the basis for the conclusions in FGE.213Rev1 requesting additional data.

S9-mix and 1 000 and 5000 µg/plate in the absence of S9-mix. In the second experiment, the concentrations were 51.2, 128, 320, 800, 2 000 and 5 000 µg/plate and the treatments in the presence of S9-mix used the pre-incubation method in all strains. In tester strain TA102 an additional lower concentration of 20.48 µg/plate was incorporated into the testing protocol in both the absence and presence of S9-mix to assess, more carefully, the toxicity observed in experiment 1. Toxicity in the form of thinning of the background lawn and/or reduction in numbers of revertants occurred at 5 000 µg/plate in strain TA102 in the absence and presence of S9-mix, and in strain TA100 only in the presence of S9-mix. The study design complied with current recommendations and an acceptable highest concentration was achieved. There was no evidence of any mutagenic effect induced by maltol in any of the strains, either in the absence or presence of S9-mix.

β-Damascone [FL-no: 07.083]

An Ames assay was conducted in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 to assess the mutagenicity of β-damascone (purity: 95 %), both in the absence and in the presence of metabolic activation by S9-mix, in three separate experiments (Bowen, 2011b). The assay was performed according to OECD Guideline 471 (1997a) and according to Good Laboratory Practice (GLP) principles.

An initial experiment was carried out both in the absence and presence of S9-mix activation in all five strains, using final concentrations of β-damascone between 0.32 and 5000 µg/plate (0.32, 1.6, 8, 40, 200, 1 000, 5 000 µg/plate), plus negative (solvent) and positive controls. Evidence of toxicity was observed through thinning of the background lawn to complete killing at concentrations of 1 000 µg/plate and greater for strains TA1535, TA1537 and TA102 and/or 5000 µg/plate for strains TA98 and TA100 in the absence and presence of S9-mix. In the second experiment the highest concentration was retained for strains TA98 and TA100 in the absence and presence of S9-mix. In all other tester strains, the highest concentration was reduced to 2500 µg/plate based on toxicity observations. In addition, more narrow concentration intervals were used, starting at either 78.13 µg/plate or 156.3 µg/plate (78.13, 156.3, 312.5, 625, 1250, 2500 µg/plate). The standard plate incorporation assay was used in the first experiment but a pre-incubation step with S9-mix activation treatment was added in the second experiment to increase the chance of detecting a positive response. Evidence of toxicity was observed in TA98 at 625 µg/plate (in the presence of S9-mix) and at concentrations of 1250 µg/plate and greater (in the absence of S9-mix). Evidence of toxicity was observed in strains TA1535, TA1537 and TA102 at 625 µg/plate in the absence and presence of S9-mix. In strain TA100 toxicity was observed at concentrations of 1250 µg/plate and greater (in the presence of S9-mix) and at concentrations of 2500 µg/plate and greater (in the absence of S9-mix).

The third experiment was conducted using strain TA98 in the presence of S9-mix activation using the pre-incubation method. The maximum test concentration was reduced to 1 250 µg/plate based on toxicity observed in the previous experiments. In addition, more narrow concentration intervals were used, covering 19.53 to 1 250 µg β-damascone/plate (19.53, 39.06, 78.13, 156.3, 312.5, 625 and 1 250 µg/plate). Evidence of toxicity was observed at the highest four concentrations in strain TA98 in the presence of S9-mix. In all three experiments, no statistically significant increases in revertant numbers were observed at any concentration, in any of the strains, either in the presence or absence of S9-mix activation.

The Panel agreed with the conclusion of the study authors that β-damascone did not induce mutations in five strains of *S. typhimurium*, when tested under the conditions of this study.

Nootkatone [FL-no: 07.089]

Nootkatone [FL-no: 07.089] was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence or presence of S9-mix (Marzin, 1998). A preliminary toxicity test to identify appropriate concentrations for the mutagenicity assays was performed in the absence and presence of S9-mix, and cytotoxicity was observed at 50 µg/plate in the absence of S9-mix and at 150 µg/plate in the presence of S9-mix. In the first mutagenicity experiment using plate incorporation methodology the concentrations tested were 0.5, 1.5, 5, 15 and 50 µg/plate in the absence of S9-mix metabolic activation and 1.5, 5, 15, 50 and 150 µg/plate in the presence of S9-mix. In the second experiment the plate incorporation method was used in the absence of S9-mix and the concentrations were 0.5, 1.5, 5, 15 and 50 µg/plate. While the pre-incubation method was used in the presence of S9-mix and

the concentrations were 0.5, 1.5, 5, 15, 50 and 150 µg/plate. Thus, the study design complied with current recommendations and an acceptable highest concentration was achieved. There was no evidence of any mutagenic effect induced by nootkatone in any of the strains, either in the absence or presence of S9-mix.

2,6,6-Trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]

2,6,6-Trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109] was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix (Bowen, 2011a). In the first experiment, the concentrations tested were 0.32, 1.6, 8, 40, 200, 1 000 and 5 000 µg/plate and plate incorporation methodology was used. In the second experiment, the concentrations were 156.3, 312.5, 625, 1 250, 2 500 and 5 000 µg/plate of 2,6,6-trimethylcyclohex-2-en-1,4-dione and treatments in the presence of S9-mix used the pre-incubation method. The test chemical elicited evidence of cytotoxicity in the form of background lawn thinning or marked reduction of the number of revertants in experiment 1 at 1 000 and/or 5 000 µg/plate in strains TA102 and TA1535 in the presence of S9-mix and in experiment 2 at 2 500 and/or 5 000 µg/plate in strain TA102 in the absence and presence of S9-mix. Thus, the study design complied with current recommendations and an acceptable highest concentration was achieved. There was no evidence of any mutagenic effect induced by 2,6,6-trimethylcyclohex-2-en-1,4-dione in any of the strains, either in the absence or presence of S9-mix.

Summary of the bacterial reverse mutation assay for all the substances is reported in Table 10.

Micronucleus Assay

β-Ionone [FL-no: 07.008]

β-Ionone [FL-no: 07.008] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat liver S9-mix fraction as an *in vitro* metabolising system. Cells were stimulated for 48 hours with phytohaemagglutinin (PHA) to produce exponentially growing cells and then treated for 3 hours (followed by a 21-hour recovery period) with 0, 30, 50 or 60 µg/ml of β-ionone in the absence of S9-mix and 0, 80, 100 or 120 µg/ml in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the highest concentrations were 52 % and 59 %, respectively.

In a parallel assay, cells were treated for 24 hours with 0, 5, 15 and 17.50 µg/ml of β-ionone in the absence of S9-mix with no recovery period. The highest concentration induced 58 % cytotoxicity. There were 2 replicate cultures per treatment and 1000 binucleate cells per replicate were scored for micronuclei. Thus, the study design complies with current recommendations (OECD Guideline 487), and acceptable levels of cytotoxicity were achieved at the highest concentrations used in all parts of the study. Treatment of cells with β-ionone for 3 hours with a 21-hour recovery period showed an increase in the frequency of micronucleated binucleated (MNBN) cells in one single replicate at concentrations of 30 and 120 µg/ml (0.9 % and 1.5 %, respectively) in the absence and presence of S9-mix, respectively. At 30 µg/ml, the lowest concentration tested in the absence of S9-mix, the increase in the frequency of MNBN cells was slightly above the 95 % confidence interval of the historical control range (0.2–0.8 %). In addition, in the presence of S9-mix, one replicate of the lowest concentration tested (80 µg/ml) had an increase in the frequency of MNBN cells at the upper limit of the 95 % confidence interval of the historical control range (0.10–1.10 %) but did not reach statistical significance. To ensure that these single occurrences are random an additional 1 000 binucleate cells were scored from the concurrent controls, 80 and 120 µg/ml cultures. The scoring of further cells resulted in overall mean frequencies of MNBN cells that were not significantly different from concurrent controls and fell below the upper 95 % confidence interval of the normal control range (recalculated due to change of stain), and therefore showed that the earlier increases were due to chance. It was concluded that β-ionone [FL-no: 07.008] did not induce micronuclei up to toxic concentrations when assayed in cultured human peripheral lymphocytes for 3 + 21 hours in the absence and presence of S9-mix or when incubated for 24 + 0 hours in the absence of S9-mix (Stone, 2011a).

