Toxicological profile for

4-(para-Hydroxyphenyl)-2-butanone

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

4-(4-Hydroxyphenyl)butan-2-one (PubChem)

1.2. Synonyms

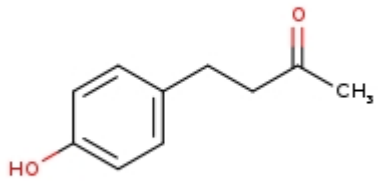
1-(p-Hydroxyphenyl)-3-butanone; 2-Butanone, 4-(4-hydroxyphenyl)-; 4-(4-Hydroxyphenyl)-2-butanone; p-Hydroxybenzyl acetone; 4-(3-Oxobutyl)phenol; EINECS 226-806-4; FEMA No. 2588; AI3-31812; Rasketone; Rheosmin; UNII-7QY1MH15BG; 1-(4-Hydroxyphenyl)-3-butanone; Frambinone; Oxyphenalon; Raspberry ketone; 2-Butanone, 4-(p-hydroxyphenyl)-; 4-(p-Hydroxyphenyl)-2-butanone; 4-08-00-00506 (Beilstein Handbook Reference); BRN 0776080; Hydroxyphenylbutanone, p-; NSC 26515; Betuligenol; HSDB 8163; 4-(4-Hydroxyphenyl)butan-2-one (ChemIDplus)

1.3. Molecular formula

C10 H12 O2

1.4. Structural Formula

ChemIDplus



1.5. Molecular weight (g/mol)

164.2028

1.6. CAS registration number

5471-51-2

1.7. Properties

1.7.1. Melting point

(°C): 80-85 (ChemSpider); 82.5 (HSDB, 2014)

1.7.2. Boiling point

(°C): 200 or 292.2 (ChemSpider); Decomposes (HSDB, 2014)

1.7.3. Solubility

13.46 g/L at 25°C (estimated) (EPISuite, 2017); 25.1 g/L 25°C (estimated) (HSDB, 2014); insoluble in water (PubChem)

1.7.4. pKa

9.51 (estimated) (HSDB, 2014)

1.7.5. Flashpoint

(°C): 122.9±13.0 (estimated) (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

Stable at normal temperatures and pressure.

1.7.10. Vapor pressure

0.0±0.6 mmHg at 25°C (estimated) (ChemSpider); $5.7x10^{-4}$ mmHg at 25°C (estimated) (HSDB, 2014); 0.000567 mmHg at 25°C (estimated) (EPISuite, 2017)

1.7.11. log Kow

0.762 (ChemSpider)

2. General information

2.1. Exposure

This ingredient is a well characterized material that has been evaluated and approved as a food additive by expert bodies including USFDA, FEMA and the CoE.

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for 4-(p-Hydroxyphenyl)-2-butanone (FEMA no. 2588):

Baked Goods	13/54
Beverages, Non-Alcoholic	3/16
Beverages, Alcoholic	250/300
Chewing Gum	71/320
Frozen Dairy	9/34

Gelatins and Puddings	14/50
Hard Candy	17/44
Soft Candy	13/36
Sweet Sauces	0.9/1

As taken from Cohen SM et al. 2020. Food Technology 74(3), 44-65. Available at https://www.femaflavor.org/sites/default/files/2020-03/GRAS%2029.pdf

Cosmetics	Yes (Cosing)	Food	Yes (Burdock, 2010)
Environment	No evidence	Pharmaceuticals	No evidence (Martindale 1993)

Raspberry ketone (CAS RN 5471-51-2) is used as a fragrance, perfuming and skin conditioning agent in cosmetics in the EU. As taken from Cosing, undated.

4-(p-Hydroxyphenyl)-2-butanone is listed as a fragrance ingredient by the US EPA InertFinder Database (2021) and IFRA.

Estimated current levels of intake in Europe and the US are 2.8 and 3.8 mg/day, respectively.

As taken from JECFA, 2001

“According to the 2006 TSCA Inventory Update Reporting data, the number of persons reasonably likely to be exposed in the industrial manufacturing, processing, and use of raspberry ketone is 1 to 99; the data may be greatly underestimated(1). [(1) US EPA; Inventory Update Reporting (IUR). Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Washington, DC: U.S. Environmental Protection Agency. Available from, as of Feb 4, 2014: http://cfpub.epa.gov/iursearch/index.cfm**PEER REVIEWED**”

“Occupational exposure to raspberry ketone may occur through inhalation and dermal contact with this compound at workplaces where raspberry ketone is produced or used. Monitoring data indicate that the general population may be exposed to raspberry ketone via inhalation from the aroma of plants and foods and from tobacco smoke, via ingestion of foods and nutritional supplements, and through dermal contact with consumer products containing raspberry ketone(SRC).”

“The average estimated daily intake of dietary raspberry ketone has been estimated to be 0.42 mg/kg/day(1). An individual daily intake of 0.01624 mg/kg/day has been reported(2). [(1) Gaunt IF; Food and Cosmetics Toxicology 8(4): 349-358 (1970) (2) Burdock GA, ed; Fenaroli's Handbook of Flavor Ingredients. 6th ed.Boca Raton, FL, p. 879 (2010)] **PEER REVIEWED**”

“Occupational exposure to raspberry ketone may occur through inhalation and dermal contact with this compound at workplaces where raspberry ketone is produced or used. Monitoring data indicate that the general population may be exposed to raspberry ketone via inhalation from the aroma of plants and foods and from tobacco smoke, via ingestion of foods and nutritional supplements, and through dermal contact with consumer products containing raspberry ketone. (SRC) **PEER REVIEWED**”

“Raspberry ketone has been detected as a component of tobacco smoke(1). [(1) Rodgman A, Perfetti TA; The Chemical Components of Tobacco and Tobacco Smoke, Second Ed, Boca Raton, FL: CRC Press, p. 260 (2013)] **PEER REVIEWED**”

“Used in fruit flavors, particularly in raspberry compositions. [Fahlbusch KG et al; Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (1999-2014). New York, NY: John Wiley & Sons. Online Posting Date: Jan 15, 2003] **PEER REVIEWED**”

“Used in perfumery, in cosmetics, and as a food additive to impart a fruity odor and taste; In the flavour industry, raspberry ketone is frequently used in products such as soft drinks, sweets, puddings and ice creams. [Beekwilder J et al; Biotechnology Journal 2(10): 1270-9 (2007)] **PEER REVIEWED**”

“Raspberry ketone has been used to relieve symptoms of gastrointestinal conditions, heart problems, menstrual discomfort, easing labor and delivery, and morning sickness of pregnancy. More recently, raspberry ketone has been promoted as a weight loss aid, promoting weight loss by increasing norepinephrine release in the body and speeding up metabolism, including increasing heart rate. [Rxlist; Definition of Red Raspberry (Raspberry ketone); RxList, The Internet Drug Index. Available from, as of Feb 10, 2014: http://www.rxlist.com/script/main/art.asp?articlekey=155766**PEER REVIEWED**”

“Raspberry ketone is a natural phenolic compound. It is used in perfumery, in cosmetics, and as a food additive to impart a fruity odor. [Jeong JB, Jeong HJ; Food Chem Toxicol. 48 (8-9): 2148-53 (2010)] **PEER REVIEWED**”

As taken from HSDB, 2014.

Raspberry ketone (CAS RN 5471-51-2) is listed as an ingredient (at given concentrations, where specified) in auto (1-5%), inside the home (0.5-5%), personal care (0.1-1%) and pet care products by the CPID.

“Raspberry ketone, 4-(4-hydroxyphenyl)-2-butanone, is the primary aroma compound (level of 0.009-4.3 mg/kg) of the fruit of raspberry (Rubus idaeus L.). It is also used as a flavouring substance. In recent years, raspberry ketone has been marketed as an ingredient in food supplements for weight loss in recommended daily doses of 100-1400 mg. European Food Safety Authority (EFSA) concluded that raspberry ketone as a flavouring substance would present no safety concern at the estimated level of intake from berries and flavourings of 2.4 mg/day (based on an estimation of Margin of Safety (MOS) equal to 2500). Other intake estimates range from 1.8-3.8 mg/day for an adult.”

As taken from Steffensen I-L, 2019

Raspberry ketone (CAS RN 5471-51-2) is used as a flavour enhancer for oral use and a fragrance ingredient for topical use in non-medicinal natural health products (Health Canada, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Report 1 (2003).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
4-(4-hydroxyphenyl)-2-butanone	6490579842	99.25	9976356678	99.58
Total ion chromatogram	6539692936	100	10018132290	100

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

Ingredient **CAS Number**	**Chemical Class**	**Mol. wt. (M)** **bp or mp (ºC)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (compound, %)**	**Max. level in smoke (µg)**

4-(p-Hydroxyphenyl)-2-butanone CAS 5471-51-2	Phenol ketone	M=164 mp 82-84	20	98	4-(p-Hydroxyphenyl)-2-butanone 96.8 4-(p-Hydroxyphenyl)-2-butanone isomer 3.0 Dihydrobenzofuran 0.1 1 Unidentified compound 0.1	10 0.3 0.01 0.01

2.3. Ingredient(s) from which it originates

0.0001 (% applied to tobacco), No evidence of presence in tobacco naturally (Stedman 1968; Lloyd et al 1976)

Found in red raspberries (Rubus idaeus) (Harada N et al., Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans; Growth Horm IGF Res. 2008, Aug; 18(4):335-44).

Reportedly found in European cranberry, raspberry, blackberry, loganberry and sea buckthorn (Hippophae rhamnoides L.) (Burdock, 2010).

“Extraction of pure raspberry ketone from raspberries is usually 1-4 mg/kg of raspberries(1). Raspberry ketone was detected in the aroma components of freshly brewed Arabica and Ethiopian coffees(2,3). Reported uses of raspberry ketone in foods: baked goods, 13.05 ppm (51.15 ppm max); chewing gum, 70.85 ppm (209.5 ppm max); frozen dairy, 9.25 ppm (30.34 ppm max); gelatins and puddings, 13.50 ppm (28.87 ppm max); hard candy, 16.74 ppm (33.69 ppm max); nonalcoholic beverages, 2.76 ppm (11.39 ppm max); soft candy, 13.42 ppm (35.99 ppm); sweet sauce, 0.90 ppm (1.4 ppm max)(4). [(1) Beekwilder J et al; Biotechnology Journal 2(10): 1270-9 (2007) (2) Akiyama M et al; J Food Sci 73(5): C335-C346 (2008) (3) Akiyama M et al; J Food Sci 72(7): C388-C366 (2007) (4) Burdock GA, ed; Fenaroli's Handbook of Flavor Ingredients. 6th ed.Boca Raton, FL, p. 880 (2010)] **PEER REVIEWED**”

“Natural occurrence: Reported found European cranberry, raspberry, blackberry, loganberry and sea buckthorn (Hippophae rhamnoides L.) [Burdock, G.A. (ed.). Fenaroli's Handbook of Flavor Ingredients. 6th ed.Boca Raton, FL 2010, p. 941] **PEER REVIEWED**”

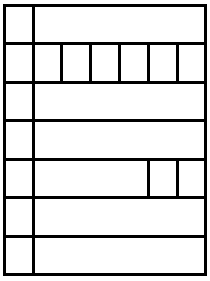
“Raspberry ketone occurs in raspberries at typical concentrations of 1-4 mg/kg of raspberries(1). Raspberry ketone has been found in numerous plant genera, such as Artemisia, Capparis, Dendrobium, Hippophae, Larix, Limonium, Pinus, Prunus, Rheum, Rubus, Saxifraga, Taxus, Vaccinium, Vanilla, and Vitis(2). Raspberry ketone is reported to occur in cranberry, blackberry, loganberry and sea buckthorn (Hippophae rhamnoides L)(3). Raspberry ketone is one of the major aromatic compounds of red raspberry (Rubus idaeus)(4). Raspberry ketone has been found in the glands of the melon fly (Dacus cucurbitae) and of the North American beaver (Castor canadensis)(2). [(1) Beekwilder J et al; Biotechnology Journal 2(10): 1270-9 (2007) (2) Zorn H et al; Appl Environ Microbiol 69(1): 367-372 (2003) (3) Burdock GA (ed); Fenaroli's Handbook of Flavor Ingredients. 6th ed.Boca Raton, FL, p. 880 (2010) (4) Takata T, Morimoto C; J Medicinal Food 17(3): 332-8 (2014)] **PEER REVIEWED**”

“Raspberry ketone has been found in the glands of the melon fly (Dacus cucurbitae) and of the North American beaver (Castor canadensis). The basidiomycete Nidula niveo-tomentosa /White Barrel Bird's Nest fungi/ is a microbial producer of 4-(4-hydroxyphenyl)-butan-2-one(1).[(1) Zorn H et al; Appl Environ Microbiol 69: 367-372 (2003). Available from, as of Jun 2, 2014: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC152476/ **PEER REVIEWED**”

As taken from HSDB, 2014.

“Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) .... is naturally occurring in raspberries (up to 4.3 mg/kg) .....” As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596

3. Status in legislation and other official guidance



In its procedure for the evaluation of food flavouring ingredients, JECFA (2001) concluded that 4-(p-hydroxyphenyl)-2-butanone was of no safety concern at the current levels of intake (estimated at 2.8 and 3.8 mg/day in Europe and the US respectively).

The compound has been designated as GRAS (generally recognized as safe) by FEMA (Hall & Oser, 1965).

Included on the US FDA’s list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and covered under 21 CFR 172.515 (synthetic flavoring substances and adjuvants) (FDA, 2021a,b).

There are REACH dossiers on 4-(4-hydroxyphenyl)butan-2-one (ECHA, 2021).

4-(4-Hydroxyphenyl)butan-2-one (CAS RN 5471-51-2) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

“4-(p-Hydroxylphenyl)-2-butanone is a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: a) they are used in the minimum quantity required to produce their intended effect, and otherwise in accordance with all the principles of good manufacturing practice, and b) they consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, prior-sanctioned for such use, or regulated by an appropriate section in this part. [21 CFR 172.515 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of March 17, 2014: http://www.ecfr.gov/cgi-bin/ECFR?page=browse **PEER REVIEWED**”

As taken from HSDB, 2014.

4-(p-Hydroxyphenyl)butan-2-one is authorised for use as a flavouring substance in all categories of flavoured food in the EU, under EU Regulation No. 872/2012 (European Commission, 2012).

4-(p-Hydroxyphenyl)-2-butanone (CAS RN 5471-51-2) is listed in the US EPA InertFinder Database (2021) as approved for fragrance use in pesticide products.

2-Butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2) is included on the US EPA’s Safer Chemical Ingredients List (US EPA, 2022).

2-Butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule).

The TSCA inventory and 2020 CDR list are available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

“Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is marketed on the Internet as a food supplement. The recommended intake is between 100 and 1400 mg per day. The substance is naturally occurring in raspberries (up to 4.3 mg/kg) and is used as a flavouring substance. Toxicological studies on raspberry ketone are limited to acute and subchronic studies in rats. When the lowest recommended daily dose of raspberry ketone (100 mg) as a food supplement is consumed, it is 56 times the established threshold of toxicological concern (TTC) of 1800 µg/day for Class 1 substances. The margin of safety (MOS) based on a NOAEL of 280 mg/kg bw/day for lower weight gain in rats is 165 at 100 mg and 12 at 1400 mg. The recommended doses are a concern taking into account the TTC and MOS. Investigations of raspberry ketone in quantitative structure-activity relationship (QSAR) models indicated potential cardiotoxic effects and potential effects on reproduction/development. Taking into account the high intake via supplements, the compound's toxic potential should be clarified with further experimental studies. In UK the pure compound is regarded as novel food requiring authorisation prior to marketing but raspberry ketone is not withdrawn from Internet sites from this country.” As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596 .

2-Butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2) is included on the New Zealand Inventory of Chemicals and does not have an individual approval but maybe used under an appropriate group standard (NZ EPA, 2006).

“Raspberry ketone was classified as a novel food by the Food Standards Agency (FSA) in UK (March 2014). It was stated that raspberry ketones other than raspberry fruit extracts prepared using water or 20% ethanol (1:4 ethanol:water) are novel and should fall within the scope of the EU legislation on novel foods. This information is not found at the homepage of FSA (November 16, 2018). Some notifications calling raspberry ketone an unauthorized novel food ingredient (newest 2016) are published on the EU RASFF (Rapid Alert System for Food and Feed) portal. The substance is not found in the Novel food catalogue (November 16, 2018). Whatever the legal status, raspberry ketone is still marketed as an ingredient in food supplements in EU; now in even higher recommended doses (up to 2000 mg/day).”

As taken from Steffensen I-L, 2019

Implementation dates: For new submissions*: February 10 2021

For existing fragrance compounds*: February 10 2022

*These dates apply to the supply of fragrance mixtures (formulas) only, not to the finished consumer products in the marketplace

Recommendation: Restriction

RESTRICTION LIMITS IN THE FINISHED PRODUCT (%):			
Category 1	0.68 %	Category 7A	0.41 %
Category 2	1.0 %	Category 7B	0.41 %
Category 3	0.27 %	Category 8	0.045 %
Category 4	1.0 %	Category 9	1.0 %
Category 5A	1.0 %	Category 10A	1.0 %
Category 5B	0.14 %	Category 10B	1.0 %
Category 5C	0.27 %	Category 11A	0.045 %
Category 5D	0.045 %	Category 11B	0.045 %
Category 6	0.82 %	Category 12	78 %

Flavour requirements: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards.

Intrinsic property driving risk management: Depigmentation

Expert panel for fragrance safety rationale / conclusion: The Expert Panel for Fragrance Safety reviewed all the available data for 4-(4-Hydroxyphenyl)butan-2-one and recommends the limits for the 12 different product categories, which are the acceptable use levels of 4-(4-Hydroxyphenyl)butan-2-one in the various product categories.

As taken from IFRA, 2020

Raspberry ketone (CAS RN 5471-51-2) is classified as an NHP [natural health product] under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

2-Butanone, 4-(4-hydroxyphenyl)- (CAS 5471-51-2) is listed on

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

p-Hydroxyphenylbutan-2-one (pHPB), the raspberry ketone, is responsible for the characteristic aroma of raspberries. The compound accumulates rapidly during the later maturation stages of the berries. The synthesis and accumulation of pHPB correlates with that of anthocyanin and soluble solids (°Brix). pHPB is synthesized in cell-free extracts of fruits and tissue cultures from p-coumaryl-CoA and malonyl-CoA in a manner similar to the synthesis of chalcones and stilbenes. The specific biosynthetic pathway for pHPB formation deviates from the general phenylpropanoid pathway at the p-coumaryl-CoA stage and it is composed of two enzymes. The first enzyme is the p-hydroxyphenylbut-3-ene-2-one synthase (pHPB-3-ene-2-one synthase) that forms p-hydroxyphenylbut-3-ene-2-one by the condensation of malonyl-CoA with p-coumaryl-CoA. The second enzyme, p-hydroxyphenylbut-3-ene-2-one reductase (pHPB-3-ene-2-one reductase),

reduces the p-hydroxyphenylbut-3-ene-2-one to p-hydroxyphenylbutan-2-one, the raspberry ketone. We detected the activity of both enzymes in crude extracts from raspberry fruits and their tissue cultures, and identified their reaction products by HPLC, crystallization to constant radioactivity and by GC-MS.

Wlodzimierz Borejsza-Wysocki and Geza Hrazdina; Biosynthesis of p-hydroxyphenylbutan-2-one in raspberry fruits and tissue cultures; Phytochemistry, Volume 35, Issue 3, February 1994, Pages 623-628; http://www.sciencedirect.com/

Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one; RK) is a major aromatic compound of red raspberry (Rubus idaeus). The structure of RK is similar to the structures of capsaicin and synephrine, compounds known to exert anti-obese actions and alter the lipid metabolism. The present study was performed to clarify whether RK helps prevent obesity and activate lipid metabolism in rodents. To test the effect on obesity, our group designed the following in vivo experiments: 1) mice were fed a high-fat diet including 0.5, 1, or 2% of RK for 10 weeks; 2) mice were given a high-fat diet for 6 weeks and subsequently fed the same high-fat diet containing1% RK for the next 5 weeks. RK prevented the high-fat-diet-induced elevations in body weight and the weights of the liver and visceral adipose tissues (epididymal, retroperitoneal, and mesenteric). RK also decreased these weights and hepatic triacylglycerol content after they had been increased by a high-fat diet. RK significantly increased norepinephrine-induced lipolysis associated with the translocation of hormone-sensitive lipase from the cytosol to lipid droplets in rat epididymal fat cells. In conclusion, RK prevents and improves obesity and fatty liver. These effects appear to stem from the action of RK in altering the lipid metabolism, or more specifically, in increasing norepinephrine-induced lipolysis in white adipocytes.

Morimoto et al.; Anti-obese action of raspberry ketone; Life Sciences, Volume 77, Issue 2, 27 May 2005, Pages 194-204 ; http://www.sciencedirect.com/

The metabolism of 4-(4-hydroxyphenyl) butan-2-one (raspberry ketone) was studied in rats, guinea-pigs and rabbits. Following intragastric dosage (1 mmol/kg) urinary metabolite excretion was nearly complete within 24 h, amounting to roughly 90% of the dose in all species. The most prominent urinary metabolites were raspberry ketone and its corresponding carbinol, both largely conjugated with glucuronic acid and/or sulphate. The extent of ketone reduction was greatest in rabbits. Oxidative metabolism included ring hydroxylation and side-chain oxidation. The latter pathway led to 1,2- and 2,3-diol derivatives. It is proposed that the latter undergo cleavage to furnish the C6-C3 and C6-C2 derivatives detected.S.Sporstøland R. R. Scheline;The metabolism of 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone) in rats, guinea-pigs and rabbits; Xenobiotica,1982, Vol. 12, No. 4, Pages 249-257 DOI: 10.3109/00498258209052463; http://informahealthcare.com/doi/abs/10.3109/00498258209052463

p-Hydroxyphenylbutan-2-one, the characteristic aroma compound of raspberries (Rubus idaeus L.), is synthesized from p-coumaryl-coenzyme A and malonyl-coenzyme A in a two-step reaction sequence that is catalyzed by benzalacetone synthase and benzalacetone reductase (W. Borejsza-Wysocki and G. Hrazdina [1994] Phytochemistry 35: 623-628). Benzalacetone synthase condenses one malonate with p-coumarate to form the pathway intermediate p-hydroxyphenylbut-3-ene-2-one (p-hydroxybenzalacetone) in a reaction that is similar to those catalyzed by chalcone and stilbene synthases. We have obtained an enzyme preparation from ripe raspberries that was preferentially enriched in benzalacetone synthase (approximately 170-fold) over chalcone synthase (approximately 14-fold) activity. This preparation was used to characterize benzalacetone synthase and to develop polyclonal antibodies in rabbits. Benzalacetone synthase showed similarity in its molecular properties to chalcone synthase but differed distinctly in its substrate specificity, response to 2-mercaptoethanol and ethylene glycol, and induction in cell-suspension cultures. The product of the enzyme, p-hydroxybenzalacetone, inhibited mycelial growth of the raspberry pathogen Phytophthora fragariae var rubi at 250 [mu]M. We do not know whether the dual activity in the benzalacetone synthase preparation is the result of a bifunctional enzyme or is caused by contamination with chalcone synthase that was also present. The rapid induction of the enzyme in

cell-suspension cultures upon addition of yeast extract and the toxicity of its product, p-hydroxybenzalacetone, to phytopathogenic fungi also suggest that the pathway may be part of a plant defense response. W. Borejsza-Wysocki and G. Hrazdina; Aromatic Polyketide Synthases (Purification, Characterization, and Antibody Development to Benzalacetone Synthase from Raspberry Fruits); PLANT PHYSIOLOGY , Vol 110, Issue 3 791-799, Copyright © 1996 by American Society of Plant Biologists; http://www.plantphysiol.org/cgi/content/abstract/110/3/791

”The metabolism of 4-(4-hydroxyphenyl)butan-2-one(raspberry ketone) was studied in rats, guinea-pigs and rabbits. 2. Following intragastric dosage (1 mmol/kg) ….. The most prominent urinary metabolites were raspberry ketone and its corresponding carbinol, both largely conjugated with glucuronic acid and/or sulphate. The extent of ketone reduction was greatest in rabbits. 4. Oxidative metabolism included ring hydroxylation and side-chain oxidation. The latter pathway led to 1,2- and 2,3-diol derivatives. It is proposed that the latter undergo cleavage to furnish the C6-C3 and C6-C2 derivatives detected. [Sporstol S, Scheline RR; Xenobiotica 12 (4): 249-57 (1982)] **PEER REVIEWED**”

“A ketone (probably acetone) was found in the urine of rats fed p-hydroxyphenylbutanone at 1% in the diet. After administration of a single 200-mg dose, rats excreted about 6% of the dose unchanged within 24 hr. A positive reaction for ketones being obtained only in urine produced between 1 and 6 hr after treatment. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**”

As taken from HSDB, 2014.