Maltol [FL-no: 07.014]

Maltol [FL-no: 07.014] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat liver S9-mix fraction as an *in vitro* metabolising system (Whitwell, 2012). Cells were stimulated for 48 hours with PHA to produce exponentially growing cells and then treated for 3 hours (followed by a 21-hour recovery period) with 0, 400, 800 or 1 262 µg/ml of maltol, the last being equivalent to 10 mM, in the absence and presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the highest concentrations were 24 % and 19 %, respectively. In a parallel assay, cells were treated for 24 hours with 0, 125, 200 and 300 µg/ml of maltol in the absence of S9-mix with no recovery period. The highest concentration induced 57 % cytotoxicity. There were 2 replicate cultures per treatment, and 1 000 binucleate cells per replicate (i.e. 2 000 cells per concentration) were scored for micronuclei. Thus, the study design complies with current recommendations (OECD Guideline 487), and acceptable highest concentrations, either 10 mM or 50–60 % toxicity, were achieved in all parts of the study. A statistically significant increase in the occurrence of MNBN cells was observed following 3 + 21 hours treatment in the presence of S9-mix at the two highest concentrations scored. Statistically significant and concentration-dependent increases in MNBN cells were seen in the 3 + 21 hours treatment groups in the absence of S9-mix, but it was noted that the increases at the two highest concentrations scored exceeded historical control ranges in only one of the two replicate cultures. No increases were observed in the frequency of MNBN cells in those that had received continuous (24 + 0 hours) treatment, but due to the cytotoxicity of maltol, lower concentrations were analysed. To further investigate these observations, fluorescence *in situ* hybridisation (FISH) analysis using human pan-centromeric probes was conducted to assess whether the mechanism of action could be attributed to chromosome loss (aneuploidy) or chromosome breakage (clastogenicity). Slides were prepared from the two highest concentrations (800 and 1 262 µg/ml) in the absence and presence of S9-mix. The FISH analysis revealed that following maltol treatment the majority (69–76 %) of micronuclei did not contain a centromere. The Panel concluded that maltol induced micronuclei *in vitro* in cultured human peripheral blood lymphocytes in the presence of rat liver metabolic activation (S9-mix) via a clastogenic mechanism of action (Whitwell, 2012). However, the Panel considered that the results observed in the absence of S9-mix were equivocal because of the fact that the increases observed (which were statistically significantly different from concurrent solvent control) were not reproduced in replicate cultures.

β-Damascone [FL-no:07.083]

β-Damascone (purity: 95 %) was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence or absence of rat S9 fraction as an *in vitro* metabolising system (Stone, 2012). Cells were stimulated for 48 hours with PHA to produce exponentially growing cells and then treated for 3 hours (followed by a 21-hour recovery period) with concentrations ranging from 2 to 30 µg/ml. For the treatment of 3 hours with a 21-hour recovery period, the concentrations of β-damascone at 8, 16 and 22 µg/ml or at 12, 16, 18 µg/ml were retained for micronuclei (MN) numeration, in the absence or in the presence of S9-mix respectively. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 59 % and 51 %, respectively. Thus, the study design complies with OECD Guideline 487 and follows GLP principles.

In a parallel assay, cells were treated for 24 hours (with no recovery period) in the absence of S9-mix with concentrations ranging from 1 to 15 µg/ml, and the concentrations of 6, 8 and 9 µg/ml of β-damascone were retained for MN numeration. The highest concentration induced 57 % cytotoxicity. There were 2 replicate cultures per treatment and 1 000 binucleate cells per replicate were scored for micronuclei. The study design complies with current recommendations (OECD Guideline 487, 2010), and acceptable levels of cytotoxicity were achieved at the highest concentrations used in all parts of the study.

Treatment of cells with β-damascone for 3 + 21 hours in the presence of S9-mix showed a statistically significant concentration-dependent increase in the induction of MNBN cells with 0.55, 2.10 and 2.70 % MNBN cells versus 0.35 % in the concurrent control and 0.1 to 1.1 % for the historical controls.

Treatment of cells with β-damascone for 3 + 21 and 24 + 0 hours in the absence of S9-mix resulted in sporadic increases in MNBN frequency. These increases were only observed in single replicates and were not concentration-related. Therefore, the effect of β-damascone was further investigated through the scoring of additional cells (2 more replicates of 1000 cells each) from the affected concentrations and concurrent controls.

Treatment of cells, in the absence of S9-mix, for 3 + 21 hours induced a statistically significant increase in the frequency of MNBN cells at 8 and 22 µg/ml (0.80 % and 0.93 %, respectively) compared with concurrent control (0.38 %), but not at the mid-dose of 16 µg/ml (0.53 % MNBN cells). The frequency of MNBN cells exceeded the historical controls (0.2–0.8 %) in 3 out of 4 replicates at the highest concentration tested (22 µg/ml). Treatment of cells for 24 hours with no recovery period in the absence of S9-mix showed a statistically significant increase in the frequency of MNBN cells at the mid-dose of 8 µg/ml (0.95 % MNBN cells) when compared with concurrent control (0.40 %) with no correlation to concentration. The frequency of MNBN cells exceeded the historical controls (0–1.1 %) in only one replicate at 8 µg/ml.

The authors considered that this result reaffirmed the sporadic nature of the induction of MNBN cells in the absence of S9-mix. It was concluded that the treatment with β-damascone for 3 + 21 hours or 24 + 0 hours (in the absence of S9-mix) induced sporadic increases in MNBN cells when compared with concurrent controls and not concentration-related; therefore, the results were considered equivocal. In the same test system, β-damascone induced micronuclei in cultured human peripheral blood lymphocytes following 3 + 21 hours treatment in the presence of S9-mix (Stone, 2012). The Panel noted that after the new reading of slides the increase in the frequency of MNBN cells was still statistically significant even at weak cytotoxic levels.

Therefore, the Panel concluded that β-damascone is genotoxic in the *in vitro* micronucleus assay on human lymphocytes with metabolic activation and equivocal without metabolic activation.

Nootkatone [FL-no: 07.089]

Nootkatone [FL-no: 07.089] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9-mix fraction as an *in vitro* metabolising system (Stone, 2011b). Cells were stimulated for 48 hours with PHA to produce exponentially growing cells and then treated for 3 hours (followed by a 21-hour recovery period) with 0, 50, 70 or 80 µg/ml of nootkatone in the absence of S9-mix and 0, 160, 180 and 185 µg/ml in the presence of S9-mix, respectively. The levels of cytotoxicity (reduction in replication index) at the highest concentrations were 60 % and 58 %, respectively. In a parallel assay, cells were treated for 24 hours with 0, 10, 15, 22 and 24 µg/ml of nootkatone in the absence of S9-mix with no recovery period. The highest concentration induced 62 % cytotoxicity. There were 2 replicate cultures per treatment and 1 000 binucleate cells per replicate (i.e. 2 000 cells per dose) were scored for micronuclei. The study design complies with current recommendations (OECD Guideline 487) and acceptable levels of cytotoxicity were achieved at the highest concentrations used in all parts of the study. No evidence of chromosomal damage or aneuploidy was observed as frequencies of MNBN cells were not significantly different from concurrent controls and fell within historical control ranges for all treatments with nootkatone in the presence or absence of S9-mix metabolic activation (Stone, 2011b).

2,6,6-Trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]

2,6,6-Trimethylcyclohex-2-en-1,4-dione was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9-mix fraction as an *in vitro* metabolising system (Lloyd, 2011). Cells were stimulated for 48 hours with PHA to produce exponentially growing cells and then treated for 3 hours (followed by a 21-hour recovery period) with 0, 500, 1 000 or 1 522 µg/ml of 2,6,6-trimethylcyclohex-2-ene-1,4-dione in the absence of S9-mix and 0, 1 000, 1 250 and 1 522 µg/ml in the presence of S9-mix, the highest concentration being equivalent to 10 mM. The levels of cytotoxicity (reduction in replication index) at the highest concentrations were 3 % and 9 %, respectively. In a parallel assay, cells were treated for 24 hours with 0, 300, 420 and 550 µg/ml of 2,6,6-trimethylcyclohex-2-ene-1,4-dione in the absence of S9-mix with no recovery period. The highest concentration induced 57 % cytotoxicity. There were 2 replicate cultures per treatment and

1 000 binucleate cells per replicate (i.e. 2 000 cells per concentration) were scored for micronuclei. The study design complies with current recommendations (OECD Guideline 487), and acceptable highest concentrations, either 10 mM or 50–60 % toxicity, were achieved in all parts of the study. No evidence of chromosomal damage or aneuploidy was observed as frequencies of MNBN cells were not significantly different from concurrent controls and fell within historical ranges for all 2,6,6-trimethylcyclohex-2-ene-1,4-dione treatments in the presence or absence of S9-mix metabolic activation (Lloyd, 2011).

The results of *in vitro* micronucleus studies are summarised in Table 10.

2.5.3. Genotoxicity *in vivo* data

In vivo Combination Assay (comet + micronucleus tests)

Since no positive results were seen in either the bacterial mutation assay or *in vitro* micronucleus tests with β-ionone [FL-no: 07.008], nootkatone [FL-no: 07.089] or 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109], no *in vivo* follow-up testing was required. To address the effects seen in the *in vitro* micronucleus assay with maltol [FL-no: 07.014] and β-damascone [FL-no: 07.083] a combination assay comprising a liver comet assay and an *in vivo* micronucleus assay in rats, after oral application, was performed to further assess the genotoxic potential for both substances. The results are summarised in Table 11.