“CONTEXT: Raspberry ketone (RK) is a natural phenolic compound of red raspberry. The dietary intake of RK has been reported to exert anti-obese actions and alter the lipid metabolism in vivo and human studies. OBJECTIVE: To elucidate a possible mechanism for anti-obese actions of RK, the effects of RK on the adipogenic and lipogenic gene expression in 3T3-L1 adipocytes were investigated. MATERIALS AND METHODS: 3T3-L1 maturing pre-adipocytes were treated from day 2 to day 8 of differentiation and mature adipocytes for 24 h on day 12 with 1, 10, 20, and 50 μM of RK. Triacylglycerols were assessed by spectrophotometry and gene expression by quantitative real-time polymerase chain reaction (qRT-PCR). RESULTS: Treatment of adipocytes with RK suppressed adipocyte differentiation and fat accumulation in a concentration-dependent manner. RK suppressed the expression of major genes involved in the adipogenesis pathway including peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT enhancer binding protein-α (C/EBPα), which led to further down-regulation of adipocyte fatty acid-binding protein-2 (aP2). In addition, treatment with 10 μM of RK also reduced mRNA levels of lipogenic genes such as acetyl-CoA carboxylase-1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD1). In mature adipocytes, RK increased the transcriptional activities of genes involved in lipolysis and the oxidative pathways including adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and carnitine palmitoyl transferase-1B (CPT1B). DISCUSSION AND CONCLUSION: These findings suggest that RK holds great promise for an herbal medicine with the biological activities altering the lipid metabolism in 3T3-L1 adipocytes.” As taken from Park KS. 2015. Pharm. Biol. 53(6), 870-5. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25429790?dopt=AbstractPlus

“Objectives: Raspberry ketone (RK) is the primary aroma compound in red raspberries and a dietary supplement for weight loss. This work aims to 1) compare RK bioavailability in male versus female, normal-weight versus obese mice; 2) characterize RK metabolic pathways. Methods: Study 1: C57BL/6J male and female mice fed a low-fat diet (LFD; 10% fat) receive a single oral gavage dose of RK (200 mg kg-1). Blood, brain, and white adipose tissue (WAT) are collected over 12 h. Study 2: Male mice are fed a LFD or high-fat diet (45% fat) for 8 weeks before RK dosing. Samples collected are analyzed by UPLC-MS/MS for RK and its metabolites. Results: RK is rapidly absorbed (Tmax ≈ 15 min), and bioconverted into diverse metabolites in mice. Total bioavailability (AUC0-12 h) is slightly lower in females than males (566 vs 675 nmol mL-1 min-1). Total bioavailability in obese mice is almost doubled that of control mice (1197 vs 679 nmol mL-1 min-1),

while peaking times and elimination half-lives are delayed. Higher levels of RK and major metabolites are found in WAT of the obese than normal-weight animals. Conclusions: RK is highly bioavailable, rapidly metabolized, and exhibits significantly different pharmacokinetic behaviors between obese and control mice. Lipid-rich tissues, especially WAT, can be a direct target of RK." As taken from Zhao D et al. 2020. Mol. Nutr. Food Res. 64(8), e1900907. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32052560/

"Raspberry ketone (RK) (4-(4-hydroxyphenyl)-2-butanone) is the major compound responsible for the characteristic aroma of red raspberries, and has long been used commercially as a flavoring agent and recently as a weight loss supplement. A targeted UHPLC-QqQ-MS/MS method was developed and validated for analysis of RK and 25 associated metabolites in mouse plasma and brain. Dispersion and projection analysis and central composite design were used for method optimization. Random effect analysis of variance was applied for validation inference and variation partition. Within this framework, repeatability, a broader sense of precision, was calculated as fraction of accuracy variance, reflecting instrumental imprecision, compound degradation and carry-over effects. Multivariate correlation analysis and principle component analysis were conducted, revealing underlying association among the manifold of method traits. R programming was engaged in streamlined statistical analysis and data visualization. Two particular phenomena, the analytes' background existence in the enzyme solution used for phase II metabolites deconjugation, and the noted lability of analytes in pure solvent at 4 ℃ vs. elevated stability in biomatrices, were found critical to method development and validation. The approach for the method development and validation provided a foundation for experiments that examine RK metabolism and bioavailability." As taken from Yuan B et al. 2020. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1149, 122146. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32474352/

"The exposure of human skin to 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone, RK) is known to cause chemical/occupational leukoderma. RK is a carbonyl derivative of 4-(4-hydroxyphenyl)-2-butanol (rhododendrol), a skin whitening agent that was found to cause leukoderma in skin of many consumers. These two phenolic compounds are oxidized by tyrosinase and the resultant products seem to cause cytotoxicity to melanocytes by producing reactive oxygen species and depleting cellular thiols through *o*-quinone oxidation products. Therefore, it is important to understand the biochemical mechanism of the oxidative transformation of these compounds. Earlier studies indicate that RK is initially oxidized to RK quinone by tyrosinase and subsequently converted to a side chain desaturated catechol called 3,4-dihydroxybenzalacetone (DBL catechol). In the present study, we report the oxidation chemistry of DBL catechol. Using UV-visible spectroscopic studies and liquid chromatography mass spectrometry, we have examined the reaction of DBL catechol with tyrosinase and sodium periodate. Our results indicate that DBL quinone formed in the reaction is extremely reactive and undergoes facile dimerization and trimerization reactions to produce multiple isomeric products by novel ionic Diels-Alder type condensation reactions. The production of a wide variety of complex quinonoid products from such reactions would be potentially more toxic to cells by causing not only oxidative stress, but also melanotoxicity through exhibiting reactions with cellular macromolecules and thiols." As taken from Sugumaran M et al. 2020. Int. J. Mol. Sci. 21(18), 6774. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32942764/

4.2. Absorption, distribution and excretion

"Urinary analyses showed ketones in the urine of all treated animals at 7 and 13 weeks. The authors reported that this effect appeared within 12 hr in rats fed a diet containing 1% 4-(para-hydroxyphenyl)-2-butanone for-7 days, and disappeared within 9 hr when the rats were returned to normal diet. The ketonuria, which was possibly due to metabolites present in the urine, was considered not to be a toxic effect [WHO Food Additives Series 46. Available from, as of January 28, 2014 http://www.inchem.org/documents/jecfa/jecmono/v46je09.htm**PEER REVIEWED**".

”The metabolism of 4-(4-hydroxyphenyl)butan-2-one(raspberry ketone) was studied in rats, guinea-pigs and rabbits. 2. Following intragastric dosage (1 mmol/kg) urinary metabolite excretion was nearly complete within 24 hr, amounting to roughly 90% of the dose in all species. 3…….. [Sporstol S, Scheline RR; Xenobiotica 12 (4): 249-57 (1982)] **PEER REVIEWED**”

“A ketone (probably acetone) was found in the urine of rats fed p-hydroxyphenylbutanone at 1% in the diet. After administration of a single 200-mg dose, rats excreted about 6% of the dose unchanged within 24 hr. A positive reaction for ketones being obtained only in urine produced between 1 and 6 hr after treatment. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**”

As taken from HSDB, 2014.

“4-(4-Hydroxyphenyl)butan-2-one [07.055] [is] mainly excreted as glucuronide and sulfate conjugates either unmodin¼üed or after reduction to the corresponding alcohol (Scheline, 1991)”.

As taken from EFSA, 2016.

4.3. Interactions

“The effect of essential oils, such as raspberry ketone, on androgen (AR) receptor was investigated using a MDA-kb2 human breast cancer cell line for predicting potential AR activity. Among them, eugenol had the highest AR antagonistic activity with its IC(50) value of 19 microM. Raspberry ketone, which has threefold higher anti-obese activity than that of capsaicin, also had AR antagonist activity with its IC(50) value of 252 microM. Based on these findings, a more precise CoMFA model was proposed as follows: pIC(50) [log (1/IC(50))]=3.77+[CoMFA field terms] (n=39, s=0.249, r(2)=0.834, s(cv)=0.507, q(2)=0.311 (three components)”. As taken from OgawaY et al. 2010. Bioorgan. Med. Chem. Lett. 20, 2111-2114. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20226658?dopt=AbstractPlus

“Inflammation is part of the host defense mechanism against harmful matters and injury; however, aberrant inflammation is associated to the development of chronic disease such as cancer. Raspberry ketone is a natural phenolic compound. It is used in perfumery, in cosmetics, and as a food additive to impart a fruity odor. In this study, we evaluated whether rheosmin, a phenolic compound isolated from pine needles regulates the expression of iNOS and COX-2 protein in LPS-stimulated RAW264.7 cells. Rheosmin dose-dependently inhibited NO and PGE(2) production and also blocked LPS-induced iNOS and COX-2 expression. Rheosmin potently inhibited the translocation of NF-kappaB p65 into the nucleus by IkappaB degradation following IkappaB-alpha phosphorylation. This result shows that rheosmin inhibits NF-kappaB activation. In conclusion, our results suggest that rheosmin inhibits LPS-induced iNOS and COX-2 expression in RAW264.7 cells by blocking NF-kappaB activation pathway”. As taken from Jeong JB & Jeong HJ. 2011. Fd Chem. Toxicol. 48, 2158-2163. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20478352

“BACKGROUND: Very few weight and fat loss supplements undergo finished-product research to examine efficacy. The purpose of this study was to determine the effects of an 8-week diet and exercise program on body composition, hip and waist girth, and adipokines and evaluate whether a dietary supplement containing raspberry ketone, capsaicin, caffeine, garlic, and Citrus aurantium enhanced outcomes. METHODS: Overweight men and women completed this randomized, placebo-controlled, double-blind study. Participants consumed 4 capsules/d of supplement (EXP; n = 18) or placebo (PLA; n = 18). Participants underwent 8 weeks of daily supplementation, calorie restriction (500 kcal < RMR [resting metabolic rate] × 1.2), and supervised progressive exercise training 3 times a week. Body composition, girth, and adipokines were assessed at baseline and postintervention (T1 and T2). RESULTS: Significant decreases in weight (-2.6 ± 0.57 kg, $p < 0.001$), fat mass (-1.8 ± 0.20 kg; $p < 0.001$), and percentage body fat (-3.7% ± 0.29%, $p < 0.001$) and a significant increase in lean body mass (LBM; 1.5 ± 0.26 kg; $p < 0.001$) were seen from T1 to T2 in both groups. For men, only those in the EXP group increased LBM from T1 to T2 (1.3 ± 0.38

kg; $p < 0.05$). Hip girth was also reduced, with the women in the EXP group (-10.7 ± 2.15 cm, $p < 0.001$) having a greater reduction. There was a time by group interaction, with significant decreases in leptin ($p < 0.001$) and significant increases in adiponectin ($p < 0.05$) in the EXP group. CONCLUSIONS: Significant improvements in adipokines and leptin support the utility of exercise, diet, and fat loss for impacting inflammatory biomarkers. The improvement in adiponectin with EXP may suggest a unique health mechanism." As taken from Arent SM et al. 2018. J. Am. Coll. Nutr. 37(2), 111-120. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29111889

"AIM: The cardioprotective role of raspberry ketone (RK) against isoproterenol (ISO)-induced myocardial infarction (MI) in rats was assessed. MATERIALS AND METHODS: Rats were randomly divided into Group I - Vehicle control; Group II - Toxic control ISO (85mg/kg, s.c.); Group III, IV and V - RK (50, 100 and 200mg/kg, respectively) with ISO; Group VI- RK (200mg/kg) alone; Group VII - Propranolol (10mg/kg) with ISO; and Group VIII - Propranolol (10mg/kg) alone. After twenty-four hours of the last dose, animals were sacrificed and creatine kinase-MB, lactate dehydrogenase, total cholesterol, triglycerides, high-density-lipoprotein, low-density-lipoprotein, very-low-density-lipoprotein, malondialdehyde, reduced glutathione, superoxide dismutase, catalase, Na^+, K^+-ATPase, nitric oxide, histopathological and immunohistochemical analysis (tumor necrosis factor-α and inducible nitric oxide synthase) were performed. KEY FINDINGS: Treatment with ISO significantly deviated the biochemical parameters from the normal levels, which were considerably restored by RK at 100 and 200mg/kg doses. 50mg/kg dose, however, did not demonstrate any significant cardioprotective action. The histopathological and immunohistochemical analysis further substantiated these findings. SIGNIFICANCE: Our study showed a dose-dependent reduction in oxidative stress, inflammation and dyslipidemia by RK in ISO-intoxicated rats, which signifies that RK from the European red raspberry plant might be a valuable entity for the management of MI." As taken from Khan V et al. 2018. Life Sci. 194, 205-212. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29225109

"AIM: Obesity is a continually growing pandemic leading to many diseases that affect the overall quality of life. The widely marketed Garcinia cambogia (GC) and Raspberry ketone (RK) were used in this study. Despite their known dietetic effect, however, the metabolomic/signaling pathways involved in this effect are not fully elucidated. Hence, our study comprehends the possible trajectories of their combination against obesity and insulin resistance in addition to exploring their combination merit. MATERIALS AND METHODS: Adult male Wistar rats were divided into 5 groups; viz., normal diet (ND), high fat fructose diet (HFFD), HFFD+GC (600mg/kg), HFFD+RK (55mg/kg) and HFFD+GC+RK. To assess our aim, we determined their effect on body weight, IPGTT, glucose homeostasis (glucose, insulin, HOMA IR), lipid profile parameters and SREBP-1c, oxidative stress markers, insulin and leptin signaling pathways (p-IRS-1/p-AKT/GLUT-4, and leptin/STAT-3), as well as liver and adipose tissue histopathology. RESULTS: GC/RK combination caused weight loss, corrected the disturbed glucose and insulin homeostasis, raised serum levels of HDL and decreased all other lipid profile parameters. They also increased Nrf-2 expression, ad GSH, as well as p-IRS-1/p-Akt/GLUT-4 cue, while they decreased MDA, leptin/STAT-3 and SREBP-1c content compared to the HFFD group. Furthermore, the GC/RK combination abolished apoptosis, fatty changes and inflammation in hepatocytes and decreased sclerotic blood vessels and congestion in adipose tissue. CONCLUSION: Our study highlights the involvement ofp-IRS-1/p-Akt/GLUT-4, leptin/STAT-3 and SREBP-1c signaling trajectories in the beneficial combination of GC and RK, besides, the efficient rebalance of the redox status, insulin resistance and tissue fat deposition confirmed histopathologically." As taken from Attia RT et al. 2019. Biomed. Pharmacother. 110, 500-509. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30530230

"The current investigation was accomplished to evaluate the hepatoprotective effect of White tea and Raspberry Ketone against toxicity induced by acrylamide in rats. Sixty adult male rats were divided randomly into group (I) control; group (II) rats received RK with dose (6 mg/kg/day); Group III: rats received 5 ml of WT extract/kg/day; Group IV rats received AA (5 mg/kg/day); Group V: rats administrated with both AA (5 mg/kg/day) and RK (6 mg/kg/day) and Group VI: rats administrated

AA (5 mg/kg/day) and 5 ml of WT extract/kg/day. The biochemical assays exhibited a significant increase in serum levels of Adiponectin, AST, ALT, ALP of the group treated with acrylamide if compared to the control group and an improvement in their levels of groups V and VI. The histopathological and immunohistochemical findings confirm the biochemical observations. In conclusion, the present investigation proved that the supplementation of WT and RK enhanced the liver histology, immunohistochemistry and biochemistry against the oxidative stress induced by acrylamide." As taken from Hamdy SM et al. 2020. Drug Chem. Toxicol. Epub ahead of print. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32482111/

"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." As taken from Marescotti D et al. 2020. Toxicol Rep 7, 67-80. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31886136/

5. Toxicity

5.1. Single dose toxicity

Type of Test	**Route of Exposure or Administration**	**Species/Test System**	**Dose Data**	**Toxic Effects**	**Reference**
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	1320 mg/kg	Behavioral - altered sleep time (including change in righting reflex) Behavioral - analgesia	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 8,349,1970
LD50 - Lethal dose, 50 percent	Intraperitoneal	Rodent - rat	350 mg/kg	Behavioral - altered sleep time (including change in righting reflex)	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year:

kill				Behavioral - analgesia	8,349,1970

As taken from RTECS, 2018.

Oral LD_{50} – Acute LD_{50} in rats 1.3 – 1.4g/kg orally (Gaunt et al 1970).

LD50 rat ip 0.7 g/kg for males and 0.35 g/kg for females /p-hydroxyphenylbutanone dissolved in propylene glycol/ [Fragrance Raw Materials Monographs; Food and Cosmetics Toxicology 16 (Supplement 1): 781-2 (1978)] **PEER REVIEWED**

LD50 rabbit acute oral and acute dermal 5 g/kg /p-hydroxyphenylbutanone dissolved in propylene glycol/ [Fragrance Raw Materials Monographs; Food and Cosmetics Toxicology 16 (Supplement 1): 781-2 (1978)] **PEER REVIEWED**

LD50 rat oral 1.32 g/kg for males and 1.40 g/kg for females /p-hydroxyphenylbutanone dissolved in propylene glycol/ [Fragrance Raw Materials Monographs; Food and Cosmetics Toxicology 16 (Supplement 1): 781-2 (1978)] **PEER REVIEWED**

As taken from HSDB, 2014.

“Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is marketed on the Internet as a food supplement. The recommended intake is between 100 and 1400 mg per day. The substance is naturally occurring in raspberries (up to 4.3 mg/kg) and is used as a flavouring substance. Toxicological studies on raspberry ketone are limited to acute and subchronic studies in rats. When the lowest recommended daily dose of raspberry ketone (100 mg) as a food supplement is consumed, it is 56 times the established threshold of toxicological concern (TTC) of 1800 µg/day for Class 1 substances. The margin of safety (MOS) based on a NOAEL of 280 mg/kg bw/day for lower weight gain in rats is 165 at 100 mg and 12 at 1400 mg. The recommended doses are a concern taking into account the TTC and MOS. Investigations of raspberry ketone in quantitative structure-activity relationship (QSAR) models indicated potential cardiotoxic effects and potential effects on reproduction/development. Taking into account the high intake via supplements, the compound's toxic potential should be clarified with further experimental studies. In UK the pure compound is regarded as novel food requiring authorisation prior to marketing but raspberry ketone is not withdrawn from Internet sites from this country.” As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596

“A change in homeostasis between food intake and energy expenditure is the hallmark of obesity. Many plant-based weight-management products are available in dietary supplement markets with no direct efficacy comparison. In this in vivo acute feed intake study in rats, the appetite suppression efficacy of well-known natural ingredients in the weight-loss market were evaluated. We tested pure caffeine, potato skin extract, Cissus quadrangularis extract, Garcinia cambogia extract, Crocus sativus extract, raspberry ketone isolated from Rubus idaeus, one commercial product (Appetrex), and one novel composition (UP601). Rats treated with potato skin extract, Crocus sativus bulb extract, and Cissus quadrangularis extracts showed statistically significant reduction in food consumption only at the 2-hour timepoint with 44.9%, 34.1%, and 44.3% reductions, respectively, after food provision at an equivalent human dosage of 2 g, 10 g, and 10 g, respectively. Garcinia cambogia fruit extract and raspberry ketone from Rubus idaeus showed statistically significant reduction in food consumption only at the 1-hour timepoint with 33.7% and 79.4% reductions, respectively, after food provision at an equivalent human dosage of 8 g and 5 g, respectively. UP601 and Appetrex were compared at 230 mg/kg. While 88.5%, 73.8%, and 63.1% reductions in food intake were observed for the UP601 treatment group, 64.2%, 27.5%, and 34.7% reductions in food intake were observed for rats treated with Appetrex at 1 h, 2 h, and 4 h after food provision. The composition UP601 demonstrated superior activity in food intake compared to any of

the dietary supplements marketed for appetite suppression tested in this study." As taken from Yimam M et al. 2019. J. Diet. Suppl. 16(1), 86-104. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29443598

"Raspberry ketone (RK; [4-(4-hydroxyphenyl)-2-butanone]) is used by the food and cosmetic industry as a flavoring agent. RK is also marketed as a dietary supplement for weight maintenance and appetite control. The purpose of the study was to characterize the acute feeding suppression with RK (64-640 mg/kg) by oral gavage in male and female C57BL/6J mice. Cumulative 24 h food intake was reduced at 200 mg/kg (24% feeding suppression) in males and reliably reduced at 640 mg/kg (49-77% feeding suppression). Feeding suppression was not associated with pica behavior over the range of doses or conditioned taste aversion. In a separate experiment, a single oral gavage of RK (640 mg/kg) resulted in approximate 43% mortality rate (6 out 14 male mice) within 2 days. Atrophy of white adipose tissue, splenic abnormalities, and thymus involution were noted after 2-4 days after oral gavage RK. Total white blood cell count, lymphocytes, monocytes, eosinophils were significantly lower, while mean red blood cells, hemoglobin, and hematocrit were significantly higher with RK treatment. Our findings indicated a dose-dependent feeding suppression with acute RK, but doses that reliable suppress food intake are associated with pathological changes." As taken from Hao L et al. 2020. Food Chem. Toxicol. 143, 111512. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32565406/

5.2. Repeated dose toxicity

A no-observed-effect level of 280 mg/kg bw/day was reported in a 13-wk oral rat study (JECFA, 2001).

"Numerous natural products are marketed and sold claiming to decrease body weight and fat, but few undergo finished product-specific research demonstrating their safety and efficacy. To determine the safety and efficacy of a multi-ingredient supplement containing primarily raspberry ketone, caffeine, capsaicin, garlic, ginger and Citrus aurantium (Prograde Metabolism [METABO]) as an adjunct to an eight-week weight loss program. Using a randomized, placebo-controlled, double-blind design, 70 obese but otherwise healthy subjects were randomly assigned to METABO or a placebo and underwent 8?weeks of daily supplementation, a calorie restricted diet, and exercise training. Subjects were tested for changes in body composition, serum adipocytokines (adiponectin, resistin, leptin, TNF-a, IL-6) and markers of health including heart rate and blood pressure. Of the 45 subjects who completed the study, significant differences were observed in: body weight (METABO -2.0% vs. placebo -0.5%, P<0.01), fat mass (METABO -7.8 vs. placebo -2.8%, P<0.001), lean mass (METABO +3.4% vs. placebo +0.8%, P<0.03), waist girth (METABO -2.0% vs. placebo -0.2%, P<0.0007), hip girth (METABO -1.7% vs. placebo -0.4%, P<0.003), and energy levels per anchored visual analogue scale (VAS) (METABO +29.3% vs. placebo +5.1%, P<0.04). During the first 4 weeks, effects/trends for maintaining elevated serum leptin (P<0.03) and decreased serum resistin (P<0.08) in the METABO group vs. placebo were also observed. No changes in systemic hemodynamics, clinical blood chemistries, adverse events, or dietary intake were noted between groups. METABO administration is a safe and effective adjunct to an eight-week diet and exercise weight loss program by augmenting improvements in body composition, waist and hip girth. Adherence to the eight-week weight loss program also led to beneficial changes in body fat in placebo. Ongoing studies to confirm these results and clarify the mechanisms (i.e., biochemical and neuroendocrine mediators) by which METABO exerts the observed salutary effects are being conducted. /Multi-ingredient supplement containing raspberry ketone/ [Lopez HL et al; J Int Soc Sports Nutr 10 (1): 22 (2013)] **PEER REVIEWED**"

"LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Groups of 15 male and 15 female SPF-derived CFE rats were given diet; containing 4-(para-hydroxyphenyl)-2-butanone (No. 728) at a concentration of 0, 0.1, 0.2, 0.4, or 1.0% for 13 weeks. The average intakes, calculated on the basis of data on body weights and food consumption, were 0, 70, 140, 280, and 700 mg/kg bw per day. No changes in appearance, behaviour, or food intake were noted at any time during the study.

A slight but statistically significant (P<0.05) reduction in weight gain was observed in male rats at the highest dose from week 5 onwards, but the decrease in weight was not associated with decreased food consumption. No significant differences were found in absolute organ weights. In males, the relative weights of the liver and kidney were increased at the two higher doses and those of the adrenal gland at the highest dose. The increases in relative organ weights may have been due to the decrease in body weight. Urinary analyses showed ketones in the urine of all treated animals at 7 and 13 weeks. The authors reported that this effect appeared within 12 hr in rats fed a diet containing 1% 4-(para-hydroxyphenyl)-2-butanone for-7 days, and disappeared within 9 hr when the rats were returned to normal diet. The ketonuria, which was possibly due to metabolites present in the urine, was considered not to be a toxic effect. Histopathological examination of rats at the highest dose showed no effect of treatment on organs of the digestive, reproductive, circulatory, or central nervous systems. The NOEL of 280 mg/kg bw per day is 10 000 times greater than the estimated daily per capita intake of 4-(para-hydroxyphenyl)-2-butanone in Europe (46 ug/kg bw per day) and the USA (63 ug/kg bw per day) from use as a flavouring substance. [WHO Food Additives Series 46. Available from, as of January 28, 2014 http://www.inchem.org/documents/jecfa/jecmono/v46je09.htm**PEER REVIEWED**"

"/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one; RK) is a major aromatic compound of red raspberry (Rubus idaeus). The structure of RK is similar to the structures of capsaicin and synephrine, compounds known to exert anti-obese actions and alter the lipid metabolism. The present study was performed to clarify whether RK helps prevent obesity and activate lipid metabolism in rodents. To test the effect on obesity, our group designed the following in vivo experiments: 1) mice were fed a high-fat diet including 0.5, 1, or 2% of RK for 10 weeks; 2) mice were given a high-fat diet for 6 weeks and subsequently fed the same high-fat diet containing 1% RK for the next 5 weeks. RK prevented the high-fat-diet-induced elevations in body weight and the weights of the liver and visceral adipose tissues (epididymal, retroperitoneal, and mesenteric). RK also decreased these weights and hepatic triacylglycerol content after they had been increased by a high-fat diet. RK significantly increased norepinephrine-induced lipolysis associated with the translocation of hormone-sensitive lipase from the cytosol to lipid droplets in rat epididymal fat cells. In conclusion, RK prevents and improves obesity and fatty liver. These effects appear to stem from the action of RK in altering the lipid metabolism, or more specifically, in increasing norepinephrine-induced lipolysis in white adipocytes.. [Morimoto C et al; Life Sci 77 (2): 194-204 (2005)] **PEER REVIEWED**"

"/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Rats fed diets containing 0.1, 0.2, 0.4 or 1.04; p-hydroxyphenylbutanone for 13 wk showed no significant differences in weight gain in either sex at or below the 0.4%, dietary level, or in females on the 1% diet. There was a slight reduction in body-weight gain in males at the 1% level. Food consumption was unaffected in all groups. There was a transient depression of hemoglobin at the 1% level, but at wk 13 all hematological values, including the hematocrit and red and white blood cell counts, were the same in the test groups as in the controls. Apart from ketonuria, which was not considered to be a sign of toxicity, no effects were found in rats on the diets containing 0.1 or 0.2% p-hydroxyphenylbutanone. The no-effect-level was therefore 0.2% of the diet, or about 100mg/kg/day. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**"

As taken from HSDB, 2014.

"Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is marketed on the Internet as a food supplement. The recommended intake is between 100 and 1400 mg per day. The substance is naturally occurring in raspberries (up to 4.3 mg/kg) and is used as a flavouring substance. Toxicological studies on raspberry ketone are limited to acute and subchronic studies in rats. When the lowest recommended daily dose of raspberry ketone (100 mg) as a food supplement is consumed, it is 56 times the established threshold of toxicological concern (TTC) of 1800 µg/day for Class 1 substances. The margin of safety (MOS) based on a NOAEL of 280 mg/kg bw/day for lower weight gain in rats is 165 at 100 mg and 12 at 1400 mg. The recommended doses are a

concern taking into account the TTC and MOS. Investigations of raspberry ketone in quantitative structure-activity relationship (QSAR) models indicated potential cardiotoxic effects and potential effects on reproduction/development. Taking into account the high intake via supplements, the compound's toxic potential should be clarified with further experimental studies. In UK the pure compound is regarded as novel food requiring authorisation prior to marketing but raspberry ketone is not withdrawn from Internet sites from this country." As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596

"A NOAEL of 128 mg/kg bw per day was identified in a 13-week study in rats (15 males/15 females each group), in which 4-(4-hydroxyphenyl) butan-2-one [07.055] was administered at dietary doses of 0%, 0.1%, 0.2% , 0.4% and 1.0% (corresponding to 0, 64, 128, 256 and 640 mg/kg bw per day, respectively). Increased relative liver and kidney weights in males were observed in the two higher doses, but these increases were not accompanied by histopathological changes in the organs. A decrease in the body weight in males was observed with the top dose (Gaunt et al., 1970)."

As taken from EFSA, 2016.

Quantitative Risk Type	Quantitative Risk Value	Product Use	Safety Evaluation Owner	POD Method	POD Value	POD Owner
Not calculated	Not calculated	Not specified	COSMOS TTC (NON-CANCER)	NOAEL	42.7	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	Original NOEL	Original LEL	Critical Sites	Critical Effects
COSMOS TTC (NON-CANCER)	128.0 mg/kg bw/day	240.0 mg/kg bw/day	• LIVER	• LIVER - WEIGHT CHANGES

Safety Evaluation Comments: no comments available.

Source Document: no source document available

POD Method	POD Value	POD Owner
NOEL	33.3	MUNRO

Lowest-observed effect

Owner	Type	Value	Sites	Effects
MUNRO	LOEL	240.0 mg/kg bw/day	• UNSPECIFIED ORGANS - WEIGHT CHANGES	• UNSPECIFIED ORGANS - WEIGHT CHANGES

No-observed effect

MUNRO: NOEL: 100.0 mg/kg bw/day

Adjustment factors

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

>Critical study: Target Organ Toxicity > Subchronic Toxicity (Rat, Oral - Dietary exposure) for 91 day

POD Method	POD Value	POD Owner
NOAEL	Not Calculated	TOXREFDB

Owner	Type	Value	Sites	Effects
TOXREFDB	LOAEL	65.0 mg/kg bw/day	• SYSTEMIC	• SYSTEMIC

TOXREFDB: NOAEL: Not established

POD Method	POD Value	POD Owner
HNEL	Not Calculated	PAFA

Lowest-observed effect

Owner	Type	Value	Sites	Effects
PAFA	LOAEL	100.0 mg/kg bw/day	• KIDNEY - WEIGHT CHANGES • LIVER - WEIGHT CHANGES • URINALYSIS PARAMETERS	• KIDNEY - WEIGHT CHANGES • LIVER - WEIGHT CHANGES • URINALYSIS PARAMETERS

PAFA: HNEL: Not established

>Critical study: Target Organ Toxicity > Subchronic Toxicity (Rat, Oral - Dietary exposure) for 91 day

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

“The animal study used for deriving the NOAEL (No Observed Adverse Effect Level) for the MOS estimation was an unpublished 90-day study in rats from 1970. No chronic/carcinogenic, reproductive or developmental studies with raspberry ketone were identified in the literature. Investigations of raspberry ketone in Quantitative Structure-Activity Relationship (QSAR) models indicated potential cardiotoxic effects and potential effects on reproduction/development that need further elucidation. MOS values for raspberry ketone intake from food supplements varied from 3-165 calculated based on daily raspberry ketone intakes of either 100 or 1400 mg for an adult (70 kg) and NOAELs (70, 100 or 280 mg/kg bw/day derived from two 90-day rat studies). The MOS values for raspberry ketone are below the MOS of ≥200 (200 instead of 100 due to limited toxicological data) that is usually considered sufficient to conclude that there would be no safety concern at the estimated level of exposure.”

As taken from Steffensen I-L, 2019

“Raspberry ketone (RK)-an aromatic compound found mostly in red raspberries (Rubus idaeus) is widely used as an over the counter product for weight loss. The present study was conducted to determine adverse effects associated with RK in obese and health-compromised obese mice. Two sets of experiments were conducted on normal obese and health-compromised obese mice treated with RK for a duration of 10 days. Obese conditions were induced by feeding mice a high fat diet for 10 weeks, while the health compromised obese mouse model was developed by a single intraperitoneal injection of a nontoxic dose of lipopolysaccharide (LPS) (6 mg/kg) to obese mice. Results showed that RK (165, 330, and 500 mg/kg) under obese as well as health-compromised condition retarded the gain in body weights as compared to the control groups. RK at doses 330 and 500 mg/kg resulted in 67.6 and 50% mortality, respectively in normal obese mice and 70% mortality was observed in health-compromised obese mice treated with RK at 500 mg/kg. At higher doses deaths were observed earlier than those given lower doses of RK. Significant elevations in

blood alanine transaminase (ALT) were also observed with RK treatment in obese mice. Blood glucose levels were significantly elevated in all groups of mice treated with RK. This study suggests that higher doses of RK may cause adverse effects in health compromised conditions. Under these conditions, prolonged use of RK, especially in high doses, may pose a health hazard." As taken from Mir TM et al. 2021. J. Diet. Suppl. 18(1), 1-6. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31603036/

There are sufficient repeated dose toxicity data on 4-(p-hydroxyphenyl)-2-butanone that can be used to support the repeated dose toxicity endpoint. An OECD 408 dietary 90-day subchronic toxicity study was conducted in Sprague Dawley rats. Groups of 10 rats/sex/dose received 4-(p-hydroxyphenyl)-2-butanone in the diet at doses of 0, 70, 275, or 700 mg/kg/day. A statistically significant decrease in bodyweight gain was observed in high-dose males with an overall reduction of 19% as compared to the controls. Food consumption was normal in all dose groups except for a slight (but statistically significant) decreases in the mid- and high-dose males during the first 2 weeks of the study only. A statistically significant increase in the relative liver weight (up to 16%) was observed at 275 and 700 mg/kg/day. Statistically significant increases were observed in alanine aminotransferase (ALT, up to 295%), aspartate aminotransferase (AST, 157%), and alkaline phosphatase (ALP, up to 69%) at 700 mg/kg/day (males and females). The increases in ALT and AST also extended to females at 275 mg/kg/day; however, liver histopathology did not show any evidence of liver degeneration or necrosis at any doses. The NOAEL was considered to be 70 mg/kg/day, based on decreased body weight and alterations in the liver (increased serum liver enzymes and liver weights) among animals of the higher dose groups (RIFM, 2004a; WHO, 2011).

In a 13-week subchronic toxicity study, groups of 15 SPF-derived CFE rats/sex/dose were fed diets containing test material, 4-(p-hydroxyphenyl)2-butanone (purity is 96%) at dose levels of 0%, 0.1%, 0.2%, 0.4%, or 1% (equivalent to 0, 50, 100, 200, and 500 mg/kg/day, as per the conversion factors for old rat, available in the JECFA Guidelines for the preparation of toxicological working papers on Food Additives; WHO, 2000) for 13 weeks. A slight but statistically significant decrease (5%) in bodyweight gain was reported in males at the 1% dose level. Since the decrease in body weight was minimal and no changes were reported in females at this dose level or in both sexes at lower dose levels, the decrease in body weight was not biologically significant. In males, the relative liver and kidney weights were increased at 0.4% and 1% (and the relative adrenals weights at 1%), but no correlation in clinical chemistry and histopathology findings were reported; therefore, these changes were not considered to be treatment-related. The NOAEL for repeated dose toxicity was considered to be 1% or 500 mg/kg/day, based on the absence of treatment-related effects up to the highest dose level tested (Gaunt et al., 1970; EFSA, 2016; WHO, 2014; NIH, 2014).As taken from Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2), 110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31756354/

5.3. Reproduction toxicity

"Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is marketed on the Internet as a food supplement. The recommended intake is between 100 and 1400 mg per day. The substance is naturally occurring in raspberries (up to 4.3 mg/kg) and is used as a flavouring substance. ..... Investigations of raspberry ketone in quantitative structure-activity relationship (QSAR) models indicated .... potential effects on reproduction/development. Taking into account the high intake via supplements, the compound's toxic potential should be clarified with further experimental studies. In UK the pure compound is regarded as novel food requiring authorisation prior to marketing but raspberry ketone is not withdrawn from Internet sites from this country." As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596

4-(p-Hydroxyphenyl)-2-butanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity,

skin sensitization, and environmental safety. The developmental and reproductive toxicity and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to 4-(p-hydroxyphenyl)-2-butanone is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively).

An OECD 408 dietary 90-day subchronic toxicity study was conducted in Sprague Dawley rats. Groups of 10 rats/ sex/dose received 4-(p-hydroxyphenyl)-2-butanone in the diet at doses of 0, 70, 275, or 700 mg/kg/day. In addition to systemic toxicity parameters, the reproductive organs were also assessed. Organ weight analysis included testes, ovaries, prostate gland, uterus with cervix, and histopathology examination of the testes, epididymides, ovaries, prostate gland, seminal vesicles, uterus with cervix, and vagina for the control and high-dose group animals. No treatment-related effects were reported in the evaluation of reproductive organs. A NOAEL for reproductive toxicity could not be derived since there were no data on spermatology and estrous cycling of the male and female animals (RIFM, 2004a; WHO, 2011).

As taken from Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2), 110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31756354/

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which 4-(para-hydroxyphenyl)-2-butanone was part of the test mixture include EMT000310 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

4-(p-Hydroxyphenyl)-2-butanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-(p-hydroxyphenyl)-2-butanone is not genotoxic.

4-(p-hydroxyphenyl)-2-butanone was assessed in the BlueScreen assay and found positive for both cytotoxicity (positive: < 80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013a). BlueScreen is a screening assay that assesses genotoxic stress through alterations in gene expressions in a human cell line. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 4-(p-hydroxyphenyl)-2-butanone has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation methods. Salmonella typhimurium strains TA98, TA100, TA102, TA1535, and TA1537 were treated with 4-(p-hydroxyphenyl)-2-butanone in dimethyl sulfoxide (DMSO) at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2003a). Under the conditions of the study, 4-(p-hydroxyphenyl)-2-butanone was not mutagenic in the Ames test.

The clastogenic activity of 4-(p-hydroxyphenyl)-2-butanone was evaluated in an in vivo micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in propylene glycol (PEG 400) via oral gavage to groups of male and female CD1 mice. Doses of 250, 500, or 1000 mg/kg body weight were administered. Mice from each dose level were euthanized at 24 or 48 h, and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2003b). Under the conditions of the study, 4-(p-hydroxyphenyl)-2-butanone was considered to be not clastogenic in the in vivo micronucleus test.

Based on the data available, 4-(p-hydroxyphenyl)-2-butanone does not present a concern for genotoxic potential.

As taken from Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2), 110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31756354/

“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

“Rhododenol or rhododendrol (RD, 4-(4-hydroxyphenyl)-2-butanol) occurs naturally in many plants along with raspberry ketone (RK, 4-(4-hydroxyphenyl)-2-butanone), a ketone derivative, which include Nikko maple tree (Acer nikoense) and white birch (Betula platyphylla). De-pigmenting activity of RD was discovered and it was used as a brightening ingredient for the skin whitening cosmetics. Recently, cosmetics containing RD were withdrawn from the market because a number of consumers developed leukoderma, inflammation and erythema on their face, neck and hands. Here, we explored the mechanism underlying the toxicity of RD and RK against melanocytes using B16F10 murine melanoma cells and human primary epidermal melanocytes. Treatment with RD or RK resulted in the decreased cell viability in a dose-dependent manner which appeared from cell growth arrest. Consistently, ROS generation was significantly increased by RD or RK as determined by DCF-enhanced fluorescence. An antioxidant enzyme, glutathione peroxidase was depleted as well. In line with ROS generation, oxidative damages and the arrest of normal cell proliferation, GADD genes (Growth Arrest and DNA Damage) that include GADD45 and GADD153, were significantly up-regulated. Prevention of ROS generation with an anti-oxidant, N-acetylcysteine (NAC) significantly rescued RD and RK-suppressed melanocyte proliferation. Consistently, up-regulation of GADD45 and GADD153 was significantly attenuated by NAC, suggesting that increased ROS and the resultant growth arrest of melanocytes may contribute to RD and RK-induced leukoderma.” As taken from Kim M et al. 2016. Toxicol. In Vitro 32, 339-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26867644

“Numerous medications are used to treat hyperpigmentation. However, several reports have indicated that repeated application of some agents, such as rhododendrol (RD), raspberry ketone (RK) and monobenzone (MB), can be toxic to melanocytes. Although these agents had severe side effects in human trials, no current in vitro methods can predict the safety of such drugs. This study

assessed the in vitro effects of five depigmentary compounds including leukoderma-inducing agents. In particular, we determined the effects of different concentrations and exposure times of different depigmentary agents on cell viability and melanogenesis in the presence and absence of ultraviolet B (UVB) radiation. Concentrations of RD, RK and MB that inhibit melanogenesis are similar to concentrations that are cytotoxic; however, concentrations of rucinol (RC) and AP736 that inhibit melanogenesis are much lower than concentrations that are cytotoxic. Furthermore, the concentrations that cause toxic effects depend on exposure duration, and prolonged exposure to RD, RK and MB had more cytotoxic effects than prolonged exposure to RC and AP736. The cytotoxic effects of RD and RK appear to be mediated by apoptosis due to increased expression of caspase-3 and caspase-8; UVB radiation increased the cytotoxicity of these agents and also increased caspase activity. Our results indicate that different leukoderma-inducing compounds have different effects on the viability of normal epidermal melanocytes and suggest that the in vitro assay used here can be used to predict whether an investigational compound that induces leukoderma may lead to adverse effects in human trials." As taken from Lee CS et al. 2016. Exp. Dermatol. 25(1), 44-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26440747

"Specific phenol compounds including rhododendrol (RD), a skin-brightening ingredient in cosmetics, are reported to induce leukoderma, inducing a social problem, and the elucidation of mechanism of leukoderma is strongly demanded. This study investigated the relationship among the cytotoxicities of six phenol compounds on B16F10 melanoma cells and HaCaT keratinocytes and generated reactive oxygen species (ROS).....although raspberry ketone (RK), RD derivative, also increased intracellular ROS in B16F10 cells, increase in ROS was suppressed by disodium dihydrogen ethylenediaminetetraacetate dehydrate (EDTA). The amounts of increased ROS with RK in HaCaT cells without melanocyte were further increased by tyrosinase. Therefore, tyrosinase, a metalloprotein having copper, was speculated to be one of causative agents allowing phenol compounds to work as a prooxidant...." As taken from Nagata T et al. 2015. Biomed. Res. Int. 2015, 479798. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25861631

"The exposure of human skin to 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone, RK) is known to cause chemical/occupational leukoderma. RK has a structure closely related to 4-(4-hydroxyphenyl)-2-butanol (rhododendrol), a skin whitening agent that was found to cause leukoderma in the skin of consumers in 2013. Rhododendrol is a good substrate for tyrosinase and causes a tyrosinase-dependent cytotoxicity to melanocytes, cells that are responsible for skin pigmentation. Therefore, it is expected that RK exerts its cytotoxicity to melanocytes through the tyrosinase-catalyzed oxidation to cytotoxic o-quinones. The results of this study demonstrate that the oxidation of RK by mushroom tyrosinase rapidly produces 4-(3-oxobutyl)-1,2-benzoquinone (RK-quinone), which is converted within 10-20 min to (E)-4-(3-oxo-1-butenyl)-1,2-benzoquinone (DBL-quinone). These quinones were identified as their corresponding catechols after reduction by ascorbic acid. RK-quinone and DBL-quinone quantitatively bind to the small thiol N-acetyl-l-cysteine to form thiol adducts and can also bind to the thiol protein bovine serum albumin through its cysteinyl residue. DBL-quinone is more reactive than RK-quinone, as judged by their half-lives (6.2 min vs 10.5 min, respectively), and decays rapidly to form an oligomeric pigment (RK-oligomer). The RK-oligomer can oxidize GSH to GSSG with a concomitant production of hydrogen peroxide, indicating its pro-oxidant activity, similar to that of the RD-oligomer. These results suggest that RK is cytotoxic to melanocytes through the binding of RK-derived quinones to thiol proteins and the pro-oxidant activity of the RK-oligomer." As taken from Ito S et al. 2017. Chem. Res. Toxicol. 30(3), 859-868. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28219012

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor	In vitro	Human - melanoma	25 mg/L/48H	In Vitro Toxicity	TIVIEQ Toxicology In Vitro. (Pergamon Press

Concentration Low				Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc. In Vitro Toxicity Studies - other assays	Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 32,339,2016
ICLo - Inhibitor Concentration Low	In vitro	Human - skin	10 mg/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 32,339,2016
ICLo - Inhibitor Concentration Low	In vitro	Mouse - fibroblast	800 umol/L/24H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	PYTOEY Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1- 1994- Volume(issue)/page/year: 31,11,2017

As taken from RTECS, 2018

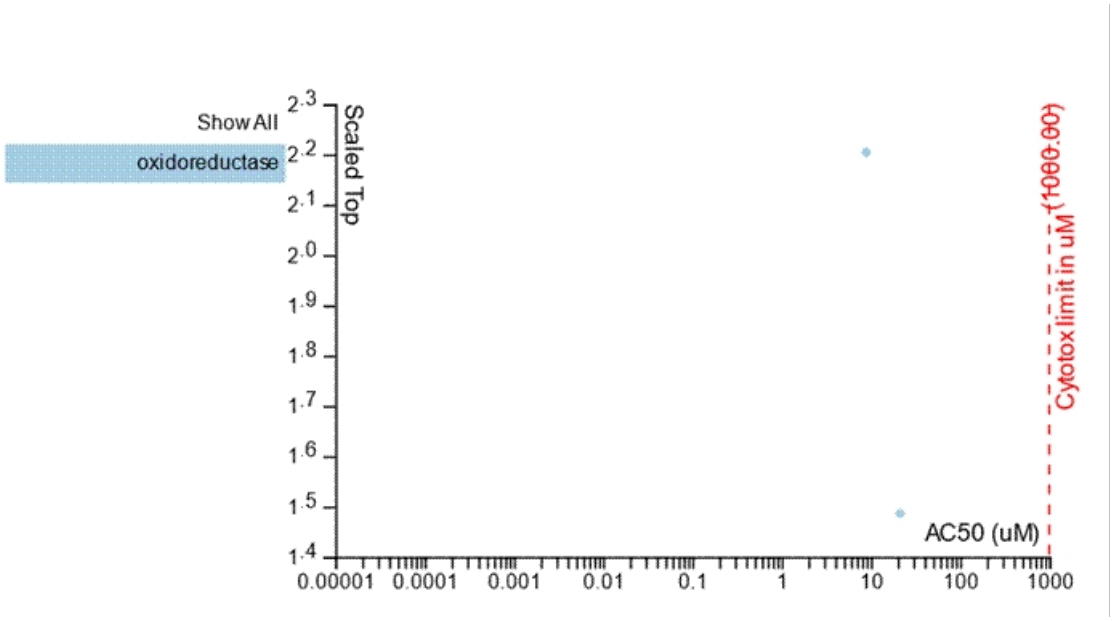
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 4-(4-hydroxyphenyl)butan-2-one 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 4-(4-hydroxyphenyl)butan-2-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



5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

Occupational leukoderma was found to be caused by exposure to depigmentation agents. This is the first report associated with the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone and its crude products. The purpose of this paper is to present three cases of occupational leukoderma in the upper extremities of workers engaged in the manufacturing of 4-(p-hydroxyphenyl)-2-butanone. Two workers had symptoms of dermatitis in the same areas before depigmentation. An epidemiological study, their clinical courses and results of phototesting suggest that these cases of leukoderma were due to exposure to 4-(p-hydroxyphenyl)-2-butanone and its crude products.