Maltol [FL-no: 07.014]

Maltol was evaluated in an *in vivo* bone marrow micronucleus assay and liver comet assay in male Han Wistar (HsdHan:WIST) rats, with 6 rats per dose group (Beevers, 2013a). The rats were administered 3 doses of 70, 350 and 700 mg/kg bw of maltol by oral gavage at time 0, 24 and 45 hours. Rats were killed and sampled at 48 hours post the initial dose. The maximum tolerable dose was estimated to be 700 mg/kg bw/day based on a range-finding study where maltol was tested at 360, 500, 700, 1 000, 1 500 and 2 000 mg/kg bw/day. Clinical observations (piloerection, ataxia, bradypnoea) and mortalities were observed at doses of 1000 mg/kg bw/day and greater. For the micronucleus assay 2 000 polychromatic erythrocytes (PCE) per rat were scored. The negative control had a normal, low frequency (0.11 %) of micronucleated polychromatic erythrocytes (MNPCEs) and a ratio of 53.7 % PCE. The positive control group resulted in a significant increase in MNPCEs (1.58 %) accompanied by some bone marrow toxicity (29.57 % PCE). Although an individual rat in the 700 mg/kg maltol dose group showed a frequency of 9 MNPCEs, which resulted in significant heterogeneity in this dose group, this was considered an outlying data point because the other 5 rats in the group were exhibiting normal control-level MNPCE frequencies (Beevers, 2013a). Overall, the results showed that there were no statistically significant increases in micronucleus frequency for any dose group after oral treatment with maltol when compared with the vehicle control group. However, in the main experiment, at the dose levels selected, no clinical signs and bone marrow toxicity were observed in any animal in the maltol-treated groups, which may reflect the possibility that the bone marrow and liver were not exposed.

In order to clarify this issue, the Panel requested plasma analysis for proof of exposure. Plasma was obtained from two satellite groups of male animals (3 + 3 animals) dosed with maltol by oral gavage at 700 mg/kg bw/day, during conduction of the main study (Beevers, 2013a). Plasma obtained from 0.5 ml blood drawn from the jugular vein from each animal was frozen in the event that analysis for proof of exposure and toxicokinetics were required. All doses of maltol were given as three administrations, at 0, 24 and 45 hours. Three samples of plasma were obtained from one group of animals at 0.5, 2 and 8 hours and three samples from the other group at 1, 4 and 24 hours from the last administration. Analysis of maltol in plasma was performed using a gas chromatography with mass selective detection (GC-MSD) method. From an analytical point of view, the Panel considered the employed approach, which was based on the use of ethylmaltol as an internal standard, as sufficient. Results showed a marked inconsistency between sampling times and animals. In samples collected at 0.5, 2 and 8 hours from last administration maltol was found in 2 out of 3 satellite animals at plasma concentrations of 265–283 µg/ml after 0.5 and 2 hours, but no longer detectable after 8 hours. In the plasma of the third animal maltol was not detectable at any time. On the other hand, in samples from another animal group (n = 3) collected at 1, 4 and 24 hours from last administration, maltol was

found at levels of 75–106 µg/l after 1 hour in all 3 animals and no longer detectable after 4 and 24 hours (Mallinson and Hough, 2014). The authors concluded that the results obtained provided evidence that maltol is present in plasma shortly after dosing. However, the Panel did not agree with this conclusion and it considered the results of the bioanalytical study as inconclusive.

In the combined comet assay, livers of rats were removed at 48 hours after the first dose (i.e. 3 hours after the final dose), cut into small pieces and forced through a bolting cloth. Single cell suspensions were embedded in low melting point agarose on slides and lysed. The DNA was unwound and subjected to electrophoresis at pH > 13 and then neutralised according to standard techniques. For each animal, 100 cells (50 cells/slide from 2 slides) were scored for comets (tail intensity and tail moment) using commercial image analysis equipment.

The comet assay did not reveal cytotoxicity, necrosis or apoptosis in the hepatocytes as assessed by cloud and halo analysis and the groups treated with maltol showed mean percentage tail intensities and tail moments that were similar to vehicle controls and fell within historical control ranges. The positive control group treated with ethyl methanesulphonate showed significant increases in both parameters (Beevers, 2013a).

Considering that maltol has been shown to induce micronuclei in mouse bone marrow after intraperitoneal injection (Hayashi et al., 1988), the Panel concludes that negative findings observed in the combined bone marrow micronucleus test and comet assay in the liver of treated rats could not rule out the concern for genotoxicity for maltol since the data provided to prove systemic availability were considered inconclusive due to the inconsistency of the data.

β-Damascone [FL-no:07.083]

A combined *in vivo* micronucleus assay and comet assay was performed after oral application of β-damascone (purity: 95.6 %) to further assess the genotoxic potential of β-damascone and damascones more generally. The results are summarised in Table 11. β-Damascone was evaluated in an *in vivo* bone marrow micronucleus assay and liver and duodenum comet assay in groups of 6 male Han Wistar (HsdHan:WIST) rats per dose group (Beevers, 2013c). Based on a range-finding study, 500 mg/kg/day was considered an appropriate estimate of the maximum tolerated dose (MTD) because doses of 750 mg/kg/day and greater induced moderate to severe clinical signs of toxicity, which included piloerection, decreased activity, hunched posture and abnormal breathing. The rats were administered 3 doses of 125, 250 and 500 mg/kg bw of β-damascone by oral gavage at time 0, 24 and 45 hours. The rats were sacrificed and sampled at 48 hours post the initial dose.

Animals administered β-damascone showed clear findings during pathological analysis. Hepatocyte vacuolation was present in animals given 500 mg/kg/day, and was characterised by scattered, occasionally shrunken hepatocytes with perinuclear cytoplasmic eosinophilia and peripheral cytoplasmic vacuolation. Single cell necrosis was present in a single animal given 500 mg/kg/day. Single cell necrosis was characterised by death of individual hepatocytes throughout the liver, with limited inflammatory cell involvement. There was a dose-related reduction in the level of glycogen vacuolation in animals given 250 or 500 mg/kg/day. Glycogen vacuolation was characterised by generally perinuclear, clear, variably sized, indistinctly defined, vacuoles. Finally, increased mitosis was present in animals from all groups given β-damascone. The greatest severity was present in animals given 250 mg/kg/day, and the lowest incidence was present in animals given 500 mg/kg/day. Increased mitosis was characterised by an increase, above the normal low background incidence, of mitotic figures within the liver parenchyma. Collectively, these findings indicate that the test animals were systemically exposed to β-damascone.

The negative control had a 0.11 % average rate of MNPCE and a ratio of 50.2 % PCE; the 125 mg/kg β-damascone treatment group had a MNPCE rate of 0.09 % and PCE ratio of 49.17 %; the 250 mg/kg treatment group had a 0.09 % MNPCE rate and 52.30 % PCE ratio; the 500 mg/kg treatment group showed 0.06 % MNPCEs and 37.63 % PCE ratio. The positive control group resulted in 1.54 % MNPCEs and a 43.17 % PCE ratio (Beevers, 2013c). The group mean frequencies observed were similar to concurrent vehicle controls for all dose groups and also were within the historical control values (mean: 0.12 %). There was a reduction in PCE ratio at the highest dose level indicating bone marrow toxicity, which demonstrates target organ exposure. These results showed that there was no statistically significant increase in micronuclei induced with β-damascone under these test conditions when compared with the negative control group. In addition, there were no statistically significant

differences among erythrocyte parameters examined in this study. It was concluded that β-damascone did not induce micronucleated erythrocytes in rat bone marrow cells following administration by oral gavage.

The comet assay in the liver tissue did not reveal cytotoxicity, necrosis or apoptosis in the hepatocytes as assessed by cloud and halo analysis. Hepatocytes of rats dosed with β-damascone were evaluated for percentage tail intensities and tail moments (± standard error of the mean, SEM); the 125 mg/kg β-damascone group had 2.45 ± 0.13 % tail intensity and 0.27 ± 0.02 % tail moment; the 250 mg/kg group had 2.99 ± 0.31 % tail intensity and 0.33 ± 0.03 tail moment; the 500 mg/kg group had 2.93 ± 0.24 % tail intensity and 0.31 ± 0.03 tail moment, which were similar to concurrent vehicle controls (tail intensity of 2.67 ± 0.26 % and 0.29 ± 0.03 tail moment) and fell within the testing laboratories historical control range for vehicle controls (0.3–8.15 % tail intensity and 0.04–0.81 tail moment). The comet arm of this study confirms that β-damascone did not induce DNA damage in the liver under the conditions of this study (Beevers, 2013c).