FUKUDA Y et al, Occupational leukoderma in workers engaged in 4-(p-hydroxyphenyl)-2-butanone manufacturing; JOURNAL OF OCCUPATIONAL HEALTH; 40 (2). 1998a. 118-122.

The authors previously reported three cases of occupational leukoderma in workers engaged in a 4-(p-hydroxyphenyl)-2-butanone (HPB. Raspberry Ketone) manufacturing process. These cases suggested that HPB might be a chemical causing the leukoderma and it had depigmenting activity. The purpose of this study is to evaluate the depigmenting activity of HPB by using laboratory animals. HPB, its two crude products and monomethyl ether of hydroquinone as the positive control were topically applied to the dorsal surface of C57 black mice. Depigmentation was shown in black mice to which HPB and its crude products were topically applied but the effect produced by these chemicals was weaker than that seen with monomethyl ether of hydroquinone. Though evidence of a reduction in melanocytes and pigmentation was not documented in the microscopic analysis, spectrophotometric assay showed a decrease in melanin content in the hair of mice to which HPB and the crude product had been applied. The results indicated a depigmenting activity of HPB and supported the conclusion that the leukoderma which we reported in a companion paper was induced by exposure to HPB and/or its crude products. Nevertheless, the potential of this depigmenting activity is so weak that the development of leukoderma due to these chemicals may be limited to those who are occupationally exposed.

FUKUDA et al.; An Experimental Study on Depigmenting Activity of 4-(p-Hydroxyphenyl)-2-Butanone in C57 Black Mice; Journal of Occupational Health, Vol.40 , No.2(1998b)pp.97-102;

http://www.journalarchive.jst.go.jp/english/jnlabstract_en.php?cdjournal=joh1996&cdvol=40&noissue=2&startpage=97

“HUMAN EXPOSURE STUDIES/ A maximization test was carried out on 25 volunteers. The material was tested at a concentration of 12% in petrolatum and produced no sensitization reactions. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ ...Tested at 12% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**”

“/LABORATORY ANIMALS: Acute Exposure/ p-Hydroxyphenylbutanone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating. [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**”

/CASE REPORTS/ Occupational leukoderma was found to be caused by exposure to depigmentation agents. This is the first report associated with the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone and its crude products. The purpose of this paper is to present three cases of occupational leukoderma in the upper extremities of workers engaged in the manufacturing of 4-(p-hydroxyphenyl)-2-butanone. Two workers had symptoms of dermatitis in the same areas before depigmentation. An epidemiological study, their clinical courses and results of phototesting suggest that these cases of leukoderma were due to exposure to 4-(p-hydroxyphenyl)-2-butanone and its crude products. [Fukuda Y et al; Journal Of Occupational Health 40 (2): 118-122 (1998)] **PEER REVIEWED**

“/IMMUNOTOXICITY/ Inflammation is part of the host defense mechanism against harmful matters and injury; however, aberrant inflammation is associated to the development of chronic disease such as cancer. Raspberry ketone is a natural phenolic compound. It is used in perfumery, in cosmetics, and as a food additive to impart a fruity odor. In this study, we evaluated whether rheosmin, a phenolic compound isolated from pine needles regulates the expression of iNOS and COX-2 protein in LPS-stimulated RAW264.7 cells. Rheosmin dose-dependently inhibited NO and PGE(2) production and also blocked LPS-induced iNOS and COX-2 expression. Rheosmin potently inhibited the translocation of NF-kappaB p65 into the nucleus by IkappaB degradation following IkappaB-alpha phosphorylation. This result shows that rheosmin inhibits NF-kappaB activation. In conclusion, our results suggest that rheosmin inhibits LPS-induced iNOS and COX-2 expression in RAW264.7 cells by blocking NF-kappaB activation pathway. [Jeong JB, Jeong HJ; Food Chem Toxicol 48 (8-9): 2148-53 (2010)] **PEER REVIEWED**”

As taken from HSDB, 2014.

4-(p-Hydroxyphenyl)-2-butanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from 4-(p-hydroxyphenyl)-2-butanone show that there are no safety concerns for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 4-(p-hydroxyphenyl)-2-butanone is not expected to be phototoxic/photoallergenic.

Based on the existing data, 4-(phydroxyphenyl)-2-butanone does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v4.1). In a murine local lymph node assay (LLNA), 4-(phydroxyphenyl)-2-butanone was not found to be sensitizing up to 50% with a Stimulation Index (SI) of 1.9 (ECHA, 2015; accessed 03/ 27/18). In a human maximization test, no skin sensitization reactions were observed (RIFM, 1974). Based on the weight of evidence (WoE) from structural analysis and animal and human studies, 4-(phydroxyphenyl)-2-butanone does not present a concern for skin sensitization under the current, declared levels of use.

As taken from Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2), 110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31756354/

5.8. All other relevant types of toxicity

Dermatitis and skin depigmentation occurred in three men occupationally exposed to the liquid or crystallised form of 4-(p-hydroxyphenyl)-2-butanone (Fukuda et al. 1998a). A subsequent mouse study involving dermal application of a 5, 10 or 20% solution of the compound (in hydrophilic ointment) confirmed its depigmenting activity (Fukuda et al. 1998b).

"In this study, we evaluated inhibitory potentials of popularly-consumed berries (bilberry, blueberry, cranberry, elderberry, and raspberry ketones) as herbal supplements on UGT1A1, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 in vitro. We also investigated the potential herb-drug interaction via UGT1A1 inhibition by blueberry in vivo. We demonstrated that these berries had only weak inhibitory effects on the five UGTs. Bilberry and elderberry had no apparent inhibitions. Blueberry weakly inhibited UGT1A1 with an IC50 value of 62.4±4.40 μg/mL and a Ki value of 53.1 μg/mL. Blueberry also weakly inhibited UGT2B7 with an IC50 value of 147±11.1 μg/mL. In addition, cranberry weakly inhibited UGT1A9 activity (IC50=458±49.7 μg/mL) and raspberry ketones weakly inhibited UGT2B7 activity (IC50=248±28.2 μg/mL). Among tested berries, blueberry showed the lowest IC50 value in the inhibition of UGT1A1 in vitro. However, the co-administration of blueberry had no effect on the pharmacokinetics of irinotecan and its active metabolite, SN-38, which was mainly eliminated via UGT1A1, in vivo. Our data suggests that these five berries are unlikely to cause clinically significant herb-drug interactions mediated via inhibition of UGT enzymes involved in drug metabolism. These findings should enable an understanding of herb-drug interactions for the safe use of popularly-consumed berries". As taken from Choi EJ et al. 2014. Food Chem. Toxicol. 72, 13-9. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24997313

"/ALTERNATIVE and IN VITRO TESTS/ The effect of essential oils, such as raspberry ketone, on androgen (AR) receptor was investigated using a MDA-kb2 human breast cancer cell line for predicting potential AR activity. Among them, eugenol had the highest AR antagonistic activity with its IC(50) value of 19 uM. Raspberry ketone, which has threefold higher anti-obese activity than that of capsaicin, also had AR antagonist activity with its IC(50) value of 252 uM. Based on these findings, a more precise CoMFA model was proposed as follows: pIC(50) [log (1/IC(50))]=3.77+[CoMFA field terms] (n=39, s=0.249, r(2)=0.834, s(cv)=0.507, q(2)=0.311 (three components)". Oqawa Y et al; Bioorg Med Chem Lett. 20 (7): 2111-4 (2010)] **PEER REVIEWED**

As taken from HSDB, 2014.

"Rhododenol or rhododendrol (RD, 4-(4-hydroxyphenyl)-2-butanol) occurs naturally in many plants along with raspberry ketone (RK, 4-(4-hydroxyphenyl)-2-butanone), a ketone derivative, which include Nikko maple tree (Acer nikoense) and white birch (Betula platyphylla). De-pigmenting activity of RD was discovered and it was used as a brightening ingredient for the skin whitening cosmetics. Recently, cosmetics containing RD were withdrawn from the market because a number of consumers developed leukoderma, inflammation and erythema on their face, neck and hands. Here, we explored the mechanism underlying the toxicity of RD and RK against melanocytes using B16F10 murine melanoma cells and human primary epidermal melanocytes. Treatment with RD or RK resulted in the decreased cell viability in a dose-dependent manner which appeared from cell growth arrest. Consistently, ROS generation was significantly increased by RD or RK as determined by DCF-enhanced fluorescence. An antioxidant enzyme, glutathione peroxidase was depleted as well. In line with ROS generation, oxidative damages and the arrest of normal cell proliferation, GADD genes (Growth Arrest and DNA Damage) that include GADD45 and GADD153, were significantly up-regulated. Prevention of ROS generation with an anti-oxidant, N-acetylcysteine (NAC) significantly rescued RD and RK-suppressed melanocyte proliferation.

Consistently, up-regulation of GADD45 and GADD153 was significantly attenuated by NAC, suggesting that increased ROS and the resultant growth arrest of melanocytes may contribute to RD and RK-induced leukoderma." As taken from Kim M et al. 2016. Toxicol. In Vitro 32, 339-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26867644

"Numerous medications are used to treat hyperpigmentation. However, several reports have indicated that repeated application of some agents, such as rhododendrol (RD), raspberry ketone (RK) and monobenzone (MB), can be toxic to melanocytes. Although these agents had severe side effects in human trials, no current in vitro methods can predict the safety of such drugs. This study assessed the in vitro effects of five depigmentary compounds including leukoderma-inducing agents. In particular, we determined the effects of different concentrations and exposure times of different depigmentary agents on cell viability and melanogenesis in the presence and absence of ultraviolet B (UVB) radiation. Concentrations of RD, RK and MB that inhibit melanogenesis are similar to concentrations that are cytotoxic; however, concentrations of rucinol (RC) and AP736 that inhibit melanogenesis are much lower than concentrations that are cytotoxic. Furthermore, the concentrations that cause toxic effects depend on exposure duration, and prolonged exposure to RD, RK and MB had more cytotoxic effects than prolonged exposure to RC and AP736. The cytotoxic effects of RD and RK appear to be mediated by apoptosis due to increased expression of caspase-3 and caspase-8; UVB radiation increased the cytotoxicity of these agents and also increased caspase activity. Our results indicate that different leukoderma-inducing compounds have different effects on the viability of normal epidermal melanocytes and suggest that the in vitro assay used here can be used to predict whether an investigational compound that induces leukoderma may lead to adverse effects in human trials." As taken from Lee CS et al. 2016. Exp. Dermatol. 25(1), 44-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26440747

"Specific phenol compounds including rhododendrol (RD), a skin-brightening ingredient in cosmetics, are reported to induce leukoderma, inducing a social problem, and the elucidation of mechanism of leukoderma is strongly demanded. This study investigated the relationship among the cytotoxicities of six phenol compounds on B16F10 melanoma cells and HaCaT keratinocytes and generated reactive oxygen species (ROS).....although raspberry ketone (RK), RD derivative, also increased intracellular ROS in B16F10 cells, increase in ROS was suppressed by disodium dihydrogen ethylenediaminetetraacetate dehydrate (EDTA). The amounts of increased ROS with RK in HaCaT cells without melanocyte were further increased by tyrosinase. Therefore, tyrosinase, a metalloprotein having copper, was speculated to be one of causative agents allowing phenol compounds to work as a prooxidant...." As taken from Nagata T et al. 2015. Biomed. Res. Int. 2015, 479798. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25861631

"The exposure of human skin to 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone, RK) is known to cause chemical/occupational leukoderma. RK has a structure closely related to 4-(4-hydroxyphenyl)-2-butanol (rhododendrol), a skin whitening agent that was found to cause leukoderma in the skin of consumers in 2013. Rhododendrol is a good substrate for tyrosinase and causes a tyrosinase-dependent cytotoxicity to melanocytes, cells that are responsible for skin pigmentation. Therefore, it is expected that RK exerts its cytotoxicity to melanocytes through the tyrosinase-catalyzed oxidation to cytotoxic o-quinones. The results of this study demonstrate that the oxidation of RK by mushroom tyrosinase rapidly produces 4-(3-oxobutyl)-1,2-benzoquinone (RK-quinone), which is converted within 10-20 min to (E)-4-(3-oxo-1-butenyl)-1,2-benzoquinone (DBL-quinone). These quinones were identified as their corresponding catechols after reduction by ascorbic acid. RK-quinone and DBL-quinone quantitatively bind to the small thiol N-acetyl-l-cysteine to form thiol adducts and can also bind to the thiol protein bovine serum albumin through its cysteinyl residue. DBL-quinone is more reactive than RK-quinone, as judged by their half-lives (6.2 min vs 10.5 min, respectively), and decays rapidly to form an oligomeric pigment (RK-oligomer). The RK-oligomer can oxidize GSH to GSSG with a concomitant production of hydrogen peroxide, indicating its pro-oxidant activity, similar to that of the RD-oligomer. These results suggest that RK is cytotoxic to melanocytes through the binding of RK-derived quinones to thiol proteins and the pro-oxidant activity of the RK-oligomer." As taken from Ito S et al. 2017. Chem. Res.

Toxicol. 30(3), 859-868. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28219012

“A change in homeostasis between food intake and energy expenditure is the hallmark of obesity. Many plant-based weight-management products are available in dietary supplement markets with no direct efficacy comparison. In this in vivo acute feed intake study in rats, the appetite suppression efficacy of well-known natural ingredients in the weight-loss market were evaluated. We tested pure caffeine, potato skin extract, Cissus quadrangularis extract, Garcinia cambogia extract, Crocus sativus extract, raspberry ketone isolated from Rubus idaeus, one commercial product (Appetrex), and one novel composition (UP601). Rats treated with potato skin extract, Crocus sativus bulb extract, and Cissus quadrangularis extracts showed statistically significant reduction in food consumption only at the 2-hour timepoint with 44.9%, 34.1%, and 44.3% reductions, respectively, after food provision at an equivalent human dosage of 2 g, 10 g, and 10 g, respectively. Garcinia cambogia fruit extract and raspberry ketone from Rubus idaeus showed statistically significant reduction in food consumption only at the 1-hour timepoint with 33.7% and 79.4% reductions, respectively, after food provision at an equivalent human dosage of 8 g and 5 g, respectively. UP601 and Appetrex were compared at 230 mg/kg. While 88.5%, 73.8%, and 63.1% reductions in food intake were observed for the UP601 treatment group, 64.2%, 27.5%, and 34.7% reductions in food intake were observed for rats treated with Appetrex at 1 h, 2 h, and 4 h after food provision. The composition UP601 demonstrated superior activity in food intake compared to any of the dietary supplements marketed for appetite suppression tested in this study.” As taken from Yimam M et al. 2019. J. Diet. Suppl. 16(1), 86-104. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29443598

“As the incidence of obesity continues to increase, identifying novel nutritional therapies to enhance weight loss are needed. Raspberry ketone (RK; 4-(4-hydroxyphenyl) butan-2-one) is a bioactive phytochemical that is marketed as a weight loss supplement in the United States, yet there is scant scientific evidence demonstrating that RK promotes weight loss. The aim of the current study was to investigate the effect of RK on accumulation of adipose mass, hepatic lipid storage, and levels of plasma adiponectin in mice fed a high-fat (HF) diet. Mice were individually housed and fed a HF control diet (45% kcal from fat) for two weeks to induce weight gain, then assigned to HF control, high-dose (1.74% wt/wt) raspberry ketone (HRK), low-dose (0.25% wt/wt) raspberry ketone (LRK), or a pair-fed group (PF) fed similar food intake to LRK mice. Following five weeks of feeding, mice fed LRK and HRK diets showed reduced food intake and body weight compared to mice maintained on control diet. When normalized to body weight, mice fed HRK diet exhibited decreased inguinal fat mass and increased liver mass compared to the control group. Hepatic steatosis was lowest in mice fed HRK diet, whereas LRK diet did not have an effect when compared to the PF group. Plasma adiponectin concentration was unaffected by RK and pair-feeding. Our findings demonstrate that RK supplementation has limited benefit to adipose loss beyond reducing energy intake in mice fed a high-fat diet. The present study supports the need for appropriate study design when validating weight-loss supplements.” As taken from Cotten BM et al. 2017. Food Funct. 8(4), 1512-1518. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28378858

“Inhibition of α-glucosidase is directly associated with treatment of type 2 diabetes. In this regard, we conducted enzyme kinetics integrated with computational docking simulation to assess the inhibitory effect of raspberry ketone (RK) on α-glucosidase. RK bound to the active site of α-glucosidase and interacted with several key residues such as ASP68, TYR71, HIS111, PHE157, PHE158, PHE177, GLN181, ASP214, THR215, ASP349, ASP408, and ARG439, as detected by protein-ligand docking simulation. Subsequently, we confirmed the action of RK on α-glucosidase as the non-competitive type of inhibition in a reversible and rapidly binding manner. The relevant kinetic parameters were IC50=6.17±0.46mM and Ki=7.939±0.211mM. Regarding the structure-activity relationship, the higher concentration of RK induced slight modulation of the shape of the active site as monitored by hydrophobic exposure. The tertiary conformational change was linked to RK inhibition, and mostly involved regional changes of the active site. Our study provides insight

into the functional role of RK due to its structural property of a hydroxyphenyl ring that interacts with the active site of α-glucosidase. We suggest that similar hydroxyphenyl ring compounds targeting the key residues of the active site might be potential α-glucosidase inhibitors." As taken from Xiong SL et al. 2018. Int. J. Biol. Macromol. 113, 212-218. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29477543

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human - melanoma	100 mg/L/48H	In Vitro Toxicity Studies - cell morphology: overgrowth of cell appendixes etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 32,339,2016
ICLo - Inhibitor Concentration Low	In vitro	Human - skin	20 mg/L/48H	In Vitro Toxicity Studies - cell morphology: overgrowth of cell appendixes etc. In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 32,339,2016
ICLo - Inhibitor Concentration Low	In vitro	Mouse - fibroblast	150 umol/L/9D	In Vitro Toxicity Studies - cell differentiation	PYTOEY Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1- 1994- Volume(issue)/page/year: 31,11,2017

As taken from RTECS, 2018

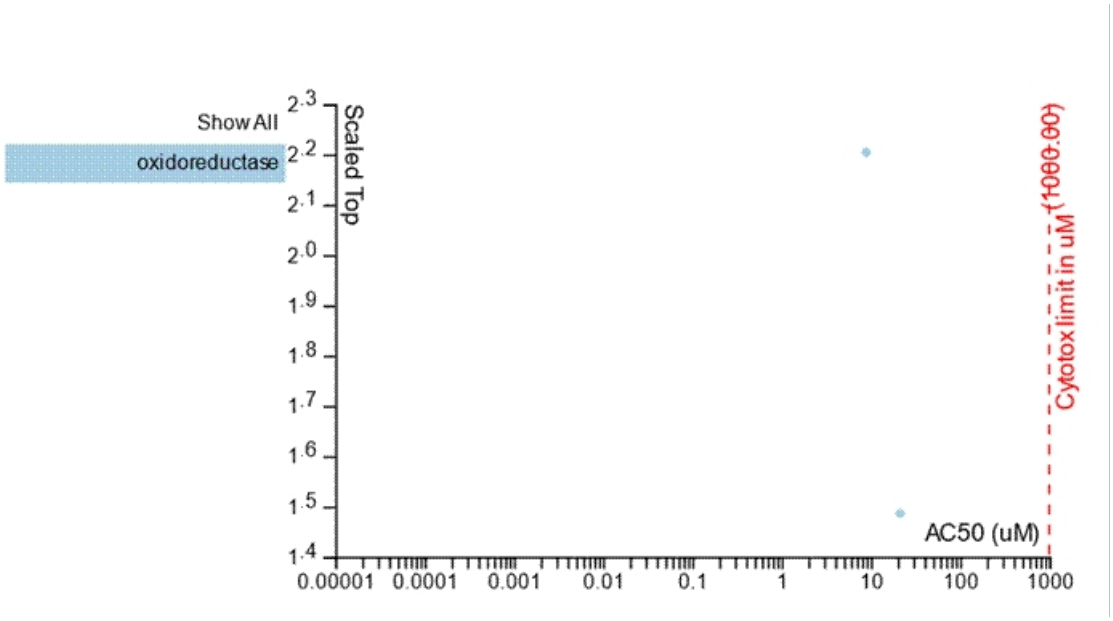
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 4-(4-hydroxyphenyl)butan-2-one 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 4-(4-hydroxyphenyl)butan-2-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



“Red raspberries (Rubus idaeus) contain numerous phenolic compounds with purported health benefits. Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is a primary raspberry flavor phenolic found in raspberries and is designated as a synthetic flavoring agent by the Food and Drug Administration. Synthetic raspberry ketone has been demonstrated to result in weight loss in rodents. We tested whether phenolic-enriched raspberry extracts, compared with raspberry ketone, would be more resilient to the metabolic alterations caused by an obesogenic diet. Male C57BL/6J mice (8 weeks old) received a daily oral dose of vehicle (VEH; 50% propylene glycol, 40% water, and 10% dimethyl sulfoxide), raspberry extract low (REL; 0.2 g/kg), raspberry extract high (REH; 2 g/kg), or raspberry ketone (RK; 0.2 g/kg). Coincident with daily dosing, mice were placed on a high-fat diet (45% fat). After 4 weeks, REH and RK reduced body weight gain (approximately 5%-9%) and white adipose mass (approximately 20%) compared with VEH. Hepatic gene expression of heme oxygenase-1 and lipoprotein lipase was upregulated in REH compared with VEH. Indirect calorimetry indicated that respiratory exchange ratio (CO2 production to O2 consumption) was lower, suggesting increased fat oxidation with all treatments. REH treatment increased total ambulatory behavior. Energy expenditure/lean mass was higher in REH compared with REL treatment. There were no treatment differences in cumulative intake, meal patterns, or hypothalamic feed-related gene expression. Our results suggest that raspberry ketone and a phenolic-enriched raspberry extract both have the capacity to prevent weight gain but differ in the preventative mechanisms for excess fat accumulation following high-fat diet exposure.” As taken from Kshatriya D et al. 2019. Nutr. Res. 68, 19-33. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31252376/

6. Functional effects on

6.1. Broncho/pulmonary system

4-(p-Hydroxyphenyl)-2-butanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. The developmental and reproductive toxicity and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to 4-(p-hydroxyphenyl)-2-butanone is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively).

As taken from Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2),

110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31756354/

6.2. Cardiovascular system

To investigate the scientific grounds for the effect of raspberry ketone bathing that is claimed to increase energy consumption by stimulating metabolism, a bathing experiment was conducted in 10 normal healthy adults. As a result, no appreciable difference was detected among tap water, CO2-enriched water and raspberry water in respect to blood pressure, pulse rate and depth thermometer readings, which suggested that bathing in warm raspberry water was safe, producing no marked load on the cardiovascular system. Changes in the skin surface temperature indicated slow elevation of body temperature, from which bathing in warm raspberry water was considered to produce no marked load on the body even if bathing lasted relatively long as compared with bathing in warm tap water or CO2-enriched warm water. From the skin tissue blood flow data, it seemed likely that the increase in blood flow caused by bathing in warm raspberry water was produced, not by vasodilatation as in CO2-enriched warm water bathing, but by such mechanisms as acceleration of metabolism. Data on insulin suggested that bathing in warm raspberry water affected the carbohydrate metabolism as compared with that in warm tap water or CO2-enriched warm water. Since there was no difference among warm water groups in changes in the adrenocortical hormone "cortisol", raspberry ketone bathing was considered not to have specific activity. Data on NK cell activity showed that bathing in warm raspberry water produced no appreciable effect on the immune system. It was suggested that measurement of .BETA.-endorphin should be performed after adjustment of psychological environments.... (author abst.)