In a satellite study the slides from the duodenum tissue samples collected in the above study (Beevers, 2013c) were analysed for comet tailing effects (Beevers, 2013b). Duodenum cells of rats dosed with β-damascone were evaluated for percentage tail intensities and tail moments (± standard error of the mean, SEM); the 125 mg/kg β-damascone group had 2.01 ± 0.43 % tail intensity and 0.32 ± 0.03 % tail moment; the 250 mg/kg group had 1.47 ± 0.15 % tail intensity and 0.16 ± 0.02 tail moment; the 500 mg/kg group had 2.03 ± 0.19 % tail intensity and 0.19 ± 0.02 tail moment, which were similar to concurrent vehicle controls (tail intensity of 2.24 ± 0.43 % and 0.23 ± 0.04 % tail moment) and fell within the testing laboratories historical control range for vehicle controls (0.3–8.15 % tail intensity and 0.04–0.81 tail moment). The duodenum comet arm of this study confirms that β-damascone did not induce DNA damage in the duodenum under the conditions of this study. The vehicle control data were within historical control ranges (95 % reference range: 0.77 to 8.32 % for tail intensity and 0.08 to 1.15 % for tail moment) and the positive control induced a clear increase in DNA damage. The study was therefore confirmed as valid. There was no evidence of duodenum toxicity as would be suggested by increases in clouds or halo cells.

The percentage tail intensity and tail moment at all dose levels were very similar to the concurrent vehicle control, thus confirming there is no test article-related DNA damage. The additional tissue sample analysis for comet tailing showed a negative result for this study (Beevers, 2013b).

The results from the combined *in vivo* micronucleus induction study and comet assay show that orally administered β-damascone did not induce micronucleated erythrocytes in rat bone marrow cells nor genotoxic events in liver and duodenum of rats.

2.5.4. Conclusion

Flavouring Group Evaluation 213 concerned 26 substances, corresponding to subgroup 2.7 of FGE.19 (see Table 4). Twenty-three of the substances are α,β-unsaturated alicyclic ketones [FL-nos: 07.008, 07.010, 07.014, 07.041, 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.083, 07.089, 07.108, 07.109, 07.117, 07.118, 07.119, 07.120, 07.127, 07.136, 07.168, 07.200 and 07.224] and three are precursors for such ketones [FL-nos: 02.106, 09.305 and 09.525].

For 11 substances [FL-nos: 07.047, 07.056, 07.057, 07.075, 07.076, 07.080, 07.117, 07.118, 07.119, 07.120 and 07.168] the Panel have ruled out concerns regarding genotoxicity in FGE.213.

In FGE.213Rev1, new data have been evaluated for the representative of the remaining substances. More specifically, data for β-ionone [FL-no: 07.008], β-damascone [FL-no: 07.083], maltol [FL-no: 07.014], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]. All these studies are fully compliant with current guidelines, and stand in contrast to earlier studies previously evaluated in FGE.213.

The combined evidence from *in vitro* and *in vivo* genotoxicity data for the selected representative substances β-ionone [FL-no: 07.008], β-damascone [FL-no: 07.083], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109] does not indicate a genotoxic potential. Therefore, these substances and the nine substances that they represent [FL-nos: 02.106, 07.010, 07.041, 07.108, 07.127, 07.136, 07.200, 07.224 and 09.305] could be evaluated through the Procedure.

For maltol [FL-no: 07.014] and maltyl isobutyrate [FL-no: 09.525], the Panel concluded that the concern for genotoxicity could not be ruled out.

3. Assessment

3.1. Additional data evaluated by the Panel in FGE.213Rev2

3.1.1. Plasma bioanalysis

Since data provided to prove the systemic exposure to maltol of the animals tested in the combined bone marrow micronucleus test and comet assay in liver (Beevers, 2013a) were considered inconclusive in FGE.213Rev1, the Flavour Industry has submitted a new plasma bioanalysis (Beevers, 2015).

Six male Han Wistar rats were dosed at 700 mg maltol/kg bw/day (determined previously as an estimate of the MTD), using the same dosing regimen employed in the combined micronucleus test and comet assay (Beevers, 2013a).

Maltol was prepared as a suspension 0.5 % (w/v) in aqueous methylcellulose and administered via oral gavage at: 0 (day 1), 24 (day 2) and 45 (day 3) hours. Whole blood was collected at 0.5, 1, 2 and 3 hours after dosing on day 3. Plasma was isolated and analysed using GC–MSD. Ethyl maltol was used as an internal standard.

Detectable levels of maltol were found in all plasma samples isolated at 0.5, 1 and 2 hours after dosing. Peak plasma levels of maltol were seen in the majority of animals at 0.5 hours after dose administration. The concentration of maltol detected in plasma was different between the animals of 2 separate cages (treated with the same dose) and the authors of the study did not identify any technical reasons that could account for this difference. However, it was concluded that data demonstrate the presence of maltol in blood and that accordingly the bone marrow could be considered exposed.

3.1.2. Additional *in vitro* data on maltol

An *in vitro* chromosomal aberration assay on maltol (not available before) is considered in the present revision of FGE.213. Maltol (purity 99 %) was tested in CHL fibroblast cell line at three concentrations: 25, 50 and 75 µg/ml. Cells were harvested for chromosomal preparations after 24 hours or 48 hours from the beginning of the treatment; metabolic activation was not included. Structural chromosomal aberrations were observed at the 2 highest concentrations tested after both 24 hours and 48 hours of treatment. These increases were concentration related, polyploidy was not observed (Ishidate, 1988). The result obtained in this study is consistent with the clastogenic effect of maltol observed in the study by Whitwell (2012), described in Section 2.5.2.

3.1.3. Additional information on *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127]

In FGE.213Rev1, the Panel concluded that the genotoxicity concern for *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] could be ruled out based on available genotoxicity data on structurally related substances and consequently could be evaluated through the Procedure in FGE.57Rev1.

During the evaluation of *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] through the Procedure, the Panel noted that the chemical structure of *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] is actually more closely related to the structure of pulegone than to the structures used for the read-across approach in FGE.213Rev1.

New information was found on genotoxicity and carcinogenicity of pulegone, based on which additional data are expected to be provided by the applicant. At present, the data available on pulegone and on the structurally related substance *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] do not rule out the concern for genotoxicity and carcinogenicity. Therefore *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] will be re-evaluated pending the submission of additional genotoxicity data.

4. Conclusion

The Panel considered the available genotoxicity studies on maltol. An *in vivo* study in mice (by intraperitoneal route) showed that maltol induced a statistically significant increase in the incidence of micronuclei in bone marrow cells. Since maltol is intended to be used as food flavouring agent, the *in vivo* study performed by gavage (combined micronucleus test and comet assay in rats) was considered more relevant, but in the previous revision of that opinion, this study did not allow conclusions on the genotoxicity of maltol to be made because exposure of target tissue was not demonstrated. Therefore, the Panel requested to investigate the systemic exposure of animals to maltol.

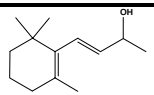
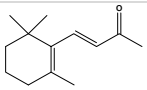
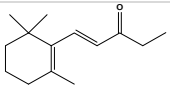
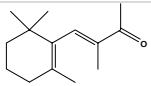
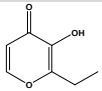
The Panel considered the new plasma bioanalysis for maltol and concluded that, based on the new data provided, it now seems justifiable to assume that the animals were systemically exposed to maltol and that the bone marrow was exposed in the *in vivo* micronucleus assay.

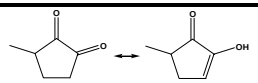
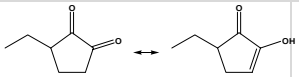
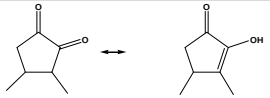
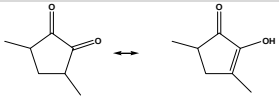
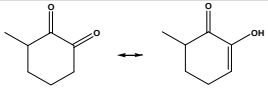
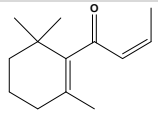
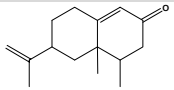
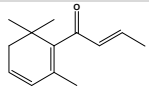
Therefore, the negative result of the *in vivo* micronucleus assay can be considered reliable and, accordingly, the concern for genotoxicity for maltol [FL-no: 07.014] and for maltyl isobutyrate [FL-no: 09.525] in food is ruled out; both substances were evaluated by JECFA before 2000 and no EFSA consideration is required.

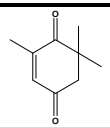
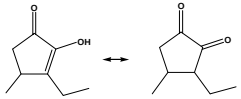
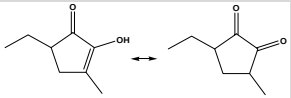
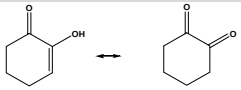
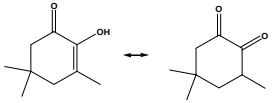
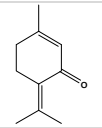
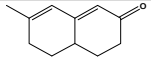
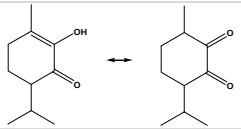
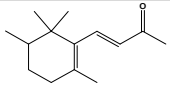
The Panel reconsidered the available data on *p*-mentha-1,4(8)-dien-3-one [FL-no: 07.127] based on new data for the structurally related substance pulegone, and concluded that additional genotoxicity data are needed for [FL-no: 07.127] to rule out the concern for genotoxicity.

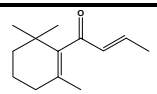
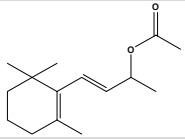
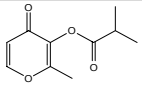
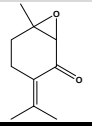
4.1. Summary of Specification for Substances in FGE.213 (JECFA 1998, 2000, 2005a, b, 2009b)

Table 4: Specification summary of the substances in FGE.213

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
02.106 **392**	4-(2,2,6-Trimethyl-1-cyclohexenyl)but-3-en-2-ol		3625 – 22029-76-1	Liquid $C_{13}H_{22}O$ 194.32	– –	107 (4 hPa) – IR 92 %	1.499 0.927–0.933
07.008 **389**	β-Ionone		2595 142 14901-07-6	Liquid $C_{13}H_{20}O$ 192.30	Insoluble 1 ml in 3 ml 70 % alcohol	239 – IR 95 %	1.517–1.522 0.940–0.947
07.010 **399**	Methyl-β-ionone		2712 144 127-43-5	Liquid $C_{14}H_{22}O$ 206.33	– –	238–242 – IR 88 %	1.503–1.508 0.930–0.935
07.014 **1480**	Maltol		2656 148 118-71-8	Solid $C_6H_6O_3$ 126.11	Very slightly soluble Soluble	– 159–162 NMR 98 %	n.a. n.a.
07.041	β-Isomethylionone		4151 650 79-89-0	Solid $C_{14}H_{22}O$ 206.32	– Freely soluble	334 62 – 95 %	n.a. n.a.
07.047 **1481**	Ethyl maltol		3487 692 4940-11-8	Solid $C_7H_8O_3$ 140.14	Soluble Soluble	– 89–93 NMR 99 %	n.a. n.a.

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
07.056 **418**	3-Methylcyclopentan-1,2-dione		2700 758 80-71-7	Solid $C_6H_8O_2$ 112.13	1 g in 72 ml water 1 g in 5 ml 90 % alcohol	– 104–108 IR 95 %	– –
07.057 **419**	3-Ethylcyclopentan-1,2-dione		3152 759 21835-01-8	Solid $C_7H_{10}O_2$ 126.16	Miscible –	78–80 (5 hPa) 36–43 IR 90 %	1.47–1.48 (25°) 1.060–1.066
07.075 **420**	3,4-Dimethylcyclopentan-1,2-dione		3268 2234 13494-06-9	Solid $C_7H_{10}O_2$ 126.16	– –	66 (1 hPa) 68–72 IR 98 %	– –
07.076 **421**	3,5-Dimethylcyclopentan-1,2-dione		3269 2235 13494-07-0	Solid $C_7H_{10}O_2$ 126.16	Insoluble –	– 87–93 MS 98 %	– –
07.080 **425**	3-Methylcyclohexan-1,2-dione		3305 2311 3008-43-3	Solid $C_7H_{10}O_2$ 126.16	Insoluble –	69–72 (1 hPa) 57–63 IR 98 %	– –
07.083 **384**	β-Damascone		3243 2340 23726-92-3	Liquid $C_{13}H_{20}O$ 192.30	– 1 ml in 10 ml 95 %	67–70 – IR 90 %	1.496–1.501 0.934–0.942 (20°)
07.089 **1398**	Nootkatone		3166 11164 4674-50-4	Liquid $C_{15}H_{22}O$ 218.35	Slightly soluble Soluble	73–103 (1 hPa) – NMR 93 %	1.510–1.523 1.003–1.032
07.108 **387**	β-Damascenone		3420 11197 23696-85-7	Liquid $C_{13}H_{18}O$ 190.28	– 1 ml in 10 ml 95 % alcohol	60 – IR 98 %	1.508–1.514 0.945–0.952 (20°)

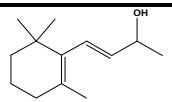
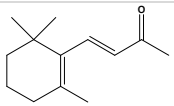
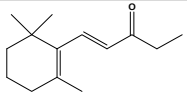
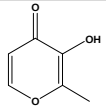
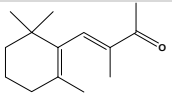
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
07.109 **1857**	2,6,6-Trimethylcyclohex-2-en-1,4-dione		3421 11200 1125-21-9	Solid $C_9H_{12}O_2$ 152.2	Slightly soluble Soluble	222 23–28 IR NMR 98 %	n.a. n.a.
07.117 **422**	3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one		3453 11077 42348-12-9	Liquid $C_8H_{12}O_2$ 140.18	Slightly insoluble Miscible	– – NMR 99 %	1.481–1.487 1.055–1.061
07.118 **423**	5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one		3454 11078 53263-58-4	Liquid $C_8H_{12}O_2$ 140.18	Slightly soluble Soluble	– – NMR 99 %	1.478–1.484 1.053–1.060
07.119 **424**	2-Hydroxycyclohex-2-en-1-one		3458 11046 10316-66-2	Solid $C_6H_8O_2$ 112.13	Soluble Soluble	53 (3 hPa) 35–38 IR 99.3 %	– –
07.120 **426**	2-Hydroxy-3,5,5-trimethylcyclohex-2-en-1-one		3459 11198 4883-60-7	Solid $C_9H_{14}O_2$ 154.21	Slightly soluble Soluble	90–100 (20 hPa) 88 IR 99 %	– –
07.127 **757**	*p*-Mentha-1,4(8)-dien-3-one		3560 11189 491-09-8	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Miscible	233 – MS 95 %	1.472–1.478 0.976–0.983
07.136 **1405**	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		3715 – 34545-88-5	Solid $C_{11}H_{14}O$ 162.23	Insoluble Soluble	n.a. 36–37 IR 99 %	n.a. n.a.
07.168 **2038**	2-Hydroxypiperitone		4143 – 490-03-9	Solid $C_{10}H_{16}O_2$ 168.24	Slightly soluble Freely soluble	233 82 NMR MS 98 %	n.a. n.a.
07.200	4-(2,5,6,6-Tetramethyl-1-cyclohexenyl)but-3-en-2-one		– – 79-70-9	Liquid $C_{14}H_{22}O$ 206.33	Practically insoluble or insoluble Freely soluble	108 (2 hPa) – MS 95 %	1.515–1.521 0.943–0.949

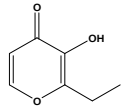
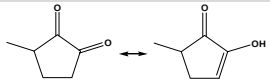
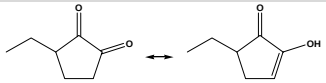
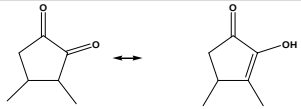
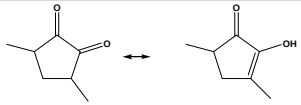
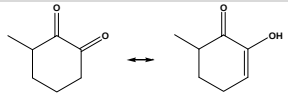
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
07.224	tr-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)but-2-en-1-one		3243 2340 23726-91-2	– – –	– –	– – – 90 %	– –
09.305 **1409**	β-Ionyl acetate		3844 10702 22030-19-9	Liquid $C_{15}H_{24}O_2$ 236.35	Insoluble Soluble	120 (3 hPa) – NMR 92 %	1.474–1.484 0.934–0.944
09.525 **1482**	Maltyl isobutyrate		3462 10739 65416-14-0	Liquid $C_{10}H_{12}O_4$ 196.20	Insoluble Soluble	100 (0.01 hPa) – IR 96 %	1.493–1.501 1.140–1.153
16.044 **1574**	Piperitenone oxide		4199 10508 35178-55-3	Solid $C_{10}H_{14}O_2$ 166.22	Soluble Soluble	– 25 NMR MS 95 %	n.a. n.a.

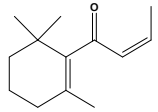
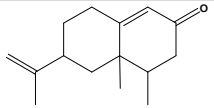
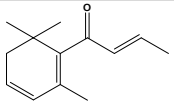
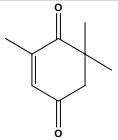
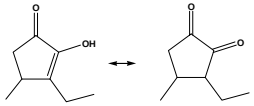
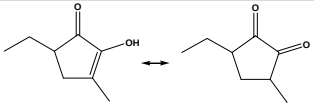
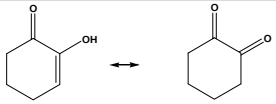
n.a.: not applicabile; (-): data not reported
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1 013.25 hPa, if not otherwise stated.
(d): At 20 °C, if not otherwise stated.
(e): At 25 °C, if not otherwise stated.