MAEDA M et al.; Effect of Raspberry Ketone Bathing on the Skin Blood Flow and Endocrine System; Journal of Japanese Association of Physical Medicine Balneology and Climatology, ISSN:0029-0343, VOL.67;NO.4;PAGE.215-224(2004); https://www.jstage.jst.go.jp/article/onki1962/67/4/67_4_215/_article

“Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) is marketed on the Internet as a food supplement. The recommended intake is between 100 and 1400 mg per day. The substance is naturally occurring in raspberries (up to 4.3 mg/kg) and is used as a flavouring substance. Toxicological studies on raspberry ketone are limited to acute and subchronic studies in rats. When the lowest recommended daily dose of raspberry ketone (100 mg) as a food supplement is consumed, it is 56 times the established threshold of toxicological concern (TTC) of 1800 µg/day for Class 1 substances. The margin of safety (MOS) based on a NOAEL of 280 mg/kg bw/day for lower weight gain in rats is 165 at 100 mg and 12 at 1400 mg. The recommended doses are a concern taking into account the TTC and MOS. Investigations of raspberry ketone in quantitative structure-activity relationship (QSAR) models indicated potential cardiotoxic effects and potential effects on reproduction/development. Taking into account the high intake via supplements, the compound's toxic potential should be clarified with further experimental studies. In UK the pure compound is regarded as novel food requiring authorisation prior to marketing but raspberry ketone is not withdrawn from Internet sites from this country.” As taken from Bredsdorff L et al. 2015. Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596

“AIM: The cardioprotective role of raspberry ketone (RK) against isoproterenol (ISO)-induced myocardial infarction (MI) in rats was assessed. MATERIALS AND METHODS: Rats were randomly divided into Group I - Vehicle control; Group II - Toxic control ISO (85mg/kg, s.c.); Group III, IV and V - RK (50, 100 and 200mg/kg, respectively) with ISO; Group VI- RK (200mg/kg) alone; Group VII - Propranolol (10mg/kg) with ISO; and Group VIII - Propranolol (10mg/kg) alone. After twenty-four hours of the last dose, animals were sacrificed and creatine kinase-MB, lactate dehydrogenase, total cholesterol, triglycerides, high-density-lipoprotein, low-density-lipoprotein, very-low-density-lipoprotein, malondialdehyde, reduced glutathione, superoxide dismutase, catalase, Na^+, K^+-ATPase, nitric oxide, histopathological and immunohistochemical analysis (tumor

necrosis factor-α and inducible nitric oxide synthase) were performed. KEY FINDINGS: Treatment with ISO significantly deviated the biochemical parameters from the normal levels, which were considerably restored by RK at 100 and 200mg/kg doses. 50mg/kg dose, however, did not demonstrate any significant cardioprotective action. The histopathological and immunohistochemical analysis further substantiated these findings. SIGNIFICANCE: Our study showed a dose-dependent reduction in oxidative stress, inflammation and dyslipidemia by RK in ISO-intoxicated rats, which signifies that RK from the European red raspberry plant might be a valuable entity for the management of MI." As taken from Khan V et al. 2018. Life Sci. 194, 205-212. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29225109

"The peroxisome proliferator-activated receptor-α (PPAR-α) controls the lipid and glucose metabolism and also affects inflammation, cell proliferation and apoptosis during cardiovascular disease. Raspberry ketone (RK) is a red raspberry (Rubusidaeus, Family-Rosaceae) plant constituent, which activates PPAR-α. This study was conducted to assess the cardioprotective action of RK against isoproterenol (ISO)-induced cardiotoxicity. Wistar rats were randomly divided into six groups (six rats/group). Rats were orally administered with RK (50, 100 and 200 mg/kg, respectively) and fenofibrate (standard, 80 mg/kg) for 28 days and ISO was administered (85 mg/kg, subcutaneously) on 27th and 28th day. Administration of ISO in rats significantly altered hemodynamic and electrocardiogram patterns, total antioxidant capacity, PPAR-α, and apolipoprotein C-III levels. These myocardial aberrations were further confirmed during infarct size, heart weight to body weight ratio and immunohistochemical assessments (caspase-3 and nuclear factor-κB). RK pretreatment (100 and 200 mg/kg) significantly protected rats against oxidative stress, inflammation, and dyslipidemia caused by ISO as demonstrated by change in hemodynamic, biochemical and histological parameters. The results so obtained were quite comparable with fenofibrate. Moreover, RK was found to have binding affinity with PPAR-α, as confirmed by docking analysis. PPAR-α expression and concentration was also found increased in presence of RK which gave impression that RK probably showed cardioprotection via PPAR-α activation, however direct binding study of RK with PPAR-α is needed to confirm this assumption." As taken from Khan V et al. 2019. Eur. J. Pharmacol. 842, 157-166. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30431010

"Raspberry ketone (RK; [4-(4-hydroxyphenyl)-2-butanone]) is a popular nutraceutical used for weight management and appetite control. We sought to determine the physiological benefits of RK on the meal patterns and cardiovascular changes associated with an obesogenic diet. In addition, we explored whether the physiological benefits of RK promoted anxiety-related behaviors. Male and female C57BL/6J mice were administered a daily oral gavage of RK 200 mg/kg, RK 400 mg/kg, or vehicle for 14 days. Commencing with dosing, mice were placed on a high-fat diet (45% fat) or low-fat diet (10% fat). Our results indicated that RK 200 mg/kg had a differential influence on meal patterns in males and females. In contrast, RK 400 mg/kg reduced body weight gain, open-field total distance travelled, hemodynamic measures (i.e., reduced systolic blood pressure (BP), diastolic BP and mean BP), and increased nocturnal satiety ratios in males and females. In addition, RK 400 mg/kg increased neural activation in the nucleus of the solitary tract, compared with vehicle. RK actions were not influenced by diet, nor resulted in an anxiety-like phenotype. Our findings suggest that RK has dose-differential feeding and cardiovascular actions, which needs consideration as it is used as a nutraceutical for weight control for obesity." As taken from Kshatriya D et al. 2020. Nutrients 12(6), 1754. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32545402/

6.3. Nervous system

"Obesity is a proven risk factor for neurodegenerative disease like Alzheimer's disease (AD). Accumulating evidences suggested that nutritional interventions provide potential for prevention and treatment of AD. The present study aimed to investigate the effect of dietary treatment of obese rats with natural Raspberry ketone (RK) and their relationship with neurodegeneration.

Obesity was first induced in 40 male Wistar rats (140-160 g) by feeding high fat diet (HFD) for 16 weeks. Obese rats were then assigned into 4 groups (n = 10 each). (O-AD) is obese induced AD group maintained on HFD for another 6 weeks. OCR is obese group received calorie restricted diet for 6 weeks. OCRRK is obese group received calorie restricted diet and RK (44 mg/kg body weight, daily, orally) for 6 weeks and OCRD is obese group received calorie restricted diet and orlistate (10 mg/kg body weight, daily orally) for 6 weeks. Another 10 normal rats received normal diet were used as normal control group (NC). Body weight, visceral white adipose tissue weight (WAT), lipid profile, oxidative stress markers, adiponectin, cholinergic activity and amyloid extracellular plaques were examined. In addition to histological changes in brain tissues were evaluated.Raspberry ketone (RK) via its antioxidant properties attenuated oxidative damage and dyslipidemia in O-AD group. It inhibited acetylcholinesterase enzyme (AchE) and hence increased acetylcholine level (Ach) in brain tissues of O-AD rats. It is also impeded the upregulation of beta-secretase-1 (BACE-1) and the accumulation of amyloid beta (Aβ) plaques which crucially involved in AD. The combination of CR diet with RK was more effective than CR diet with orlistate (antiobese drug) in abrogating the neurodegenerative changes induced by obesity. Results from this study suggested that concomitant supplementation of RK with calorie restricted regimen effectively modulate the neurodegenerative changes induced by obesity and delay the progression of AD." As taken from Mohamed HE et al. 2018. Biomed. Pharmacother. 107, 1166-1174. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30257330

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

Sensory neurons release calcitonin gene-related peptide (CGRP) on activation. We recently reported that topical application of capsaicin increases facial skin elasticity and promotes hair growth by increasing dermal insulin-like growth factor-I (IGF-I) production through activation of sensory neurons in mice and humans. Raspberry ketone (RK), a major aromatic compound contained in red raspberries (Rubus idaeus), has a structure similar to that of capsaicin. Thus, it is possible that RK activates sensory neurons, thereby increasing skin elasticity and promoting hair growth by increasing dermal IGF-I production. In the present study, we examined this possibility in mice and humans. RK, at concentrations higher than 1 microM, significantly increased CGRP release from dorsal root ganglion neurons (DRG) isolated from wild-type (WT) mice and this increase was completely reversed by capsazepine, an inhibitor of vanilloid receptor-1 activation. Topical application of 0.01% RK increased dermal IGF-I levels at 30 min after application in WT mice, but not in CGRP-knockout mice. Topical application of 0.01% RK increased immunohistochemical expression of IGF-I at dermal papillae in hair follicles and promoted hair re-growth in WT mice at 4 weeks after the application. When applied topically to the scalp and facial skin, 0.01% RK promoted hair growth in 50.0% of humans with alopecia (n=10) at 5 months after application and increased cheek skin elasticity at 2 weeks after application in 5 females (P<0.04). These observations strongly suggest that RK might increase dermal IGF-I production through sensory neuron activation, thereby promoting hair growth and increasing skin elasticity.

Harada N et al., Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans; Growth Horm IGF Res. 2008, Aug; 18(4):335-44.

The aim of this study is to investigate the enzymatic properties and the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone (HPB) in vitro. The activity of HPB as a substrate of tyrosinase, its effect on tyrosinase enzymatic reactions, and its inhibition of the growth and the melanogenesis of cultured melanoma cells were examined. The HPB-tyrosinase reaction and the effect of HPB on tyrosine-tyrosinase and dopa-tyrosinase reactions were followed spectrophotometrically. Fifty percent growth inhibition concentrations (IC50) of several chemicals for melanoma cells and non-pigmented cells were measured. Melanogenic activities in HPB-treated melanoma cells were assayed. The results showed that HPB was oxidized by tyrosinase and stimulated tyrosine-tyrosinase and dopa-tyrosinase reactions. The IC50 of HPB for melanoma cells was higher than

those of the established depigmenting agents but it was lower than that of HPB for non-melanotic cells. Tyrosine hydroxylase in HPB-t

FUKUDA Y et al., In vitro studies on the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone; JOURNAL OF OCCUPATIONAL HEALTH; 40 (2). 1998c. 137-142.

“The protective effect of raspberry ketone against nonalcoholic steatohepatitis (NASH) was tested by using a high-fat diet-induced NASH model, and its mechanism was explored. Forty Sprague-Dawley rats with a 1:1 male to female ratio were randomly divided into five groups: the normal control (NC) group (n=8) fed normal diet for 8 weeks, the model control (MC) group (n=8) fed high-fat diet (82% standard diet, 8.3% yolk powder, 9.0% lard, 0.5% cholesterol, and 0.2% sodium taurocholate), and the raspberry ketone low-dose (0.5%) (RKL) group (n=8), the raspberry ketone middle-dose (1%) (RKM) group (n=8), and the raspberry ketone high-dose (2%) (RKH) group (n=8) fed high-fat diet for 4 weeks. After 8 weeks of experiment, all the rats were sacrificed, and blood lipid parameters (total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]), liver function parameters (serum alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase [ALP]), leptin (LEP), free fatty acid (FFA), tumor necrosis factor α (TNF-α), blood glucose (GLU), and insulin (INS) with calculated INS resistance index (IRI) and INS-sensitive index (ISI) were measured in rats. Therefore, we determined the peroxisome proliferator-activated receptor (PPAR)-α activity in liver homogenate and the levels of low-density lipoprotein receptor (LDLR), high-sensitivity C-reactive protein (hs-CRP), adiponection (APN), superoxide dismutase, and malondialdehyde (MDA). The liver tissues of rats in each group were imaged by electron microscopy with hematoxylin-eosin as the staining agent. The levels of TG, TC, LDL-C, ALT, AST, ALP, GLU, INS, IRI, FFA, LEP, TNF-α, MDA, and hs-CRP of MC rats were significantly increased (P<.05, P<.01). Therefore, the levels of HDL-C, ISI, PPAR-α, LDLR, and APN were significantly decreased (P<.05, P<.01). Compared with the MC group, each parameter in the RKL, RKM, and RKH groups was significantly improved (P<.05, P<.01). Thus raspberry ketone was an effective intervention for NASH in rats. It was believed that raspberry ketone had a dual effect of liver protection and fat reduction, and the mechanism was probably mediated by alleviation of fatty degeneration of liver cells, decreased liver inflammation, correction of dyslipidemia, reversal of LEP and INS resistance, and improved antioxidant capacity”. As taken from Wang L et al. 2012. J. Med. Food 15, 495-503. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22551412

“Raspberry ketone (RK) is a natural phenolic compound of the red raspberry. The dietary administration of RK to male mice has been reported to prevent high-fat diet-induced elevation in body weight and to increase lipolysis in white adipocytes. To elucidate a possible mechanism for the antiobesity action of RK, its effects on the expression and the secretion of adiponectin, lipolysis, and fatty acid oxidation in 3T3-L1 were investigated. Treatment with 10 μM of RK increased lipolysis significantly in differentiated 3T3-L1 cells. An immunoassay showed that RK increased both the expression and the secretion of adiponectin, an adipocytokine mainly expressed and secreted by adipose tissue. In addition, treatment with 10 μM of RK increased the fatty acid oxidation and suppressed lipid accumulation in 3T3-L1 adipocytes. These findings suggest that RK holds great promise as an herbal medicine since its biological activities alter the lipid metabolism in 3T3-L1 adipocytes”. As taken from Park KS. 2010. Planta Med. 76, 1654-1658. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20425690

“BACKGROUND: Numerous natural products are marketed and sold claiming to decrease body weight and fat, but few undergo finished product-specific research demonstrating their safety and efficacy. OBJECTIVE: To determine the safety and efficacy of a multi-ingredient supplement containing primarily raspberry ketone, caffeine, capsaicin, garlic, ginger and Citrus aurantium (Prograde MetabolismTM [METABO]) as an adjunct to an eight-week weight loss program. METHODS: Using a randomized, placebo-controlled, double-blind design, 70 obese but otherwise healthy subjects were randomly assigned to METABO or a placebo and underwent 8 weeks of daily supplementation, a calorie restricted diet, and exercise training. Subjects were tested for changes

in body composition, serum adipocytokines (adiponectin, resistin, leptin, TNF-alpha, IL-6) and markers of health including heart rate and blood pressure. RESULTS: Of the 45 subjects who completed the study, significant differences were observed in: body weight (METABO -2.0% vs. placebo -0.5%, P<0.01), fat mass (METABO -7.8 vs. placebo -2.8%, P<0.001), lean mass (METABO +3.4% vs. placebo +0.8%, P<0.03), waist girth (METABO -2.0% vs. placebo -0.2%, P<0.0007), hip girth (METABO -1.7% vs. placebo -0.4%, P<0.003), and energy levels per anchored visual analogue scale (VAS) (METABO +29.3% vs. placebo +5.1%, P<0.04). During the first 4 weeks, effects/trends for maintaining elevated serum leptin (P<0.03) and decreased serum resistin (P<0.08) in the METABO group vs. placebo were also observed. No changes in systemic hemodynamics, clinical blood chemistries, adverse events, or dietary intake were noted between groups. CONCLUSIONS: METABO administration is a safe and effective adjunct to an eight-week diet and exercise weight loss program by augmenting improvements in body composition, waist and hip girth. Adherence to the eight-week weight loss program also led to beneficial changes in body fat in placebo. Ongoing studies to confirm these results and clarify the mechanisms (i.e., biochemical and neuroendocrine mediators) by which METABO exerts the observed salutary effects are being conducted". As taken from Lopez HL et al. 2013. J. Int. Soc. Sports Nutr. 10(1), 22. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23601452

"The decrease in the bone mass associated with osteoporosis caused by ovariectomy, aging, and other conditions is accompanied by an increase in bone marrow adipose tissue. The balance between osteoblasts and adipocytes is influenced by a reciprocal relationship. The development of modalities to promote local/systemic bone formation by inhibiting bone marrow adipose tissue is important in the treatment of fractures or metabolic bone diseases such as osteoporosis. In this study, we examined whether raspberry ketone [4-(4-hydroxyphenyl)butan-2-one; RK], which is one of the major aromatic compounds of red raspberry and exhibits anti-obesity action, could promote osteoblast differentiation in C3H10T1/2 stem cells. Confluent C3H10T1/2 stem cells were treated for 6 days with 10-100 μg/mL of RK in culture medium containing 10 nM all-trans-retinoic acid (ATRA) or 300 ng/mL recombinant human bone morphogenetic protein (rhBMP)-2 protein as an osteoblast-differentiating agent. RK in the presence of ATRA increased alkaline phosphatase (ALP) activity in a dose-dependent manner. RK in the presence of rhBMP-2 also increased ALP activity. RK in the presence of ATRA also increased the levels of mRNAs of osteocalcin, α1(I) collagen, and TGF-βs (TGF-β1, TGF-β2, and TGF-β3) compared with ATRA only. RK promoted the differentiation of C3H10T1/2 stem cells into osteoblasts. However, RK did not affect the inhibition of early-stage adipocyte differentiation. Our results suggest that RK enhances the differentiation of C3H10T1/2 stem cells into osteoblasts, and it may promote bone formation by an action unrelated to adipocyte differentiation". As taken from Takata T and Morimoto C. 2014. J. Med. Food. 17(3), 332-8. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24404978

"/ENDOCRINE MODULATION/ Sensory neurons release calcitonin gene-related peptide (CGRP) on activation. We recently reported that topical application of capsaicin increases facial skin elasticity and promotes hair growth by increasing dermal insulin-like growth factor-I (IGF-I) production through activation of sensory neurons in ... humans. Raspberry ketone (RK), a major aromatic compound contained in red raspberries (Rubus idaeus), has a structure similar to that of capsaicin. Thus, it is possible that RK activates sensory neurons, thereby increasing skin elasticity and promoting hair growth by increasing dermal IGF-I production.... When applied topically to the scalp and facial skin, 0.01% RK promoted hair growth in 50.0% of humans with alopecia (n=10) at 5 months after application and increased cheek skin elasticity at 2 weeks after application in 5 females (P<0.04). These observations strongly suggest that RK might increase dermal IGF-I production through sensory neuron activation, thereby promoting hair growth and increasing skin elasticity. [Harada N et al; Growth Horm IGF Res. 18 (4): 335-44 (2008)] **PEER REVIEWED**"

"/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ The protective effect of raspberry ketone against nonalcoholic steatohepatitis (NASH) was tested by using a high-fat diet-induced NASH model, and its mechanism was explored. Forty Sprague-Dawley rats with a 1:1 male to

female ratio were randomly divided into five groups: the normal control (NC) group (n=8) fed normal diet for 8 weeks, the model control (MC) group (n=8) fed high-fat diet (82% standard diet, 8.3% yolk powder, 9.0% lard, 0.5% cholesterol, and 0.2% sodium taurocholate), and the raspberry ketone low-dose (0.5%) (RKL) group (n=8), the raspberry ketone middle-dose (1%) (RKM) group (n=8), and the raspberry ketone high-dose (2%) (RKH) group (n=8) fed high-fat diet for 4 weeks. After 8 weeks of experiment, all the rats were sacrificed, and blood lipid parameters (total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]), liver function parameters (serum alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase [ALP]), leptin (LEP), free fatty acid (FFA), tumor necrosis factor alpha (TNF-alpha), blood glucose (GLU), and insulin (INS) with calculated INS resistance index (IRI) and INS-sensitive index (ISI) were measured in rats. Therefore, we determined the peroxisome proliferator-activated receptor (PPAR)-alpha activity in liver homogenate and the levels of low-density lipoprotein receptor (LDLR), high-sensitivity C-reactive protein (hs-CRP), adiponection (APN), superoxide dismutase, and malondialdehyde (MDA). The liver tissues of rats in each group were imaged by electron microscopy with hematoxylin-eosin as the staining agent. The levels of TG, TC, LDL-C, ALT, AST, ALP, GLU, INS, IRI, FFA, LEP, TNF-alpha, MDA, and hs-CRP of MC rats were significantly increased (P<.05, P<.01). Therefore, the levels of HDL-C, ISI, PPAR-alpha, LDLR, and APN were significantly decreased (P<.05, P<.01). Compared with the MC group, each parameter in the RKL, RKM, and RKH groups was significantly improved (P<.05, P<.01). Thus raspberry ketone was an effective intervention for NASH in rats. It was believed that raspberry ketone had a dual effect of liver protection and fat reduction, and the mechanism was probably mediated by alleviation of fatty degeneration of liver cells, decreased liver inflammation, correction of dyslipidemia, reversal of LEP and INS resistance, and improved antioxidant capacity. [Wang L et al; J Med Food 15 (5): 495-503 (2012)] **PEER REVIEWED**"

"/ALTERNATIVE and IN VITRO TESTS/ Raspberry ketone (RK) is a natural phenolic compound of the red raspberry. The dietary administration of RK to male mice has been reported to prevent high-fat diet-induced elevation in body weight and to increase lipolysis in white adipocytes. To elucidate a possible mechanism for the antiobesity action of RK, its effects on the expression and the secretion of adiponectin, lipolysis, and fatty acid oxidation in 3T3-L1 were investigated. Treatment with 10 uM of RK increased lipolysis significantly in differentiated 3T3-L1 cells. An immunoassay showed that RK increased both the expression and the secretion of adiponectin, an adipocytokine mainly expressed and secreted by adipose tissue. In addition, treatment with 10 uM of RK increased the fatty acid oxidation and suppressed lipid accumulation in 3T3-L1 adipocytes. These findings suggest that RK holds great promise as an herbal medicine since its biological activities alter the lipid metabolism in 3T3-L1 adipocytes" [Park KS; Planta Med 76 (15): 1654-8 (2010)] **PEER REVIEWED**"

"Melanogenesis inhibition by raspberry ketone (RK) from Rheum officinale was investigated both in vitro in cultivated murine B16 melanoma cells and in vivo in zebrafish and mice. In B16 cells, RK inhibited melanogenesis through a post-transcriptional regulation of tyrosinase gene expression, which resulted in down regulation of both cellular tyrosinase activity and the amount of tyrosinase protein, while the level of tyrosinase mRNA transcription was not affected. In zebrafish, RK also inhibited melanogenesis by reduction of tyrosinase activity. In mice, application of a 0.2% or 2% gel preparation of RK applied to mouse skin significantly increased the degree of skin whitening within one week of treatment. In contrast to the widely used flavoring properties of RK in perfumery and cosmetics, the skin-whitening potency of RK has been demonstrated in the present study. Based on our findings reported here, RK would appear to have high potential for use in the cosmetics industry. [Lin CH et al; Int J Mol Sci 12 (8): 4819-35 (2011)] **PEER REVIEWED**"

"The decrease in the bone mass associated with osteoporosis caused by ovariectomy, aging, and other conditions is accompanied by an increase in bone marrow adipose tissue. The balance between osteoblasts and adipocytes is influenced by a reciprocal relationship. The development of modalities to promote local/systemic bone formation by inhibiting bone marrow adipose tissue is important in the treatment of fractures or metabolic bone diseases such as osteoporosis. In this

study, we examined whether raspberry ketone [4-(4-hydroxyphenyl)butan-2-one: RK], which is one of the major aromatic compounds of red raspberry and exhibits anti-obesity action, could promote osteoblast differentiation in C3H10T1/2 stem cells. Confluent C3H10T1/2 stem cells were treated for 6 days with 10-100 ug/mL of RK in culture medium containing 10 nM all-trans-retinoic acid (ATRA) or 300 ng/mL recombinant human bone morphogenetic protein (rhBMP)-2 protein as an osteoblast-differentiating agent. RK in the presence of ATRA increased alkaline phosphatase (ALP) activity in a dose-dependent manner. RK in the presence of rhBMP-2 also increased ALP activity. RK in the presence of ATRA also increased the levels of mRNAs of osteocalcin, alpha1(I) collagen, and TGF-betas (TGF-beta1, TGF-beta2, and TGF-beta3) compared with ATRA only. RK promoted the differentiation of C3H10T1/2 stem cells into osteoblasts. However, RK did not affect the inhibition of early-stage adipocyte differentiation. Our results suggest that RK enhances the differentiation of C3H10T1/2 stem cells into osteoblasts, and it may promote bone formation by an action unrelated to adipocyte differentiation. [Takata T, Morimoto C; J Med Food 17(3):332-8 (2014)] **PEER REVIEWED**"

As taken from HSDB, 2014.

"CONTEXT: Raspberry ketone (RK) is a natural phenolic compound of red raspberry. The dietary intake of RK has been reported to exert anti-obese actions and alter the lipid metabolism in vivo and human studies. OBJECTIVE: To elucidate a possible mechanism for anti-obese actions of RK, the effects of RK on the adipogenic and lipogenic gene expression in 3T3-L1 adipocytes were investigated. MATERIALS AND METHODS: 3T3-L1 maturing pre-adipocytes were treated from day 2 to day 8 of differentiation and mature adipocytes for 24 h on day 12 with 1, 10, 20, and 50 μM of RK. Triacylglycerols were assessed by spectrophotometry and gene expression by quantitative real-time polymerase chain reaction (qRT-PCR). RESULTS: Treatment of adipocytes with RK suppressed adipocyte differentiation and fat accumulation in a concentration-dependent manner. RK suppressed the expression of major genes involved in the adipogenesis pathway including peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT enhancer binding protein-α (C/EBPα), which led to further down-regulation of adipocyte fatty acid-binding protein-2 (aP2). In addition, treatment with 10 μM of RK also reduced mRNA levels of lipogenic genes such as acetyl-CoA carboxylase-1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD1). In mature adipocytes, RK increased the transcriptional activities of genes involved in lipolysis and the oxidative pathways including adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and carnitine palmitoyl transferase-1B (CPT1B). DISCUSSION AND CONCLUSION: These findings suggest that RK holds great promise for an herbal medicine with the biological activities altering the lipid metabolism in 3T3-L1 adipocytes." As taken from Park KS. 2015. Pharm. Biol. 53(6), 870-5. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25429790?dopt=AbstractPlus

"The exposure of human skin to 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone, RK) is known to cause chemical/occupational leukoderma. RK has a structure closely related to 4-(4-hydroxyphenyl)-2-butanol (rhododendrol), a skin whitening agent that was found to cause leukoderma in the skin of consumers in 2013. Rhododendrol is a good substrate for tyrosinase and causes a tyrosinase-dependent cytotoxicity to melanocytes, cells that are responsible for skin pigmentation. Therefore, it is expected that RK exerts its cytotoxicity to melanocytes through the tyrosinase-catalyzed oxidation to cytotoxic o-quinones. The results of this study demonstrate that the oxidation of RK by mushroom tyrosinase rapidly produces 4-(3-oxobutyl)-1,2-benzoquinone (RK-quinone), which is converted within 10-20 min to (E)-4-(3-oxo-1-butenyl)-1,2-benzoquinone (DBL-quinone). These quinones were identified as their corresponding catechols after reduction by ascorbic acid. RK-quinone and DBL-quinone quantitatively bind to the small thiol N-acetyl-l-cysteine to form thiol adducts and can also bind to the thiol protein bovine serum albumin through its cysteinyl residue. DBL-quinone is more reactive than RK-quinone, as judged by their half-lives (6.2 min vs 10.5 min, respectively), and decays rapidly to form an oligomeric pigment (RK-oligomer). The RK-oligomer can oxidize GSH to GSSG with a concomitant production of hydrogen peroxide, indicating its pro-oxidant activity, similar to that of the RD-oligomer. These results suggest

that RK is cytotoxic to melanocytes through the binding of RK-derived quinones to thiol proteins and the pro-oxidant activity of the RK-oligomer." As taken from Ito S et al. 2017. Chem. Res. Toxicol. 30(3), 859-868. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28219012

"This study aimed to determine the antiobesity effects of raspberry ketone (RK), one of the major aromatic compounds contained in raspberry, and its underlying mechanisms. During adipogenesis of 3T3-L1 cells, RK (300 μM) significantly reduced lipid accumulation and downregulated the expression of CCAAT/enhancer-binding protein α (C/EBPα), peroxisome proliferation-activated receptor γ (PPARγ), fatty acid-binding protein 4 (FABP4), and fatty acid synthase (FAS). RK also reduced the expression of light chain 3B (LC3B), autophagy-related protein 12 (Atg12), sirtuin 1 (SIRT1), and phosphorylated-tuberous sclerosis complex 2 (TSC2), whereas it increased the level of p62 and phosphorylated-mammalian target of rapamycin (mTOR). Daily administration of RK decreased the body weight (ovariectomy [Ovx] + RK, 352.6 ± 5 vs Ovx, 386 ± 5.8 g; $P < 0.05$), fat mass (Ovx + RK, 3.2 ± 0.05 vs Ovx, 5.0 ± 0.4 g; $P < 0.05$), and fat cell size (Ovx + RK, 6.4 ± 0.6 vs Ovx, 11.1 ± 0.7 × 103 μm2; $P < 0.05$) in Ovx-induced obesity in rats. The expression of PPARγ, C/EBPα, FAS, and FABP4 was significantly reduced in the Ovx + RK group compared with that in the Ovx group. Similar patterns were observed in autophagy-related proteins and endoplasmic reticulum stress proteins. These results suggest that RK inhibited lipid accumulation by regulating autophagy in 3T3-L1 cells and Ovx-induced obese rats." As taken from Leu SY et al. 2017. J. Agric. Food Chem. 65(50), 10907-10914. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29164883

"BACKGROUND: Obesity is caused by excessive accumulation of body fat and is closely related to complex metabolic diseases. Raspberry ketone (RK), a major aromatic compound in red raspberry, was recently reported to possess anti-obesity effects. However, its mechanisms are unclear. AIM: Adipogenesis plays a critical role in obesity and, therefore, this study aimed to investigate the effect and mechanisms of action of RK on adipogenesis in 3T3-L1 preadipocytes. MATERIALS AND METHODS: 3T3-L1 preadipocytes were differentiated in medium containing insulin, dexamethasone, and 1-methyl-3-isobutylxanthine. Adipocyte lipid contents were determined using oil-red O staining while adipogenic transcription factor and lipogenic protein expressions were determined using western blotting. RESULTS: RK (300-400μM) strongly inhibited lipid accumulation during 3T3-L1 preadipocyte differentiation into adipocytes. RK reduced the CCAAT/enhancer-binding protein-α (C/EBP-α), peroxisome proliferation-activated receptor-γ (PPAR-γ), fatty acid synthase (FAS), and fatty acid-binding protein 4 (FABP4) expressions and increased heme oxygenase-1 (HO-1), Wnt10b, and β-catenin expressions in 3T3-L1 adipocytes. Additionally, RK inhibited lipid accumulation, and adipogenic transcription factor and lipogenic protein expressions were all decreased by inhibiting HO-1 or β-catenin using tin protoporphyrin (SnPP) or β-catenin short-interfering RNA (siRNA), respectively. Furthermore, Wnt10b and β-catenin expressions were negatively regulation by SnPP. CONCLUSION: RK may exert anti-adipogenic effects through modulation of the HO-1/Wnt/beta-catenin signaling pathway." As taken from Tsai YC et al. 2017. Phytomedicine 31, 11-17. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28606512

"BACKGROUND: Very few weight and fat loss supplements undergo finished-product research to examine efficacy. The purpose of this study was to determine the effects of an 8-week diet and exercise program on body composition, hip and waist girth, and adipokines and evaluate whether a dietary supplement containing raspberry ketone, capsaicin, caffeine, garlic, and Citrus aurantium enhanced outcomes. METHODS: Overweight men and women completed this randomized, placebo-controlled, double-blind study. Participants consumed 4 capsules/d of supplement (EXP; n = 18) or placebo (PLA; n = 18). Participants underwent 8 weeks of daily supplementation, calorie restriction (500 kcal < RMR [resting metabolic rate] × 1.2), and supervised progressive exercise training 3 times a week. Body composition, girth, and adipokines were assessed at baseline and postintervention (T1 and T2). RESULTS: Significant decreases in weight (-2.6 ± 0.57 kg, $p < 0.001$), fat mass (-1.8 ± 0.20 kg; $p < 0.001$), and percentage body fat (-3.7% ± 0.29%, $p < 0.001$)

and a significant increase in lean body mass (LBM; 1.5 ± 0.26 kg; p < 0.001) were seen from T1 to T2 in both groups. For men, only those in the EXP group increased LBM from T1 to T2 (1.3 ± 0.38 kg; p < 0.05). Hip girth was also reduced, with the women in the EXP group (-10.7 ± 2.15 cm, p < 0.001) having a greater reduction. There was a time by group interaction, with significant decreases in leptin (p < 0.001) and significant increases in adiponectin (p < 0.05) in the EXP group. CONCLUSIONS: Significant improvements in adipokines and leptin support the utility of exercise, diet, and fat loss for impacting inflammatory biomarkers. The improvement in adiponectin with EXP may suggest a unique health mechanism." As taken from Arent SM et al. 2018. J. Am. Coll. Nutr. 37(2), 111-120. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29111889

"Promoting white adipose tissue (WAT) to acquire brown-like characteristics is a promising approach for obesity treatment. Although raspberry ketone (RK) has been reported to possess antiobesity activity, its effects on the formation of brown-like adipocytes remain unclear. Therefore, we investigated the effects and underlying mechanism of RK on WAT browning in 3T3-L1 adipocytes and rats with ovariectomy (Ovx)-induced obesity. RK (100 μM) significantly induced browning of 3T3-L1 cells by increasing mitochondrial biogenesis and the expression of browning-specific proteins (PR domain containing 16, PRDM16; peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PGC-1α; uncoupling protein-1, UCP-1) and lipolytic enzymes (hormone-sensitive lipase and adipose triglyceride lipase). RK significantly reduced the expression of the autophagy-related protein Atg12 and increased the expression of p62 and heme oxygenase 1 (HO-1). Additionally, these effects of RK were reversed by the HO-1 inhibitor SnPP (20 μM). In addition, RK (160 mg/kg, gavage, for 8 weeks) significantly reduced body weight gain (Ovx+RK, 191.8 ± 4.6 g vs. Ovx, 223.6 ± 5.9; P < .05), food intake, the amount of inguinal adipose tissue (Ovx+RK, 9.05 ± 1.1 g vs Ovx, 12.9 ± 0.92 g; P < .05) and the size of white adipocytes in Ovx rats. Moreover, compared to expression in the Ovx group, the levels of browning-specific proteins were significantly higher and the levels of autophagy-related proteins were significantly lower in the Ovx+RK group. Therefore, this study elucidated the mechanism associated with RK-induced WAT browning and thus provides evidence to support the clinical use of RK for obesity treatment." As taken from Leu SY et al. 2018. J. Nutr. Biochem. 56, 116-125. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29525531

"Obesity constitutes a major worldwide problem in which hyperlipidemia and insulin resistance represents adverse metabolic consequences of it. The present study was conducted to elucidate the role of raspberry ketones (RKs) in controlling body weight gain, hyperlipidemia and insulin resistance in male obese rats through affecting the expression of various adipocytokines. As Aquaporin-7 is co-related with the expression of various adipocytokines and has recently emerged as a modulator of adipocyte metabolism, the present study evaluated the effect of RKs on adipose tissue expression of aquaporin-7(AQP7) in high-fat (HF) diet-fed rats. Groups of male rats were assigned to normal, HF diet-fed control rats and RKs-treated (250 and 500 mg/kg) groups. RKs administration effectively abrogated hyperlipidemia and oxidative burden and enhanced insulin sensitivity. In addition, treatment with RKs ameliorated adipose tissue and liver indices and the reduced adipocyte diameters. Moreover, administration of the low dose of RKs ameliorated the expression of apelin and its receptor, and visfatin with upregulating adiponectin expression compared to HF diet control rats. However, both doses effectively downregulated leptin expression. It was obvious that both RKs doses revealed effectiveness in upregulating the AQP7 expression. The present data suggest the promising therapeutic role of RKs in HF diet-induced obesity that is likely attributable, at least in part, to upregulation of AQP7 expression." As taken from Mehanna ET et al. 2018. Eur. J. Pharmacol. 832, 81-89. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29787773

"Over the past 20 years the use of dietary supplements as adjuvant therapy for weight loss gained growing favor among consumers and dietician–nutritionists, with the subsequent astounding increase in health costs. Despite the reassuring label of natural remedy for losing weight, dietary supplements contain a wide variety of ingredients on which available information is rather scanty and scientifically incomplete. Currently, there is little evidence that weight-loss supplements offer

effective aids to reduce weight and meet criteria for recommended use. Robust, randomized, placebo-controlled studies to provide clear-cut scientific evidence of their efficacy and potential side effects in clinical practice are still lacking. Understanding the evidence for the efficacy, safety, and quality of these supplements among nutritionists and physicians is critical to counsel patients appropriately, especially considering the risk of serious adverse effects and interference with concomitant therapies. Detailed information on the efficacy and safety of the most commonly used weight-loss dietary supplements has been recently published by the National Institutes of Health (NIH). However, in this report the thorny issue that may result from drug interactions with weight-loss dietary supplements has been not sufficiently addressed. The aim of this review was to provide a synthetic, evidence-based report on efficacy and safety of the most commonly used ingredients in dietary supplements marketed for weight loss, particularly focusing on their possible drug interactions." As taken from Barrea L et al. 2019. Int. J. Obes. Suppl. 9(1), 32–49. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31391923/

"Obesity management regimens suffer from discomfort, undesirable side effects and/or unsuccessful outcomes. This study compared normal diet (ND) regimen with raspberry ketone (RK) supplementation and calorie restriction (CR). 40-MaleWistar rats received high fat diet (HFD) for 16 weeks. Obese control group was maintained on HFD for another 6 weeks (n=10). Other obese groups (n=10 each) received ND, CR diet or ND with RK oral supplement (44mg/kg) for 6 weeks. HFD increased body and visceral white adipose tissue (WAT) weights, serum glucose, total cholesterol, TG, LDL-C, inflammatory markers (MCP-1, IL-6 and TNF-α) in WAT, while decreased serum HDL-C and WAT adiponectin. Like CR diet, ND with RK supplementation restored the changes attained by HFD. Conclusion: RK supplement with ND regimen effectively attenuated obesity-related changes. Such approach could be an alternative to CR in the management of obesity, thereby overcome the side effects of applying CR for long time." As taken from Asker ME et al. 2018. Journal of Molecular and Cellular Biology Forecast 1(2), Article 1015. Available at https://scienceforecastoa.com/Articles/JMCBF-V1-E2-1015.pdf

"Chemical leukoderma is a patchy hypopigmentation in the skin. Phenol derivatives such as raspberry ketone have been reported to cause the development of occupationally induced leukoderma. Recently, 2% (w/w) rhododenol, a reduced form of raspberry ketone used in a skin-lightning agent, also caused the development of leukoderma in >16,000 users, about 2% of all users, in Asian countries including Japan. However, a method for assessing the risk of leukoderma caused by 2% rhododenol has not been established despite the fact that the development of leukoderma caused by 30% rhododenol was previously shown in animal experiments. Establishment of a novel technique for risk assessment of leukoderma in humans caused by external treatment with chemicals is needed to prevent a possible future chemical disaster. This study demonstrated that external treatment with 2% rhododenol and the same concentration of raspberry ketone caused the development of leukoderma in murine tail skin without exception with significant decreases in the amount of melanin and number of melanocytes in the epidermis. Thus, a novel in vivo technique that can assess the risk of leukoderma caused by 2% rhododenol was developed. The unique technique using tail skin has the potential to prevent chemical leukoderma in the future." As taken from Iida M et al. 2019. Chemosphere 235, 713-718. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31279121/

"Introduction: Clinical and epidemiological studies suggest that patients who are overweight or obese are more at risk in developing glucose intolerance (G/I) and insulin resistance (I/R) leading to type 2 diabetes (T2DM) and cardiovascular disease.
Aim of work: Assess the dynamic contribution of visfatin in the development of obesity and/or diabetes and demonstrate their possible molecular mechanism(s) from side and from another side, modulate role of Raspberry ketone (RK) as weight management supplement and illustrate their possible molecular mechanism(s).
Materials and Methods: Eighty adult rats were divided into eight groups (10 rats for each group, G); G1: Normal Control Group (Normal diet); G2: Diabetic Control Group (received streptozotocin 35 mg/kg); G3: Obese Control Group (received high fat diet, HFD); G4: Obese Diabetic Control Group,

G5: Raspberry ketone Control Group (received 500 mg/kg), G6: Diabetic rats treated with Raspberry ketone; G7: Obese rats treated with Raspberry ketone and G8: Obese Diabetic rats treated with Raspberry ketone to assess the study's aims, their effect was determined on body weight, OGTT, glucose homeostasis (glucose, insulin, HOMA-IR), oxidative stress markers, cytoglobin, visfatin and liver histopathology.
Results: RK caused weight loss, corrected the disturbed glucose and insulin homeostasis, Furthermore, RK increased hepatic content of glutathione (GSH), while decreased hepatic content of malonaldialdehyde (MDA). RK also up regulated hepatic protein expression of cytoglobin, while down regulated hepatic mRNA expression of visfatin.
Conclusion: This study assessed the involvement of visfatin and cytoglobin in obese diabetic rats and modulated the role of RK through the efficient rebalance of glucose homeostasis, I/R, the redox status and liver histopathology." As taken from Hussein HT et al. 2020. Bulletin of Pharmaceutical Sciences 43(1), 53-72. Available at https://bpsa.journals.ekb.eg/article_93587.html

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 4-(para-Hydroxyphenyl)-2-butanone 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 4-(para-Hydroxyphenyl)-2-butanone. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	31	Carmines, 2002 & Rustemeier et al., 2002

	23	Baker et al., 2004a
	0.13 0.65 130 150	JTI KB Study Report(s)
	7.800	Gaworski et al., 2011 & Coggins et al., 2011e
	6	Roemer et al, 2014
In vitro genotoxicity	31	Carmines, 2002 & Roemer et al., 2002
	23	Baker et al., 2004c
	0.13	Renne et al., 2006
	0.13 0.65 150	JTI KB Study Report(s)
	40	fGLH Study Report (2010)
	7.800	Gaworski et al., 2011 & Coggins et al., 2011e
	6	Roemer et al, 2014
In vitro cytotoxicity	31	Carmines, 2002 & Roemer et al., 2002
	23	Baker et al., 2004c
	0.13 0.65 150	JTI KB Study Report(s)

	40	fGLH Study Report (2010)
	7.800	Gaworski et al., 2011 & Coggins et al., 2011e
	6	Roemer et al, 2014
Inhalation study	<0.1	Gaworski et al., 1998
	31	Carmines, 2002 & Vanscheeuwijck et al., 2002
	23	Baker et al., 2004c
	0.13	Renne et al., 2006
	0.13 0.65 150	JTI KB Study Report(s)
	6	Schramke et al, 2014
Skin painting	<0.1	Gaworski et al., 1999
	0.13 0.65	JTI KB Study Report(s)
In vivo genotoxicity	6 150	Schramke et al, 2014 JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing 4-(para-Hydroxyphenyl)-2-butanone 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	1600	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	1600	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	1600	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 4-(para-Hhydroxyphenyl)-2-butanone 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 (ppm)	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 4-(para-Hydroxyphenyl)-2-butanone was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.62	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	0.62	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	0.62	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

According to the Ecological Categorization results from the Canadian Domestic Substances List, 4-(4-hydroxyphenyl)-2-butanone 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	0.478
TOPKAT probability of biodegradation	0.888
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.2359

Data accessed December 2016 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 :	5.53E-010 atm-m3/mole (5.60E-005 Pa-m3/mole)
Group Method:	1.44E-010 atm-m3/mole (1.46E-005 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.149E-008 atm-m3/mole (1.165E-003 Pa-m3/mole) VP: 0.000716 mm Hg (source: MPBPVP) WS: 1.35E+004 mg/L (source: WSKOWWIN)

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

Log Kow used:	1.48 (KowWin est)
Log Kaw used:	-7.646 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	9.126
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.8467
Biowin2 (Non-Linear Model) :	0.8468
Biowin3 (Ultimate Survey Model):	2.7953 (weeks)
Biowin4 (Primary Survey Model) :	3.5598 (days-weeks)
Biowin5 (MITI Linear Model) :	0.4326
Biowin6 (MITI Non-Linear Model):	0.4780
Biowin7 (Anaerobic Linear Model):	-0.0322
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	0.268 Pa (0.00201 mm Hg)
Log Koa (Koawin est):	9.126

Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.12E-005 0.000328

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.000404
Mackay model:	0.000895
Octanol/air (Koa) model:	0.0256

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	45.3494 E-12 cm3/molecule-sec
Half-Life =	0.236 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	2.830 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.000649 (Junge-Pankow, Mackay avg) 0.0256 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	216.7 L/kg (MCI method)
Log Koc:	2.336 (MCI method)

Koc :	127.7 L/kg (Kow method)
Log Koc:	2.106 (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: 1.44E-010 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	5.21E+006 hours (2.171E+005 days)
Half-Life from Model Lake:	5.684E+007 hours (2.368E+006 days)

Removal In Wastewater Treatment:

Total removal:	1.97 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.87 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.00256	5.66	1000
Water	17.7	360	1000

Soil	82.1	720	1000
Sediment	0.179	3.24e+003	0

Persistence Time: 762 hr

Environmental Fate/Exposure Summary:

“Raspberry ketone's production and use in perfumery, in cosmetics, and as a food additive to impart a fruity odor and taste in products such as soft drinks, sweets, puddings and ice creams may result in its release to the environment through various waste streams. Raspberry ketone occurs in raspberries and in numerous plant genera, such as Artemisia, Capparis, Dendrobium, Hippophae, Larix, Limonium, Pinus, Prunus, Rheum, Rubus, Saxifraga, Taxus, Vaccinium, Vanilla, Vitis and in cranberry, blackberry, loganberry and sea buckthorn (Hippophae rhamnoides L). If released to air, an estimated vapor pressure of 5.7X10-4 mm Hg at 25 deg C indicates raspberry ketone will exist in both the vapor and particulate phases in the atmosphere. Vapor-phase raspberry ketone will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 8.5 hours. Particulate-phase raspberry ketone will be removed from the atmosphere by wet and dry deposition. Raspberry ketone contains chromophores that absorb at wavelengths >290 nm and, therefore, may be susceptible to direct photolysis by sunlight. If released to soil, raspberry ketone is expected to have moderate mobility based upon an estimated Koc of 217. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of 5.5X10-10 atm-cu m/mole. Although raspberry ketone's estimated vapor pressure of 5.7X10-4 mm Hg suggests that volatilization from dry soil may not occur, raspberry ketone has a strong odor reminiscent of raspberries which suggests some volatilization may occur. Although data specific to the environmental biodegradation of raspberry ketone were not available, raspberry ketone is a mono-substituted phenol with a structure suggesting that it may be readily biodegradable in the soil and water environments. If released into water, raspberry ketone may adsorb moderately to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 4.4 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions (pH 5 to 9). Photo-oxidation via peroxy and hydroxyl radicals may have some importance in natural waters exposed to sunlight… (SRC) **PEER REVIEWED**”

Artificial Pollution Sources:

“Raspberry ketone's production and use in perfumery, in cosmetics, and as a food additive to impart a fruity odor in products such as soft drinks, sweets, puddings and ice creams(1) may result in its release to the environment through various waste streams(SRC). [(1) Beekwilder J et al; Biotechnology Journal 2(10): 1270-9 (2007)] **PEER REVIEWED**”

Environmental Fate:

“TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 217(SRC), determined from a structure estimation method(2), indicates that raspberry ketone is expected to have moderate mobility in soil(SRC). Volatilization of raspberry ketone from moist soil surfaces is not expected to be an important fate process(SRC) given an estimated Henry's Law constant of 5.5X10-10 atm-cu m/mole(SRC), using a fragment constant estimation method(2). Although raspberry ketone's estimated vapor pressure of 5.7X10-4 mm Hg(SRC), determined from a fragment constant method(2), suggests that volatilization from dry soil may not occur(SRC),

raspberry ketone has a strong odor reminiscent of raspberries(3) which suggests some volatilization may occur(SRC). Aliphatic ketones, such as methyl ethyl ketone and dimethyl ketone, absorb UV-light at wavelengths >290 nm and are susceptible to direct photolysis in sunlight(4,5); therefore, raspberry ketone may be susceptible to direct photolysis on soil surfaces exposed to sunlight(SRC). Data specific to the environmental biodegradation of raspberry ketone were not available(SRC 2014). However, raspberry ketone is a mono-substituted phenol with a structure suggesting that it may be biodegradable(SRC) in the environment(6). Analagous compounds, phenol and p-ethylphenol, present at 100 mg/L, reached 85-90% of their theoretical BODs in 4 weeks using the Japanese MITI test which classified them as readily biodegradable(7). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (3) Fahlbusch KG et al; Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (1999-2014). New York, NY: John Wiley & Sons. Online Posting Date: Jan 15, 2003. (4) Atkinson R; Atmos Environ 34: 2063-101 (2000) (5) NIST; NIST Chemistry WebBook. Acetone (67-64-1). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available from, as of Feb 4, 2014: http://webbook.nist.gov (6) Howard P; pp. 286, 298 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (7) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Feb 10, 2014: http://www.safe.nite.go.jp/english/db.html **PEER REVIEWED**"

"AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 217(SRC), determined from a structure estimation method(2), indicates that raspberry ketone may adsorb moderately to suspended solids and sediment(SRC). Volatilization from water surfaces is not expected(3) based upon an estimated Henry's Law constant of 5.5X10-10 atm-cu m/mole(SRC), developed using a fragment constant estimation method(2). According to a classification scheme(4), an estimated BCF of 4.4(SRC), from an estimated log Kow of 1.48(2) and a regression-derived equation(2), suggests the potential for bioconcentration in aquatic organisms is low. Aliphatic ketones, such as methyl ethyl ketone and dimethyl ketone, absorb UV-light at wavelengths >290 nm and are susceptible to direct photolysis in sunlight(5,6); therefore, raspberry ketone may be susceptible to direct photolysis on water surfaces exposed to sunlight(SRC). Raspberry ketone contains a phenol function, and phenols are susceptible to photo-oxidation via peroxy and hydroxyl radicals in natural waters exposed to sunlight with a half-life on the order of two weeks at the water's surface(7). Raspberry ketone is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under environmental conditions(3). Data specific to the environmental biodegradation of raspberry ketone were not available(SRC 2014). However, raspberry ketone is a mono-substituted phenol with a structure suggesting that it may be biodegradable(SRC) in the environment(8). Analagous compounds, phenol and p-ethylphenol, present at 100 mg/L, reached 85-90% of their theoretical BODs in 4 weeks using the Japanese MITI test which classified them as readily biodegradable(9). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm(3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5, 15-1 to 15-29 (1990) (4) Franke C et al; Chemosphere 29: 1501-14 (1994) (5) Atkinson R; Atmos Environ 34: 2063-101 (2000) (6) NIST; NIST Chemistry WebBook. Acetone (67-64-1). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available from, as of Feb 4, 2014: http://webbook.nist.gov(7) Mill T; p. 368 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (8) Howard P; pp. 286, 298 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (9) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Feb 10, 2014: http://www.safe.nite.go.jp/english/db.html**PEER REVIEWED**"

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), raspberry ketone, which has an estimated vapor pressure of 5.7X10-4 mm Hg at 25 deg C(SRC), determined from a fragment constant method(2), will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase raspberry ketone is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 8.5 hours(SRC), calculated from its rate constant of 4.5X10-11 cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(2). Particulate-phase raspberry ketone may be removed from the air by wet and dry deposition(SRC). Aliphatic ketones, such as methyl ethyl ketone and dimethyl ketone, absorb UV-light at wavelengths >290 nm and are susceptible to direct photolysis in sunlight(3,4); therefore, raspberry ketone may be susceptible to direct photolysis(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (3) Atkinson R; Atmos Environ 34: 2063-101 (2000) (4) NIST; NIST Chemistry WebBook. Acetone (67-64-1). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available from, as of Feb 4, 2014: http://webbook.nist.gov **PEER REVIEWED**"

Environmental Biodegradation:

"AEROBIC: Data specific to the environmental biodegradation of raspberry ketone were not available(SRC, 2014). However, raspberry ketone is a mono-substituted phenol with a structure suggesting that it may be biodegradable(SRC) in the environment(1). For example, phenol and p-ethylphenol, present at 100 mg/L, reached 85-90% of their theoretical BODs in 4 weeks using an activated sludge inoculum at 30 mg/L in the Japanese MITI test which classified them as readily biodegradable(2). [(1) Howard P; pp. 286, 298 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (2) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Feb 10, 2014: http://www.safe.nite.go.jp/english/db.html **PEER REVIEWED**"

Environmental Abiotic Degradation:

"The rate constant for the vapor-phase reaction of raspberry ketone with photochemically-produced hydroxyl radicals has been estimated as 4.5X10-11 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 8.5 hours at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). Raspberry ketone is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under environmental conditions(2). Aliphatic ketones, such as methyl ethyl ketone and dimethyl ketone, absorb UV-light at wavelengths >290 nm and are susceptible to direct photolysis in sunlight(3,4). The rate of photolysis of methyl ethyl ketone has been approximated as 4 days(3); therefore, direct photolysis of raspberry ketone, which contains a methyl ethyl ketone function, may have some importance as an environmental fate process(SRC). Raspberry ketone also contains a phenol function, and phenols are susceptible to photo-oxidation via peroxy and hydroxyl radicals in natural waters exposed to sunlight with a half-life on the order of two weeks at the water's surface(5). [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5 (1990) (3) Atkinson R; Atmos Environ 34: 2063-101 (2000) (4) NIST; NIST Chemistry WebBook. Acetone (67-64-1). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available frpm, as of Feb 4, 2014: http://webbook.nist.gov (5) Mill T; p. 368 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000)] **PEER REVIEWED**"

Environmental Bioconcentration:

“An estimated BCF of 4.4 was calculated in fish for raspberry ketone(SRC), using an estimated log Kow of 1.48(1) and a regression-derived equation(1). According to a classification scheme(2), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm(2) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**”

Soil Adsorption/Mobility:

“Using a structure estimation method based on molecular connectivity indices(1), the Koc of raspberry ketone can be estimated to be 217(SRC). According to a classification scheme(2), this estimated Koc value suggests that raspberry ketone is expected to have moderate mobility in soil. [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (2) Swann RL et al; Res Rev 85: 17-28 (1983)] **PEER REVIEWED**”

Volatilization from Water/Soil:

“The Henry's Law constant for raspberry ketone is estimated as 5.5X10-10 atm-cu m/mole(SRC) using a fragment constant estimation method(1). This Henry's Law constant indicates that raspberry ketone is expected to be essentially nonvolatile from water surfaces(2). Raspberry ketone's Henry's Law constant indicates that volatilization from moist soil surfaces is not expected to be an important fate process(SRC). Although raspberry ketone's estimated vapor pressure of 5.7X10-4 mm Hg(SRC), determined from a fragment constant method(1), suggests that volatilization from dry soil may not occur(SRC), raspberry ketone has a strong odor reminiscent of raspberries(3) which suggests some volatilization may occur(SRC). [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Feb 4, 2014: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (3) Fahlbusch KG et al; Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (1999-2014). New York, NY: John Wiley & Sons. Online Posting Date: Jan 15, 2003] **PEER REVIEWED**”

As taken from HSDB, 2014.

10.2. Aquatic toxicity

According to the Ecological Categorization results from the Canadian Domestic Substances List, 4-(4-hydroxyphenyl)-2-butanone is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	276.7
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	276.7
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	50.432
Toxicity to fish (LC50 in mg/l) as predicted by Aster	169.271547
Toxicity to fish (LC50 in mg/l) as predicted by PNN	10.93444

Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	4.7
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	235.282
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.25E+002

Data accessed December 2016 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

ECOSAR Version 1.11 provides the following aquatic toxicity data for CAS RN 5471-51-2:

Values used to Generate ECOSAR Profile

Log Kow: 1.484 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.346E+004 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Phenols

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Phenols :	Fish	96-hr	LC50	70.409
Phenols :	Daphnid	48-hr	LC50	16.845
Phenols :	Green Algae	96-hr	EC50	81.464
Phenols :	Fish		ChV	6.590
Phenols :	Daphnid		ChV	3.207
Phenols :	Green Algae		ChV	38.521
Phenols :	Fish (SW)	96-hr	LC50	34.876
Phenols :	Lemna gibba	7-day	EC50	57.239

Neutral Organic SAR :	Fish	96-hr	LC50	392.353	
(Baseline Toxicity) :	Daphnid	48-hr	LC50	212.409	
	Green Algae	96-hr	EC50	129.919	
	Fish		ChV	36.254	
	Daphnid		ChV	18.141	
	Green Algae		ChV	30.596	

melanogenesis by reduction of tyrosinase activity. ….. In contrast to the widely used flavoring properties of RK in perfumery and cosmetics, the skin-whitening potency of RK has been demonstrated in the present study. Based on our findings reported here, RK would appear to have high potential for use in the cosmetics industry. [Lin CH et al; Int J Mol Sci 12 (8): 4819-35 (2011)] **PEER REVIEWED**"

As taken from HSDB, 2014.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR Version 1.11 provides the following terrestrial toxicity data for CAS RN 5471-51-2:

Values used to Generate ECOSAR Profile

Log Kow: 1.484 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.346E+004 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Phenols

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Phenols :	Earthworm	14-day	LC50	248.030

10.5. All other relevant types of ecotoxicity

According to the Ecological Categorization results from the Canadian Domestic Substances List, 4-(4-hydroxyphenyl)-2-butanone is not bioaccumulative in the environment:

Log Kow predicted by KowWin	1.48
Log BAF T2MTL predicted by Gobas	0.453991343723849
Log BCF 5% T2LTL predicted by Gobas	0.396036468800647
Log BCF Max predicted by OASIS	1.43843459438671
Log BCF predicted by BCFWIN	0.442

Data accessed December 2016 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.646 (BCF = 4.425 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.4063 days (HL = 0.03924 days)
Log BCF Arnot-Gobas method (upper trophic):	0.427 (BCF = 2.672)
Log BAF Arnot-Gobas method (upper trophic):	0.427 (BAF = 2.672)
log Kow used:	1.48 (estimated)

"/OTHER TOXICITY INFORMATION/ Hyphal growth of two strains of the heterothallic fungus Choanephora trispora was slightly inhibited by 0.3% p-hydroxyphenylbutanone but was unaffected at a concentration of 0.03% . Formation of sporangia was strongly inhibited at 0.3% in both strains; at 0.03% and 0.003%, strain A-9216 was unaffected while strain A-9159 showed stimulation." [Food and Cosmetics Toxicology 16: 781-2 (1978)] **PEER REVIEWED**

As taken from HSDB, 2014.

11. References

- AICIS (Undated) Australian Goverment. Department of Health. Australian Inventory of Industrial Chemicals. Available at https://www.industrialchemicals.gov.au/chemicals/2-butanone-4-4-hydroxyphenyl
- Aomori T et al. (2018). Effect of a dietary supplement containing raspberry ketone on CYP3A activity in healthy women. Pharmaceutica Analytica Acta 9(6), 1000587. DOI:

10.4172/2153-2435.1000587. Available at https://pdfs.semanticscholar.org/c236/b41a4747def342caa6e5668df63597190235.pdf
- Api AM et al. (2019). RIFM fragrance ingredient safety assessment, 4-(p-hydroxyphenyl)-2-butanone, CAS Registry Number 5471-51-2. Food Chem. Toxicol. 134(Suppl. 2), 110948. DOI: 10.1016/j.fct.2019.110948. PubMed, 2020 available at
- Arent SM et al. (2018). The combined effects of exercise, diet, and a multi-ingredient dietary supplement on body composition and adipokine changes in overweight adults. J. Am. Coll. Nutr. 37(2), 111-120. DOI: 10.1080/07315724.2017.1368039. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29111889
- Ash M (1995). Handbook of food additives: an international guide to more than 7000 compounds by trade name, chemical, function and manufacture. Gower Publishing Ltd. ISBN 0-566-07592-x.
- Asker ME et al. (2018). Raspberry ketone versus calorie restriction on modulating metabolic disorders in obese rats. Journal of Molecular and Cellular Biology Forecast 1(2), Article 1015. Available at https://scienceforecastoa.com/Articles/JMCBF-V1-E2-1015.pdf
- Attia RT et al. (2019). Raspberry ketone and Garcinia cambogia rebalanced disrupted insulin resistance and leptin signaling in rats fed high fat fructose diet. Biomed. Pharmacother. 110, 500-509. DOI: 10.1016/j.biopha.2018.11.079. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30530230
- Baker R and Bishop L (2004). The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrolysis 71, 223–311.
- Baker R et al. (2004a). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. Food and Chemical Toxicology 42s, S3-S37.
- Baker R et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food and Chemical Toxicology 42s, S53-S83.
- Barrea L et al. (2019). Nutritionist and obesity: brief overview on efficacy, safety, and drug interactions of the main weight-loss dietary supplements Int. J. Obes. Suppl. 9(1), 32–49. DOI: 10.1038/s41367-019-0007-3. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31391923/
- Borejsza-Wysocki W and Hrazdina G; Aromatic Polyketide Synthases (Purification, Characterization, and Antibody Development to Benzalacetone Synthase from Raspberry Fruits); PLANT PHYSIOLOGY , Vol 110, Issue 3 791-799, Copyright © 1996 by American Society of Plant Biologists; http://www.plantphysiol.org/cgi/content/abstract/110/3/791
- Bredsdorff L et al. (2015). Raspberry ketone in food supplements--High intake, few toxicity data--A cause for safety concern? Regul. Toxicol. Pharmacol. 73(1), 196-200. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26160596
- Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. 6th Ed. CRC Press.
- Carmines E (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1. Cigarette design, testing approach, and review of results. Food and Chemical Toxicology, 40, 77-91.
- ChemIDplus. Accessed June 2021. Available at: https://chem.nlm.nih.gov/chemidplus/
- ChemSpider. Record for raspberry ketone (CAS RN 5471-51-2). Undated, accessed June 2021. Available at http://www.chemspider.com/Chemical-Structure.20347.html
- Choi EJ et al. (2014). Evaluation of the in vitro/in vivo potential of five berries (bilberry, blueberry, cranberry, elderberry, and raspberry ketones) commonly used as herbal supplements to inhibit uridine diphospho-glucuronosyltransferase. Food Chem. Toxicol. 72, 13-9. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24997313
- Coggins CRE et al. (2011e). A comprehensive evaluation of the toxicology of cigarette ingredients: aromatic carbonyl compounds. Inhalation toxicology, 23 (S1); 90-101.
- Cohen SM et al. (2020). GRAS 29 Flavoring Substances. Food Technology 74(3), 44-65. Available at https://www.femaflavor.org/sites/default/files/2020-03/GRAS 29.pdf

- CosIng. Cosmetic substances and ingredients database. Record for raspberry ketone. Undated. Available at https://ec.europa.eu/growth/tools-databases/cosing/
- COSMOS Database (undated). Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety. Record for 4-(p-hydroxyphenyl)-2-butanone (CAS RN 5471-51-2). Accessed June 2021. Available at https://ng.cosmosdb.eu/
- Cotten BM et al. (2017). Raspberry ketone fails to reduce adiposity beyond decreasing food intake in C57BL/6 mice fed a high-fat diet. Food Funct. 8(4), 1512-1518. DOI: 10.1039/c6fo01831a. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28378858
- CPID (undated). Consumer Product Information Database. Record for raspberry ketone (CAS RN 5471-51-2). Accessed June 2021. Available at https://www.whatsinproducts.com/
- DOI: 10.1016/j.jchromb.2020.122146. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32474352/
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at http://legacy.library.ucsf.edu/tid/thy03c00
- Doull, J et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes.Http://legacy.library.ucsf.edu/tid/wzp67e00.
- ECHA (2021). European Chemicals Agency. Information on Chemicals. Records for 4-(4-hydroxyphenyl)-2-butanone. Last updated 24 May 2021. Available at https://echa.europa.eu/information-on-chemicals/registered-substances
- ECHA (2022). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 31 May 2022. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR (undated). Record for 2-butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2). Accessed December 2016. (ECOSAR content has not been updated since 2012, version 1.11.) Available to download, through EPISuite, at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EFSA (2016). EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Safety and efficiency of aromatic ketones, secondary alcohols and related esters belonging to chemical group 21 when used as flavourings for all animal species. EFSA Journal 14(8), 4557. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4557/epdf
- EPISuite (2017). Record for 2-butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- EPISuite (undated). Record for 2-butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2). Accessed December 2016. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download via
- European Commission (2012). Database of Food Flavourings. Record for 4-(p-hydroxyphenyl)butan-2-one. Last modified 17 September 2012. Available at https://webgate.ec.europa.eu/foods_system/
- FDA (2022a). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 17 May 2022. . Available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2022b). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR) Title 21. Current as of 29 March 2022. Available at https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- fGLH Study Report (2010)
- Fukuda Y et al (1998a). Occupational leukoderma in workers engaged in 4-(p-hydroxyphenyl)-2-butanone manufacturing. Journal of Occupational Health, 40, 118-122.

- Fukuda Y et al (1998b). An experimental study on depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone in C57 black mice. Journal of Occupational Health, 40, 97-102
- FUKUDA Y et al. (1998c). In vitro studies on the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone; JOURNAL OF OCCUPATIONAL HEALTH; 40 (2). 137-142
- Gaunt et al. (1970). Fd. Cosm. Tox. 8, 349.
- Gaworski C L et al (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 134-week inhalation exposure in rats. Inhalation Toxicology, 10, 357-381.
- Gaworski C L et al (1999). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology, 139, 1-17
- Gaworski CL et al. (2011a). An evaluation of the toxicity of 95 ingredients added individually to experimental cigarettes: approach and methods. Inhalation Toxicology, 23 (S1), 1-12.
- Gaworski CL et al. (2011b). Insights from a multi-year program designed to test the impact of ingredients on mainstream cigarette smoke toxicity. Inhalation Toxicology, 23 (S1), 172-183.
- Hall R L & Oser B L (1965). Recent progress in the consideration of flavouring ingredients under the food additives amendment. III. GRAS substances. Food Technology, 19, 151-197.
- Hamdy SM et al. (2020). Hepatoprotective effect of Raspberry ketone and white tea against acrylamide-induced toxicity in rats. Drug Chem. Toxicol. Epub ahead of print. DOI: 10.1080/01480545.2020.1772279. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32482111/
- Hao L et al. (2020). Acute feeding suppression and toxicity of raspberry ketone [4-(4-hydroxyphenyl)-2-butanone] in mice. Food Chem. Toxicol. 143, 111512. DOI: 10.1016/j.fct.2020.111512. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32565406/
- Harada N et al., Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans; Growth Horm IGF Res. 2008, Aug; 18(4):335-44.
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Record for raspberry ketone (CAS RN 5471-51-2). Last updated 4 May 2021. Accessed June 2021. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=1767&lang=eng
- HSDB (2014). Record for Raspberry ketone. Hazardous Substances Databank Number: 8163. Last revision date 4 September 2014. Available at https://www.toxinfo.io/
- Hung PH et al. (2020). In vitro and in silico genetic toxicity screening of flavor compounds and other ingredients in tobacco products with emphasis on ENDS. J. Appl. Toxicol. 40(11), 1566-1587. DOI: 10.1002/jat.4020. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32662109/
- Hussein HT et al. (2020). The role of visfatin and cytoglobin in obese diabetic rats: the modulatory effects of raspberry ketone. Bulletin of Pharmaceutical Sciences 43(1), 53-72. DOI: 10.21608/BFSA.2020.93587. Available at https://bpsa.journals.ekb.eg/article_93587.html
- IFRA (2020). International Fragrance Association. IFRA Standard, 49th amendment. Record for 4-(4-hydroxyphenyl)butan-2-one. Accessed June 2021. Available at: https://ifrafragrance.org/pdf/web/viewer.html?file=/standards/IFRA_STD_217.pdf
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Available at https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
- Iida M et al. (2019). A unique system that can sensitively assess the risk of chemical leukoderma by using murine tail skin. Chemosphere 235, 713-718. DOI: 10.1016/j.chemosphere.2019.06.185. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31279121/

- Ito S et al. (2017). Tyrosinase-catalyzed oxidation of the leukoderma-inducing agent raspberry ketone produces (E)-4-(3-oxo-1-butenyl)-1,2-benzoquinone: implications for melanocyte toxicity. Chem. Res. Toxicol. 30(3), 859-868. DOI: 10.1021/acs.chemrestox.7b00006. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28219012
- JECFA (2001). 55th Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 46. http://www.inchem.org/documents/jecfa/jecmono/v46je09.htm
- Jeong JB & Jeong HJ (2011). Rheosmin, a naturally occurring phenolic compound inhibits LPS-induced iNOS and COX-2 expression in RAW264.7 cells by blocking NF-kappaB activation pathway. Fd Chem. Toxicol. 48, 2158-2163. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20478352
- JTI KB Study Report (s)
- JTI Report 1 (2003).
- Khan V et al. (2018). Raspberry ketone protects against isoproterenol-induced myocardial infarction in rats. Life Sci. 194, 205-212. DOI: 10.1016/j.lfs.2017.12.013. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29225109
- Khan V et al. (2019). Suppression of isoproterenol-induced cardiotoxicity in rats by raspberry ketone via activation of peroxisome proliferator activated receptor-α. Eur. J. Pharmacol. 842, 157-166. DOI: 10.1016/j.ejphar.2018.10.034. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30431010
- Kim M et al. (2016). Rhododenol and raspberry ketone impair the normal proliferation of melanocytes through reactive oxygen species-dependent activation of GADD45. Toxicol. In Vitro 32, 339-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26867644
- Kshatriya D et al. (2019). Phenolic-enriched raspberry fruit extract (Rubus idaeus) resulted in lower weight gain, increased ambulatory activity, and elevated hepatic lipoprotein lipase and heme oxygenase-1 expression in male mice fed a high-fat diet. Nutr. Res. 68, 19-33. DOI: 10.1016/j.nutres.2019.05.005. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31252376/
- Kshatriya D et al. (2020). Raspberry Ketone [4-(4-Hydroxyphenyl)-2-Butanone] Differentially Effects Meal Patterns and Cardiovascular Parameters in Mice. Nutrients 12(6), 1754. DOI: 10.3390/nu12061754. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32545402/
- Labstat International Inc. (2020a) Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2020b) Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Labstat International Inc. (2021) Characterization of E-cigarette Aerosol. Analytical Test Report.
- Labstat International Inc. (2021a). Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2021b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Labstat International Inc. (2022) Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Collected Matter (ACM) and Mainstream Gas Vapor Phase (GVP) of Electronic Cigarette Products.Biological Activity Test Report.

- Lee CS et al. (2016). Different effects of five depigmentary compounds, rhododendrol, raspberry ketone, monobenzone, rucinol and AP736 on melanogenesis and viability of human epidermal melanocytes. Exp. Dermatol. 25(1), 44-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26440747
- Leu SY et al. (2017). Raspberry ketone reduced lipid accumulation in 3T3-L1 cells and ovariectomy-induced obesity in Wistar rats by regulating autophagy mechanisms. J. Agric. Food Chem. 65(50), 10907-10914. DOI: 10.1021/acs.jafc.7b03831. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29164883
- Leu SY et al. (2018). Raspberry ketone induces brown-like adipocyte formation through suppression of autophagy in adipocytes and adipose tissue. J. Nutr. Biochem. 56, 116-125. DOI: 10.1016/j.jnutbio.2018.01.017. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29525531
- Lloyd R A et al (1976). Flue-cured tobacco flavour. 1. Essence and essential oil components.Tobacco Science, 20, 40-48
- Logic (2019a). G.5. Nonclinical Evaluation Summary - Logic Power (PMTA) and G.5. Nonclinical Evaluation Summary - Logic Pro (PMTA)
- Logic (2019b). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- Lopez HL et al (2013). Eight weeks of supplementation with a multi-ingredient weight loss product enhances body composition, reduces hip and waist girth, and increases energy levels in overweight men and women. J. Int. Soc. Sports Nutr. 10(1), 22. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23601452
- MAEDA M et al.; Effect of Raspberry Ketone Bathing on the Skin Blood Flow and Endocrine System; Journal of Japanese Association of Physical Medicine Balneology and Climatology, ISSN:0029-0343, VOL.67;NO.4;PAGE.215-224(2004); https://www.jstage.jst.go.jp/article/onki1962/67/4/67_4_215/_article
- Marescotti D et al. (2020). Systems toxicology assessment of a representative E-liquid formulation using human primary bronchial epithelial cells. Toxicol. Rep. 7, 67-80. DOI: 10.1016/j.toxrep.2019.11.016. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31886136/
- Martindale (1993). The Extra Pharmacopoeia. Edited by J E F Reynolds. Thirteenth edition. The Pharmaceutical Press. ISBN 0-85369-300-5.
- Mehanna ET et al. (2018). An optimized dose of raspberry ketones controls hyperlipidemia and insulin resistance in male obese rats: Effect on adipose tissue expression of adipocytokines and Aquaporin 7. Eur. J. Pharmacol. 832, 81-89. DOI: 10.1016/j.ejphar.2018.05.028. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29787773
- Merck (1996). The Merck Index. An encyclopaedia of chemicals, drugs and biologicals. Twelfth edition. Budavari, S et al ed. Merck and Co., Inc.; Whitehouse Station, New Jersey, USA.
- Mir TM et al. (2021). Effect of raspberry ketone on normal, obese and health-compromised obese mice: A preliminary study. J. Diet. Suppl. 18(1), 1-6. DOI: 10.1080/19390211.2019.1674996. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31603036/
- Mohamed HE et al. (2018). Raspberry ketone preserved cholinergic activity and antioxidant defense in obesity induced Alzheimer disease in rats. Biomed. Pharmacother. 107, 1166-1174. DOI: 10.1016/j.biopha.2018.08.034. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30257330
- Morimoto et al.; Anti-obese action of raspberry ketone; Life Sciences, Volume 77, Issue 2, 27 May 2005, Pages 194-204 ; http://www.sciencedirect.com/
- Nagata T et al. (2015). The mechanism of melanocytes-specific cytotoxicity induced by phenol compounds having a prooxidant effect, relating to the appearance of leukoderma.