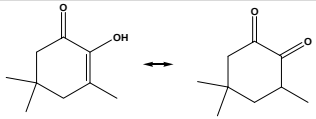
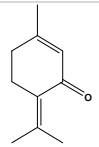
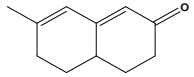
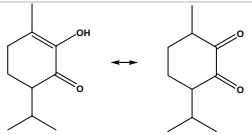
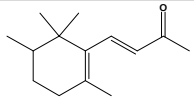
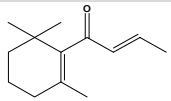
4.2. Summary of Safety Evaluation Applying the Procedure (JECFA 1999, 2001, 2006a, b, 2009a)

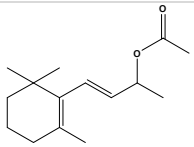
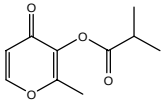
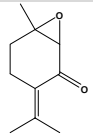
Table 5: Summary of safety evaluation applying the procedure

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/capita/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (genotoxicity)
02.106 **392**	4-(2,2,6-Trimethyl-1-cyclohexenyl)but-3-en-2-ol		0.73 0.1	Class I A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.008 **389**	β-Ionone		130 100	Class I A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.010 **399**	Methyl-β-ionone		5.4 0.2	Class I A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.014 **1480**	Maltol		3 060 2 898	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	(d)	Evaluated in FGE.213Rev2, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.041	β-Isomethylionone		0.011	Not evaluated by the JECFA		Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.12Rev5.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/capita/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (genotoxicity)
07.047 **1481**	Ethyl maltol		1 580 6 692	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.83Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
07.056 **418**	3-Methylcyclopentan-1,2-dione		570 710	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.057 **419**	3-Ethylcyclopentan-1,2-dione		32 23	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.075 **420**	3,4-Dimethylcyclopentan-1,2-dione		30 2	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.076 **421**	3,5-Dimethylcyclopentan-1,2-dione		35 29	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.080 **425**	3-Methylcyclohexan-1,2-dione		1.3 8	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/capita/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (genotoxicity)
07.083 **384**	β-Damascone		37 10	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.089 **1398**	Nootkatone		130 20	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.87Rev2.
07.108 **387**	β-Damascenone		73 5	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.109 **1857**	2,6,6-Trimethylcyclohex-2-en-1,4-dione		50	Class II No evaluation		Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.09Rev5.
07.117 **422**	3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one		ND 0.17	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.118 **423**	5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one		ND 0.38	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.119 **424**	2-Hydroxycyclohex-2-en-1-one		0.049 0.76	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA

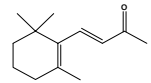
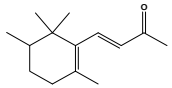
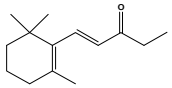
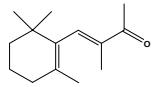
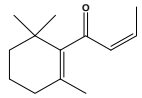
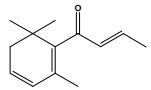
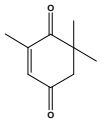
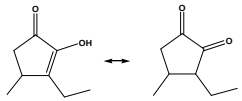
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/capita/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (genotoxicity)
						before 2000. No EFSA consideration required.
07.120 **426**	2-Hydroxy-3,5,5-trimethylcyclohex-2-en-1-one		1.2 2	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
07.127 **757**	*p*-Mentha-1,4(8)-dien-3-one		0.012 0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Evaluated in FGE.213Rev1 and FGE.213Rev2, genotoxicity concern could not be ruled out. Additional data are requested.
07.136 **1405**	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		ND 0.04	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.87Rev2.
07.168 **2038**	2-Hydroxypiperitone		0.0012	Class III A3: Intake below threshold	(d)	Evaluated in FGE.213, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.11Rev2. No safety concern at the estimated level of intake based on the MSDI approach.
07.200	4-(2,5,6,6-Tetramethyl-1-cyclohexenyl)but-3-en-2-one		0.012	Class I No evaluation		Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.12Rev5.
07.224	tr-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)but-2-en-1-one		100	No evaluation		Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated

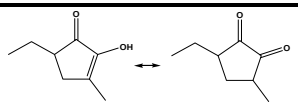
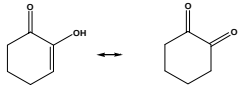
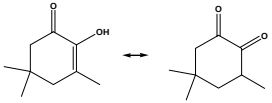
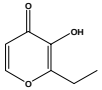
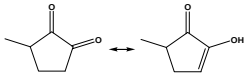
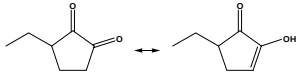
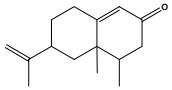
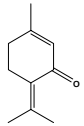
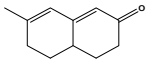
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/capita/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (genotoxicity)
						using the Procedure in FGE.12Rev5.
09.305 **1409**	β-Ionyl acetate		ND 9	Class I A3: Intake below threshold	(d)	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure in FGE.73Rev3. MSDI based on USA production figure.
09.525 **1482**	Maltyl isobutyrate		20 38	Class II A3: Intake below threshold	(d)	Evaluated in FGE.213Rev2, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No EFSA consideration required.
16.044 **1574**	Piperitenone oxide		0.012 0.2	Class III A3: Intake below threshold	(d)	Evaluated in FGE.213, additional genotoxicity data required. The substance is not supported by the Flavour Industry any longer. No further evaluation.

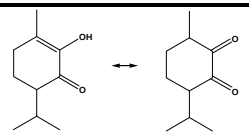
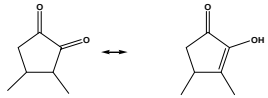
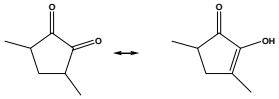
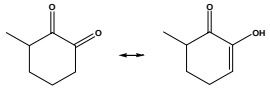
ND: no data
(a): EU MSDI: Amount added to food as flavour in (kg/year) x 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/capita/day
(b): Thresholds of concern: class I = 1800 μg/person/day, class II = 540 μg/person/day, Class III = 90 μg/person/day
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot
(d): No safety concern based on intake calculated by the MSDI approach of the named compound
(e): Data must be available on the substance or closely related substances to perform a safety evaluation

4.3. (Q)SAR Predictions on Mutagenicity in Five Models for 22 Ketones from Subgroup 2.7

Table 6: (Q)SAR predictions on mutagenicity for 22 alicyclic ketones from subgroup 2.7

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
07.008 389	β-Ionone		NEG	NEG	NEG	NEG	EQU
07.200	4-(2,5,6,6-Tetramethyl-1-cyclohexenyl)but-3-en-2-one		NEG	NEG	NEG	NEG	EQU
07.010 399	Methyl-β-ionone		NEG	NEG	OD	OD	EQU
07.041	β-Isomethylionone		NEG	EQU	NEG	NEG	NEG
07.083 384	β-Damascone		OD	NEG	OD	OD	EQU
07.108 387	β-Damascenone		OD	NEG	OD	OD	EQU
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione		OD	NEG	OD	NEG	EQU
07.117 422	3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one		OD	NEG	NEG	OD	NEG

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
07.118 423	5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one		OD	NEG	NEG	NEG	NEG
07.119 424	2-Hydroxycyclohex-2-en-1-one		OD	OD	NEG	OD	NEG
07.120 426	2-Hydroxy-3,5,5-trimethylcyclohex-2-en-1-one		OD	NEG	NEG	OD	NEG
07.014 1480	Maltol		OD	OD	NEG	OD	POS
07.047 1481	Ethyl maltol		OD	OD	NEG	OD	POS
07.056 418	3-Methylcyclopentan-1,2-dione		OD	NEG	NEG	OD	NEG
07.057 419	3-Ethylcyclopentan-1,2-dione		OD	NEG	NEG	OD	NEG
07.089 1398	Nootkatone		OD	NEG	NEG	NEG	POS
07.127 757	*p*-Mentha-1,4(8)-dien-3-one		OD	NEG	OD	NEG	NEG
07.136 1405	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		OD	NEG	NEG	NEG	OD

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
07.168 -	2-Hydroxypiperitone		OD	NEG	NEG	NEG	NEG
07.075 420	3,4-Dimethylcyclopentan-1,2-dione		OD	NEG	NEG	OD	NEG
07.076 421	3,5-Dimethylcyclopentan-1,2-dione		OD	NEG	NEG	NEG	NEG
07.080 425	3-Methylcyclohexan-1,2-dione		OD	NEG	NEG	OD	NEG

OD, out of applicability domain: not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physicochemical, structural, biological etc.
(a): Structure group 2.7: α,β-unsaturated ketones.
(b): Local model on aldehydes and ketones, Ames TA100. (NEG: Negative; POS: Positive; OD*: out of domain).
(c): MultiCASE Ames test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(d): MultiCASE Mouse Lymphona test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(e): MultiCASE Chromosomal aberration in CHO (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(f): MultiCASE Chromosomal aberration in CHL (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).

4.4. Genotoxicity data (*in vitro*) considered by the Panel in FGE.213

Table 7: Genotoxicity (*in vitro*)

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments [(d)]
β-Ionone [07.008]	Gene mutation (preincubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–180 µg/plate	Negative [(a)]	Mortelmans et al., 1986	Valid.
	Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 mmol/plate	Negative [(a)]	Florin et al., 1980	Insufficient validity (spot test, not according to OECD guideline, methods and results insufficiently reported).
3-Methylcyclopentan-1,2-dione [07.056]	Reverse mutation	*S. typhimurium* TA1535	10 000 µg/plate	Negative [(b)]	Heck et al., 1989	Validity cannot be evaluated (result not reported in detail).
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg/plate	Negative [(b)]	Heck et al., 1989	Validity cannot be evaluated (result not reported in detail).
Maltol [07.014]	Reverse mutation	*S. typhimurium* TA100	4.44 µmol/plate (560 µg/plate)	Negative [(c)]	Kim et al., 1987	Insufficient validity (only one concentration was tested with only one bacterial strain without metabolic activation). The main purpose of the study was to investigate antimutagenic effects.
	Reverse mutation	*S. typhimurium* TA98 and TA100	Up to 3 mg/plate (3 000 µg/plate)	Positive [(a)]	Bjeldanes and Chew, 1979	Valid.
	Reverse mutation	*S. typhimurium* TA92, TA98, TA100 and TA104	1.5 to 11 µmol/plate (189 to 1 387 µg/plate)	Negative	Gava et al., 1989	Limited validity (data not reported in detail).
	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100 and TA1537	33 to 10 000 µg/plate	Positive [(b)]	Mortelmans et al., 1986	Valid.
	Reverse mutation	*S. typhimurium* TA97 and TA102	0.1, 0.5, 1, 5, or 10 mg/plate (100, 500, 1 000, 5 000, or 10 000 µg/plate)	Weak Positive [(a)]	Fujita et al., 1992	Result is considered equivocal. Limited validity (the use of only two strains is not according to OECD guideline).
	DNA damage (SOS Chromotest)	*Escherichia coli* PQ37	5 mM (631 µg/ml)	Negative	Ohshima et al., 1989	The test system used is considered inappropriate, due to insufficient validity.

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments [(d)]
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 1.5 μmol/ml (12.6 to 189 μg/ml)	Positive [(c)]	Gava et al., 1989	Validity cannot be evaluated (insufficiently reported: number of cells analysed not reported. Statistical test used not reported). SCEs were reported as SCE per chromosome. Effect was less than twofold compared to control.
	Sister chromatid exchange	Human lymphocytes	Up to 1.0 mM (126.11 μg/ml)	Positive	Jansson et al., 1986	Validity cannot be evaluated. Relevance of test system for the evaluation of genotoxicity uncertain.
Ethyl maltol [07.047]	Reverse mutation	*S. typhimurium* TA 1535, TA1537, TA1538, TA98 and TA100	5 concentrations up to cytotoxicity, or max. 3 600 μg/plate	Negative [(a)]	Wild et al., 1983	Limited validity (result not reported in details, no TA102 or E. Coli).
	Reverse mutation	*S. typhimurium* TA98 and TA100	Up to 2 mg/plate (2 000 μg/plate)	Positive [(a)]	Bjeldanes and Chew, 1979	Valid.

(a): With and without metabolic activation
(b): With metabolic activation
(c): Without metabolic activation
(d): Validity of genotoxicity studies:
- Valid
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or limited documentation)
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system)
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

4.5. Genotoxicity data (*in vivo*) considered by the Panel in FGE.213

Table 8: Genotoxicity (*in vivo*)

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments [(a)]
Maltol [07.014]	Micronucleus formation	ddY Mouse bone marrow cells	Intraperitoneal	125, 250, or 500 mg/kg	Positive	Hayashi et al., 1988	Valid. The induction of micronuclei was up to about 10-fold compared with control
	Sex-linked Recessive Lethal Mutation	*Drosophila melanogaster*	Feed	6 000 ppm (6000 µg/ml)	Equivocal	Zimmering et al., 1989	Limited validity (only one exposure level tested). Test system considered of limited relevance.
	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	Feed	10 000 ppm (10 000 µg/ml)	Negative	Mason et al., 1992	Valid, however, test system considered of limited relevance.
	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	Injection	0.2 – 0.3 µl, 10 000 ppm (10 000 µg/ml)	Negative	Mason et al., 1992	Valid, however, test system considered of limited relevance.
Ethyl maltol [07.047]	Micronucleus formation	NMRI Mouse bone marrow cells	Intraperitoneal	420, 700, or 980 mg/kg	Negative	Wild et al., 1983	Limited validity (injected twice; only analysis at one time point; no PCE/NCE ratio reported).
	Micronucleus formation	NMRI mouse bone marrow cells	Intraperitoneal	980 mg/kg	Negative	Wild et al., 1983	Limited validity (single injection, analysis at three time points, no PCE/NCE ratio reported).
	Sex-linked recessive lethal mutation (Basc test)	*Drosophila melanogaster*	Feed	14, 25 or 50 mM	Negative	Wild et al., 1983	Limited validity (limited reporting, test system considered of limited relevance).

(a): Validity of genotoxicity studies:

- Valid
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or limited documentation)
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system)
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too few experimental details provided)

4.6. Carcinogenicity studies considered by the Panel in FGE.213

Table 9: Carcinogenicity studies

Chemical name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	Results	Reference	Comments [(a)]
Ethyl maltol [07.047]	Rats; Male, Female 25/sex/group	Diet	0, 50, 100 and 200 mg/kg bw/day	2 years	Males: No increase in tumour incidences Females: No increase in tumour incidences	Gralla et al., 1969	Valid. The study was performed before the introduction of OECD guidelines but is, however, considered valid. The NOAEL was 200 mg/kg bw/day, the highest dose tested
3-Ethylcyclopentan-1,2-dione [07.057]	Rats; Male, Female 50/sex/group	Diet	0, 30, 80 and 200 mg/kg bw/day	2 years	Males: No increase in tumour incidences Females: No increase in tumour incidences	King et al., 1979	Valid. The study was performed before the introduction of OECD guidelines but is, however, considered valid. The NOAEL was 200 mg/kg bw/day, the highest dose tested

(a): Validity of genotoxicity studies:

- Valid
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or limited documentation)
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system)
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too few experimental details provided)

4.7. Genotoxicity data (*in vitro*) considered by the Panel in FGE.213Rev1 and FGE.213Rev2

Table 10: Summary of additional *in vitro* genotoxicity data for FGE.213Rev1

Chemical name [FL-no]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
β-Ionone [07.008]	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	0.32–5000 µg/plate (a, b)	Negative	Ballantyne, 2011	Evidence of toxicity was observed in all strains at concentrations of 1000 µg/plate and greater in the absence and in the presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
		S. typhimurium TA98, TA100, TA102, TA1535 and TA1537	10.24–1000 µg/plate (b, d) or (c, e)	Negative		Evidence of toxicity was observed in all strains at 1 000 µg/plate in the absence and presence of S9-mix, and in most cases these toxic effects also extended down to concentrations of 160 or 400 µg/plate. Study design complied with current recommendations.
	Micronucleus assay	Human peripheral blood lymphocytes	30–60 µg/ml (d, f) 80–120 µg/ml (e, f) 5–17.5 µg/ml (d, g)	Negative	Stone, 2011a	The top concentrations induced 50–60 % toxicity. The MNBN cell frequencies in all treated cultures fell within the normal range. Study design complies with OECD Guideline 487.

Chemical name [FL-no]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
Maltol [07.014]	Reverse mutation	*S. typhimurium* TA98, TA100 and TA102, TA1535 and TA1537	0.32–5000 µg/plate [a, b]	Negative	Ballantyne, 2012	Evidence of toxicity was observed in TA102 at concentrations of 1000 and 5000 µg/plate in the absence of S9-mix and at concentrations of 200 µg/plate and greater in the presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
		S. typhimurium TA98, TA100, TA1535 and TA1537	51.2–5000 µg/plate (b, d) or (c, e)	Negative		Toxicity was observed at 5000 µg/plate in strain TA100 only in the presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
		S. typhimurium TA102	20.48–5000 µg/plate (b, d) or (c, e)	Negative		Evidence of toxicity was observed at 5 000 µg/plate in the absence and presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
	Micronucleus assay	Human peripheral blood lymphocytes	400–1262 µg/ml [d, f] 400–1262 µg/ml [e, f] 125–300 µg/ml [d, g]	Equivocal Positive Negative	Whitwell, 2012	The top concentrations in the 3 + 21 hours treatments in the absence and presence of S9-mix induced, respectively, 24 % and 19 % of toxicity. The top concentration in the 24 + 0 hours treatment in the absence of S9-mix induced 57 % toxicity. There was evidence of micronuclei induction when tested for 3 + 21 hours in the presence of S9-mix, while in absence of S9-mix the data were considered equivocal. However, no induction of micronuclei was observed in the continuous exposure test. Study design complies with OECD Guideline 487.
	Chromosomal aberration test	CHL cells	25, 50, 75 µg/ml (d, g) or (d, h)	Positive	Ishidate, 1988	Structural chromosomal aberrations were observed at the 2 highest concentrations tested.

Chemical name [FL-no]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
β-Damascone [07.083]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.32–5000 µg/plate (a, b)	Negative	Bowen, 2011b	Toxicity was observed at 1 000 and/or 5 000 µg/plate across all strains in the absence and presence of S9-mix; no clear evidence of toxicity in TA100 in the presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium, TA1535, TA1537 and TA102 *S. typhimurium* TA98, TA100	78.13–2500 µg/plate (b, d) or (c, e) 156.3–5000 µg/plate (b, d) or (c, e)	Negative Negative		Evidence of toxicity was observed at the highest three or four concentrations across all strains in the absence and presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix
		S. typhimurium TA98	19.3–1250 µg/plate (c, e)	Negative		Evidence of toxicity was observed at the highest four concentrations in strain TA98 in the presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, in the presence of S9-mix.
	Micronucleus assay	Human peripheral blood lymphocytes	8–22 µg/ml (d, f) 12–18 µg/ml (e, f) 6–9 µg/ml (d, g)	Equivocal(d, f) Positive (e, f) Equivocal(d, g)	Stone, 2012	Positive result was obtained in the 3 + 21 hour treatment in the presence of S9-mix. Study design complies with OECD Guideline 487.
Nootkatone [07.089]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.5–50 µg/plate (b, d) 1.5.5–150 µg/plate (b, e) 0.5–50 µg/plate (b, d) 0.5–150 µg/plate (c, e)	Negative	Marzin, 1998	Evidence of toxicity was observed at 50 µg/plate in all strains in the absence of S9-mix and at 150 µg/plate in all strains in the presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
	Micronucleus assay	Human peripheral blood lymphocytes	50–80 µg/ml (d, f) 160–185 µg/ml (e, f) 10–24 µg/ml (d, g)	Negative	Stone, 2011b	The top concentrations in all parts of the study induced >50 % toxicity. The MNBN cell frequencies in all treated cultures fell within the normal range. Study design complies with OECD Guideline 487.

Chemical name [FL-no]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
2,6,6-Trimethylcyclohex-2-en-1,4-dione [07.109]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.32–5000 µg/plate (a, b)	Negative	Bowen, 2011a	Evidence of toxicity was observed at 1000 and/or 5000 µg/plate in strains TA102 and TA1535 in the presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	156.3–5000 µg/plate (b, d) or (c, e)	Negative		Evidence of toxicity was observed in TA102 at 2500 and 5000 µg/plate. Study design complied with current recommendations. Acceptable top concentration was achieved.
	Micronucleus Assay	Human peripheral blood lymphocytes	500–1522 µg/ml (d, f) 1000–1522 µg/ml (e, f) 300–550 µg/ml (d, g)	Negative	Lloyd, 2011	The top concentrations in the 3 + 21 hours in the absence and presence of S9-mix were 10 mM. The top concentration in the 24 + 0 hours in the absence of S9-mix induced 57 % toxicity. The MNBN cell frequencies in all treated cultures fell within the normal range. Study design complies with OECD Guideline 487.

(a): With and without S9-mix metabolic activation
(b): Plate incorporation method
(c): Without S9-mix metabolic activation
(d): Pre-incubation method
(e): With S9-mix metabolic activation
(f): 3-hour incubation with a 21-hour recovery period
(g): 24-hour incubation with no recovery period
(h): 48-hour incubation with no recovery period

4.8. Genotoxicity data (*in vivo*) considered by the Panel in FGE.213Rev1 and FGE.213Rev2

Table 11: Summary of additional *in vivo* genotoxicity data submitted for FGE.213Rev1 and FGE.213Rev2

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments
Maltol [07.014]	Micronucleus assay	Han Wistar Rat; M	Gavage	70, 350, 700 mg/kg bw/day [(a)]	Negative	Beevers, 2013a	The average MNPCE appearance frequency and ratio of PCE at all dose levels fell within concurrent and historical control ranges. However, evidence of target tissue exposure was inconclusive. The study was performed in compliance with OECD Guideline 474. A further plasma analysis was performed (Beevers, 2015) showing the systemic exposure of animals to maltol. Based on the new bioanalysis, results of the micronucleus assay were considered as negative.
	Comet assay	Han Wistar rat; M	Gavage		Negative		Mean percentage tail intensity and mean tail moment were within historical control range at all test doses. The study was performed in compliance with recommendations of the comet and IWGT workshop, Japanese Centre for the Validation of Alternative Methods (JaCVAM) and current literature.
β-Damascone [07.083]	Micronucleus assay	Han Wistar rat; M	Gavage	125, 250 and 500 mg/kg bw/day [(a)]	Negative	Beevers, 2013b,c	The average MNPCE appearance frequency and ratio of PCE at all dose levels fell within concurrent and historical control ranges. The study was performed in compliance with OECD Guideline 474.
	Comet assay	Han Wistar rat; M	Gavage		Negative		Mean% tail intensity and mean tail moment were within historical control range at all test doses. The study was performed in compliance with recommendations of the comet and IWGT workshop, Japanese Centre for the Validation of Alternative Methods (JaCVAM) and current literature.

(a): Administered via gavage in 3 doses at times 0, 24 and 45 hours with sacrifice and harvest at 48 hours

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Abbreviations

bw	Body Weight
CAS	Chemical Abstracts Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary (cells)
CHL	Chinese Hamster Lung (cells)
CoE	Council of Europe
EC	European Commission
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FISH	Fluorescence In Situ Hybridisation
FLAVIS (FL)	Flavour Information System (database)
GC–MSD	gas chromatography–mass selective detection
GLP	Good Laboratory Practice
ID	Identity
IOFI	International Organization of the Flavor Industry
IR	Infrared Spectroscopy
IWGT	International Workshops on Genotoxicity Testing
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MN	Micronuclei
MNBN	MicroNucleated BiNucleate cells
MNPCE	Micronucleated Polychromatic Erythrocytes
MS	Mass Spectrometry
MSDI	Maximised Survey-derived Daily Intake
MTD	Maximum Tolerated Dose
NCE	NormoChromatic Erythrocytes
NMR	Nuclear Magnetic Resonance
No	Number
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic Erythrocytes
PHA	Phytohaemagglutinin
(Q)SAR	(Quantitative) Structure Activity Relationship
SCE	Sister Chromatid Exchange
WHO	World Health Organization

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.0
Revision Date 18.09.2019
Print Date 08.10.2022

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Ethyl maltol

Product Number : W348708
Brand : Aldrich
REACH No. : 01-2120758795-36-XXXX
CAS-No. : 4940-11-8

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 number

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
Acute toxicity, Oral (Category 4), H302

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)
H302 Harmful if swallowed.

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Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Ethyl maltol 2-Ethyl-3-hydroxy-4H-pyran-4-one
Formula	:	$C_7H_8O_3$
Molecular weight	:	140.14 g/mol
CAS-No.	:	4940-11-8
EC-No.	:	225-582-5

Component	Classification	Concentration
2-Ethyl-3-hydroxy-4-pyrone		
	Acute Tox. 4; H302	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

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SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.Normal measures for preventive fire protection.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

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

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

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Personal protective equipment

Eye/face protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

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: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: solid
b)	Odour	No data available

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c)	Odour Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	Melting point/range: 85 - 95 °C - lit.
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - No data available
In the event of fire: see section 5

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SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - 1,150 mg/kg
LD50 Dermal - Rabbit - > 5,000 mg/kg

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

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

Additional Information
RTECS: UQ0840000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

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12.6 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number
ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)
ADR/RID: - IMDG: - IATA: -

14.4 Packaging group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user
No data available

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

International Chemical Weapons Convention (CWC) Schedules of Toxic Chemicals and Precursors	: Neither banned nor restricted
Restrictions on the marketing and use of certain dangerous substances and preparations	: Neither banned nor restricted
Regulation (EC) No 649/2012 of the European Parliament and the Council concerning the export and import of dangerous chemicals	: Neither banned nor restricted
Candidate List of Substances of Very High Concern for Authorisation	: Neither banned nor restricted

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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.

H302	Harmful if swallowed.

Further information

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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