Biomed. Res. Int. 2015, 479798. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25861631

- Natural sources of flavourings. Report No 1. Council of Europe, 2000. ISBN 92-871-4324-2.
- NZ EPA (2006). New Zealand Environmental Protection Authority Inventory of Chemicals. Record for 2-butanone, 4-(4-hydroxyphenyl)- (CAS RN 5471-51-2). Date added to inventory: 1 December 2006. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/D61E4720-3B52-4374-8C93-95892F956D6C
- OECD (undated). Organisation for Economic Co-operation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). 4-(4-hydroxyphenyl)-2-butanone. Accessed December 2016. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Ogawa Y et al. (2010). Effect of essential oils, such as raspberry ketone and its derivatives, on antiandrogenic activity based on in vitro reporter gene assay. Bioorgan. Med. Chem. Lett. 20, 2111-2114. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20226658?dopt=AbstractPlus
- Park KS (2010). Raspberry ketone increases both lipolysis and fatty acid oxidation in 3T3-L1 adipocytes. Planta Med. 76, 1654-1658. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20425690
- Park KS. (2015). Raspberry ketone, a naturally occurring phenolic compound, inhibits adipogenic and lipogenic gene expression in 3T3-L1 adipocytes. Pharm. Biol. 53(6), 870-5. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25429790?dopt=AbstractPlus
- PubChem (2022). Record for 4-(4-hydroxyphenyl)-2-butanone (CAS RN 5471-51-2). Created 26 March 2005. Modified 28 May 2022. Available at https://pubchem.ncbi.nlm.nih.gov/compound/21648
- Renne RA et al., (2006). Effects of flavoring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. Inhal Toxicol;18(9):685-706
- Roemer E et al (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology, 40, 105-111.
- Roemer E et al., (2014). Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regulatory Toxicology and Pharmacology 70; S66-80. (SC, Ames, NRU, MLA)
- RTECS (2018). Registry of Toxic Effects of Chemical Substances. Record for 2-butanone, 4-(p-hydroxyphenyl)- (CAS RN 5471-51-2). Last updated December 2018.
- Rustemeier K et al (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- Schramke H et al., (2014). Toxicological assessment of kretek cigarettes Part 7: The impact of ingredients added to kretek cigarettes on inhalation toxicity. Regulatory Toxicology and Pharmacology 70; S81-89. (Inhalation, in vivo micronucleus)
- Sheftel V O (2000). Indirect food additives and polymers: migration and toxicology. CRC Press LLC. ISBN 1-56670-499-5.
- Sporstøl S and Scheline RR; The metabolism of 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone) in rats, guinea-pigs and rabbits; Xenobiotica, 1982, Vol. 12, No. 4, Pages 249-257, DOI: 10.3109/00498258209052463; http://informahealthcare.com/doi/abs/10.3109/00498258209052463
- Stedman, R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207.
- Steffensen I-L (2019). Nordic Council of Ministers. Nordic Working Papers. Safer food supplements in the Nordic countries. Report from a Nordic workshop November 21-22, 2018. NA2019:901. ISSN 2311-0562. DOI: 10.6027/NA2019-901. Available at http://www.diva-portal.org/smash/get/diva2:1287921/FULLTEXT02.pdf

- Sugumaran M et al. (2020). Oxidative Oligomerization of DBL Catechol, a potential Cytotoxic Compound for Melanocytes, Reveals the Occurrence of Novel Ionic Diels-Alder Type Additions. Int. J. Mol. Sci. 21(18), 6774. DOI: 10.3390/ijms21186774. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32942764/
- Takata T and Morimoto C (2014). Raspberry Ketone Promotes the Differentiation of C3H10T1/2 Stem Cells into Osteoblasts. J. Med. Food. 17(3) 332-8. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24404978
- Tsai YC et al. (2017). Heme oxygenase-1 mediates anti-adipogenesis effect of raspberry ketone in 3T3-L1 cells. Phytomedicine 31, 11-17. DOI: 10.1016/j.phymed.2017.05.005. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28606512
- US EPA (2020). Safer Chemical Ingredients List. Last updated 16 March 2022. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA 2020 CDR list (Chemical Data Reporting Rule). Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA InertFinder Database (2021). Record for 4-(p-hydroxyphenyl)-2-butanone. Last updated 21 May 2021. Accessed June 2021. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA ToxCast. Available via US EPA CompTox Chemistry Dashboard at https://comptox.epa.gov/dashboard
- US EPA TSCA inventory. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- Vanscheeuwijck P M et al (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. Food and Chemical Toxicology, 40, 113-131.
- Wang L et al. (2012). Raspberry ketone protects rats fed high-fat diets against nonalcoholic steatohepatitis J. Med. Food 15, 495-503. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22551412
- Wlodzimierz Borejsza-Wysocki and Geza Hrazdina; Biosynthesis of p-hydroxyphenylbutan-2-one in raspberry fruits and tissue cultures; Phytochemistry, Volume 35, Issue 3, February 1994, Pages 623-628; http://www.sciencedirect.com/
- Xiong SL et al. (2018). Inhibitory effect of raspberry ketone on α-glucosidase: Docking simulation integrating inhibition kinetics. Int. J. Biol. Macromol. 113, 212-218. DOI: 10.1016/j.ijbiomac.2018.02.124. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29477543
- Yimam M et al. (2019). Evaluation of natural product compositions for appetite suppression. J. Diet. Suppl. 16(1), 86-104. DOI: 10.1080/19390211.2018.1429518. PubMed, 2019 available at http://www.ncbi.nlm.nih.gov/pubmed/29443598
- Yuan B et al. (2020). UHPLC-QqQ-MS/MS method development and validation with statistical analysis: Determination of raspberry ketone metabolites in mice plasma and brain. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1149, 122146.
- Zhao D et al. (2020). Influence of diet-induced obesity on the bioavailability and metabolism of raspberry ketone (4-(4-hydroxyphenyl)-2-Butanone) in mice. Mol. Nutr. Food Res. 64(8), e1900907. DOI: 10.1002/mnfr.201900907. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32052560/

12. Other information

- Borejsza-Wysocki W et al. (1992). (p-Hydroxyphenyl)butan-2-one levels in raspberries determined by chromatographic and organoleptic methods. Journal of Agricultural and Food Chemistry 40, 1176.

- Cadby P (1996). Estimating intakes of flavouring substances. Food Additives and Contaminants 13, 453.
- Cadby P (2004). Novel estimates of the exposure to flavouring substances. Toxicology Letters 149, 215.
- Council of Europe (2000). Chemically-defined flavouring substances. Partial Agreement in the Social and Public Health Field, Strasbourg. 4th Edition, revised.
- Hall RL Ford RA (1999). Comparison of two methods to assess the intake of flavouring substances. Food Additives and Contaminants 16, 481.
- Japanese Ministry of Health Labour and Welfare (MHLW) (2017). In vitro mammalian chromosome aberration test for 4-(p-hydroxyphenyl)-2-butanone (CAS RN 5471-51-2). Available at: http://dra4.nihs.go.jp/mhlw_data/jsp/FileListPageENG.jsp?parameter_csno=5471-51-2
- MacCallum R et al. (2017). Raspberry ketone ingestion in dogs. Vet. Rec. 180(1), 23-24. DOI: 10.1136/vr.j37. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28062780
- Opdyke DLJ (1978). Monographs on Fragrance Raw Materials: 4-(para-Hydroxyphenyl)-2 butanone. Food and Cosmetics Toxicology 16, 781.

13. Last audited

June 2022

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

4-(p-HYDROXYPHENYL)-2-BUTANONE

COE No.:	755
FEMA No.:	2588
JECFA No.:	728
Chemical names:	4-(4-HYDROXYPHENYL)BUTAN-2-ONE
Synonyms:	p-HYDROXYBENZYLACETONE; 1-p-HYDROXYPHENYL-3-BUTANONE; RASPBERRY KETONE; RASTONE; OXYPHENYLON; OXANONE
Functional class:	FLAVOURING AGENT
Latest evaluation:	2000
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 901-JECFA 55/44
Specifications:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/172
Tox monograph:	FAS 46-JECFA 55/165

12 Nov 01

See Also:
Toxicological Abbreviations

Inhalation Toxicology, 18:685–706, 2006

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370600742680

Taylor & Francis
Taylor & Francis Group

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

Roger A. Renne
Battelle, Toxicology Northwest, Richland, Washington, USA

Hiroyuki Yoshimura
Japan Tobacco, Inc., Tokyo, Japan

Kei Yoshino
Japan Tobacco, Inc., Kanagawa, Japan

George Lulham
JTI Macdonald Corp., Toronto, Canada

Susumu Minamisawa
Japan Tobacco, Inc., Tokyo, Japan

Albrecht Tribukait
Japan Tobacco, Inc., Germany, Cologne, Germany

Dennis D. Dietz, Kyeonghee Monica Lee, and R. Bruce Westerberg
Battelle, Toxicology Northwest, Richland, Washington, USA

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

Received 2 January 2006; accepted 31 March 2006.
The authors are grateful to the following staff for their valuable contributions to this work: J. C. Blessing, M. L. Clark, K. M. Gideon, B. K. Hayden, J. D. Penner, J. T. Pierce, B. L. Thomas, and R. L. Thomas.
Address correspondence to Roger Renne, PO Box 999, Richland, WA 99352, USA. E-mail: renne@battelle.org

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

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

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

MATERIALS AND METHODS

Cigarette Design

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

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

TABLE 1
Blend composition of prototype cigarettes

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

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

Cigarette Performance

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

In Vitro Study Design

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

TABLE 2
Ingredients added to test cigarettes in study 1

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

(Continued on next page)

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

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

(Continued on next page)

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

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

(Continued on next page)

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

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

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

Inhalation Toxicity Study Design

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

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

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

Smoke Generation and Exposure System

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

TABLE 3
Ingredients added to study 2 test cigarettes

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

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

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

Exposure Atmosphere Characterization

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

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

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

Animals and Animal Care

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

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

Body Weight and Clinical Observations

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

Respiratory Function Measurements

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

Carboxyhemoglobin and Plasma Nicotine Determinations

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

Clinical Pathology

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

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

Necropsy and Tissue Collection

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

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

Histopathology

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

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

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

Statistical Methods

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

RESULTS

Cigarette Performance

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

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

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

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

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

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

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

Note. Cig, cigarette.

Exposure Atmosphere Characterization

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

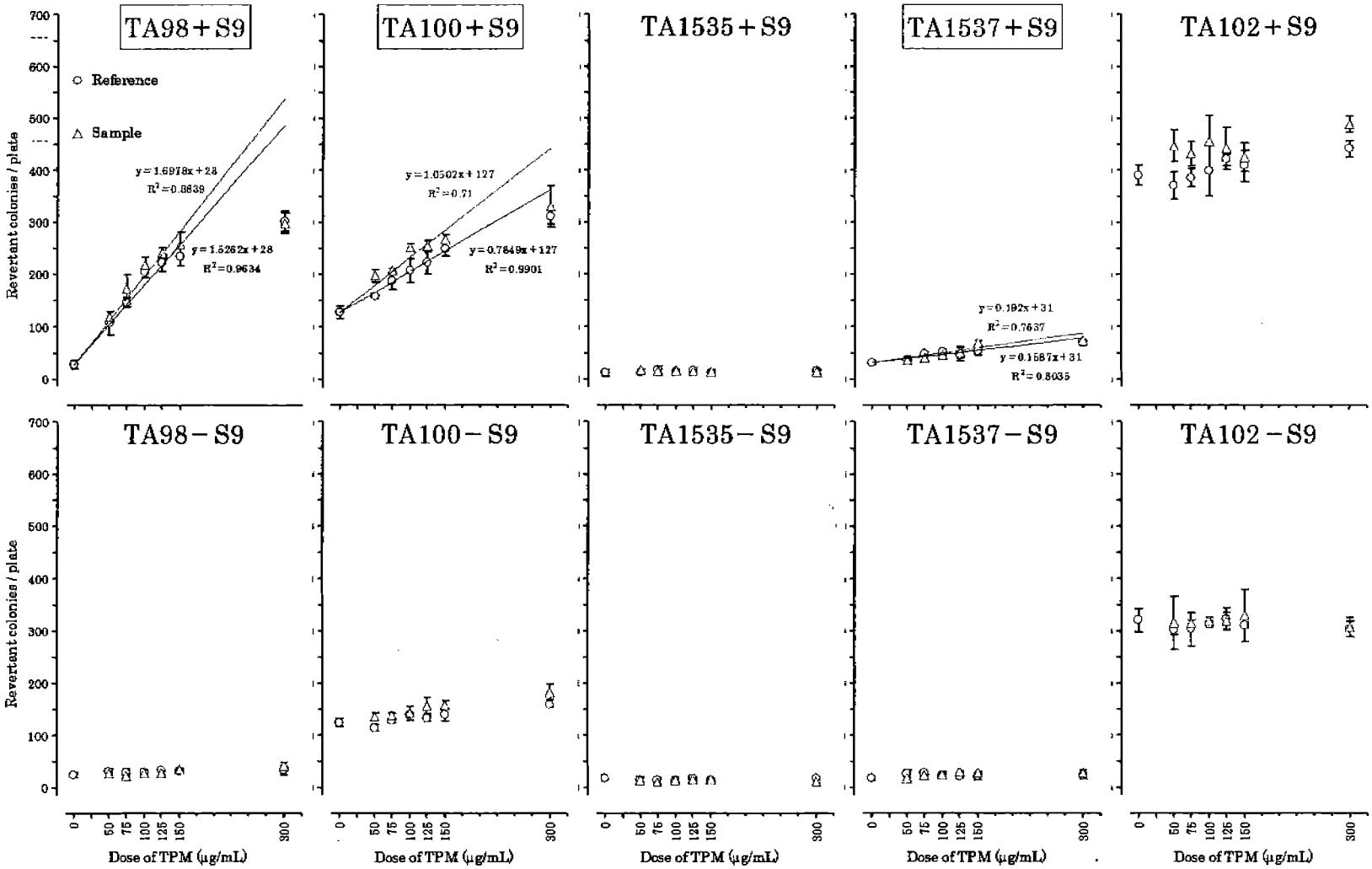
Body Weights and Clinical Observations

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

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

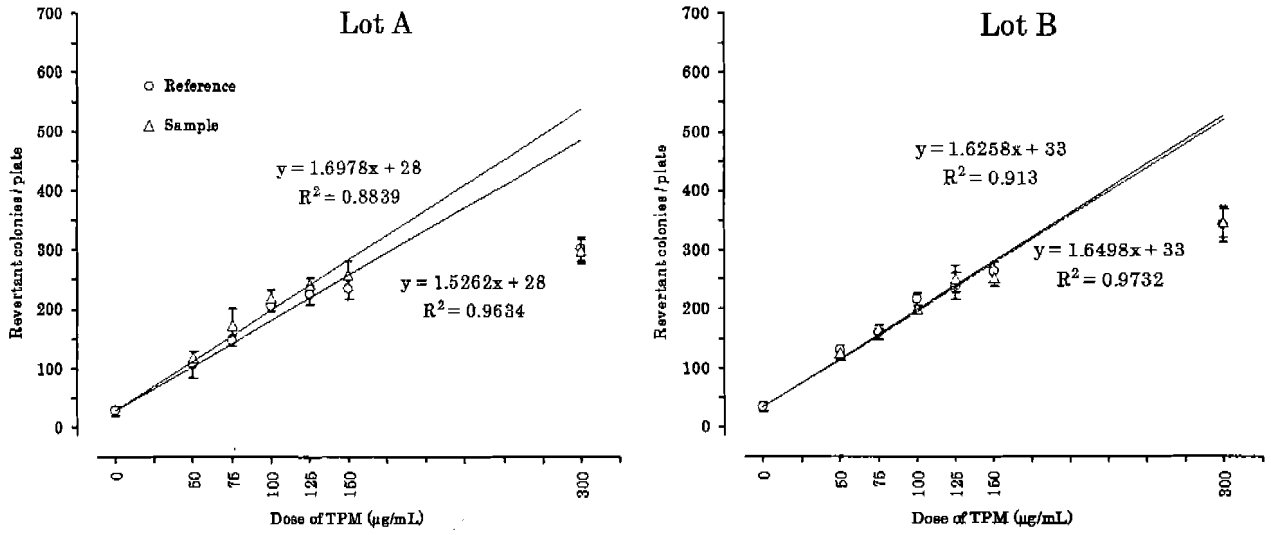
Organ Weights

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



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

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



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

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

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

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

Respiratory Physiology

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

Clinical Pathology

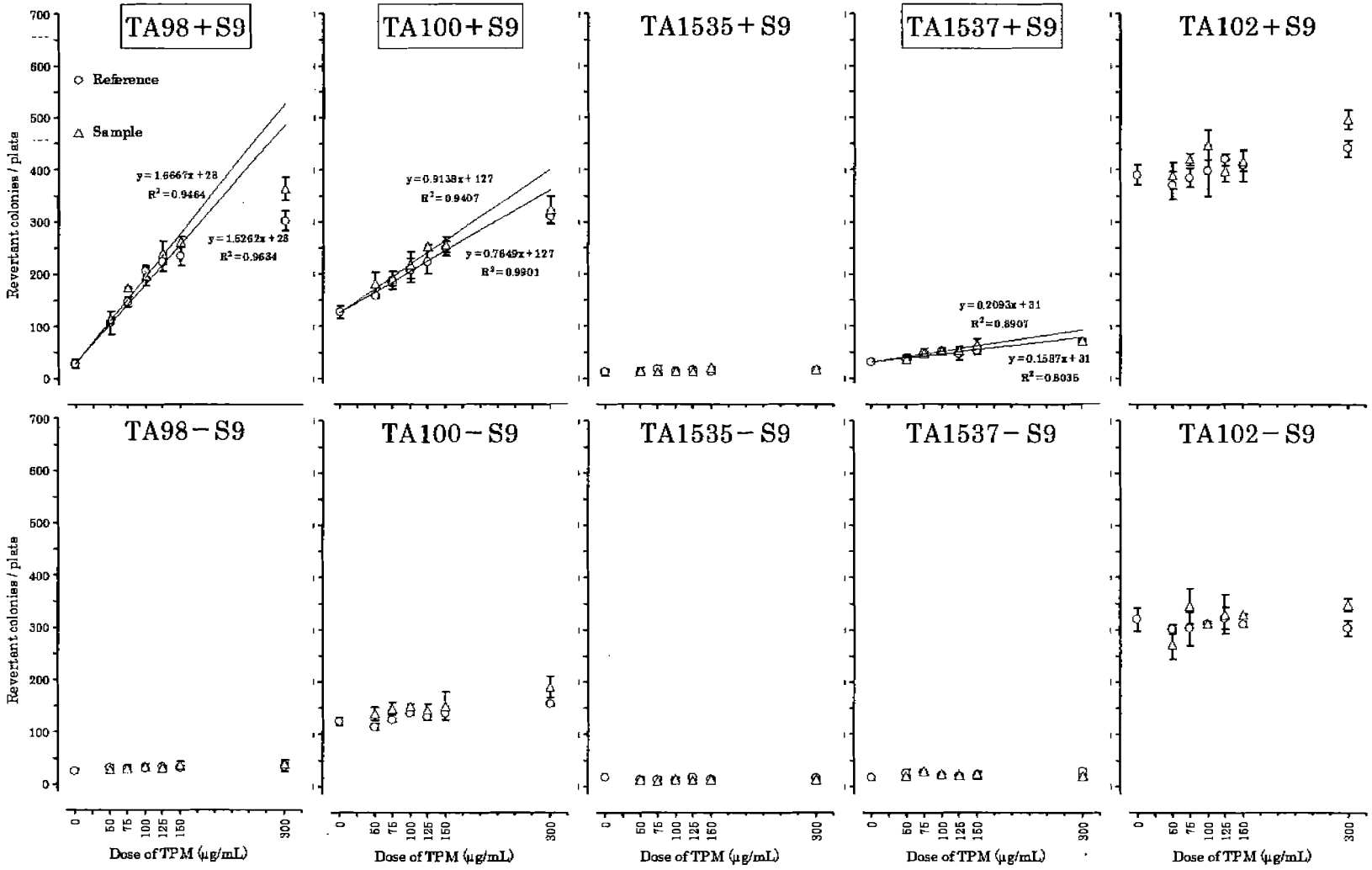
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2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

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

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

Pathology

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



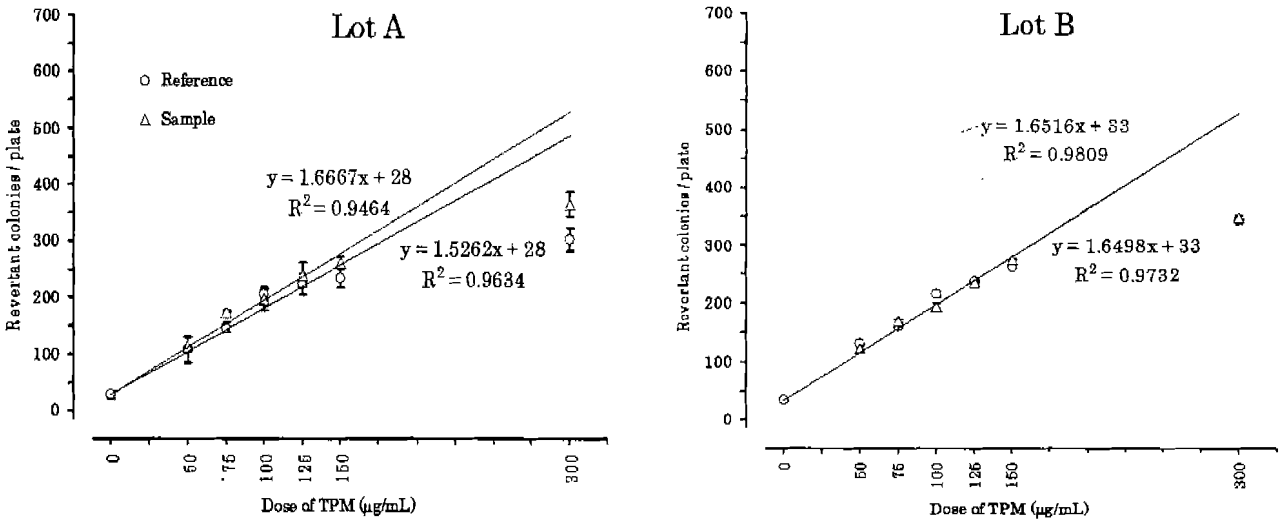
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

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

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

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



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

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

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

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

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

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

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

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

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

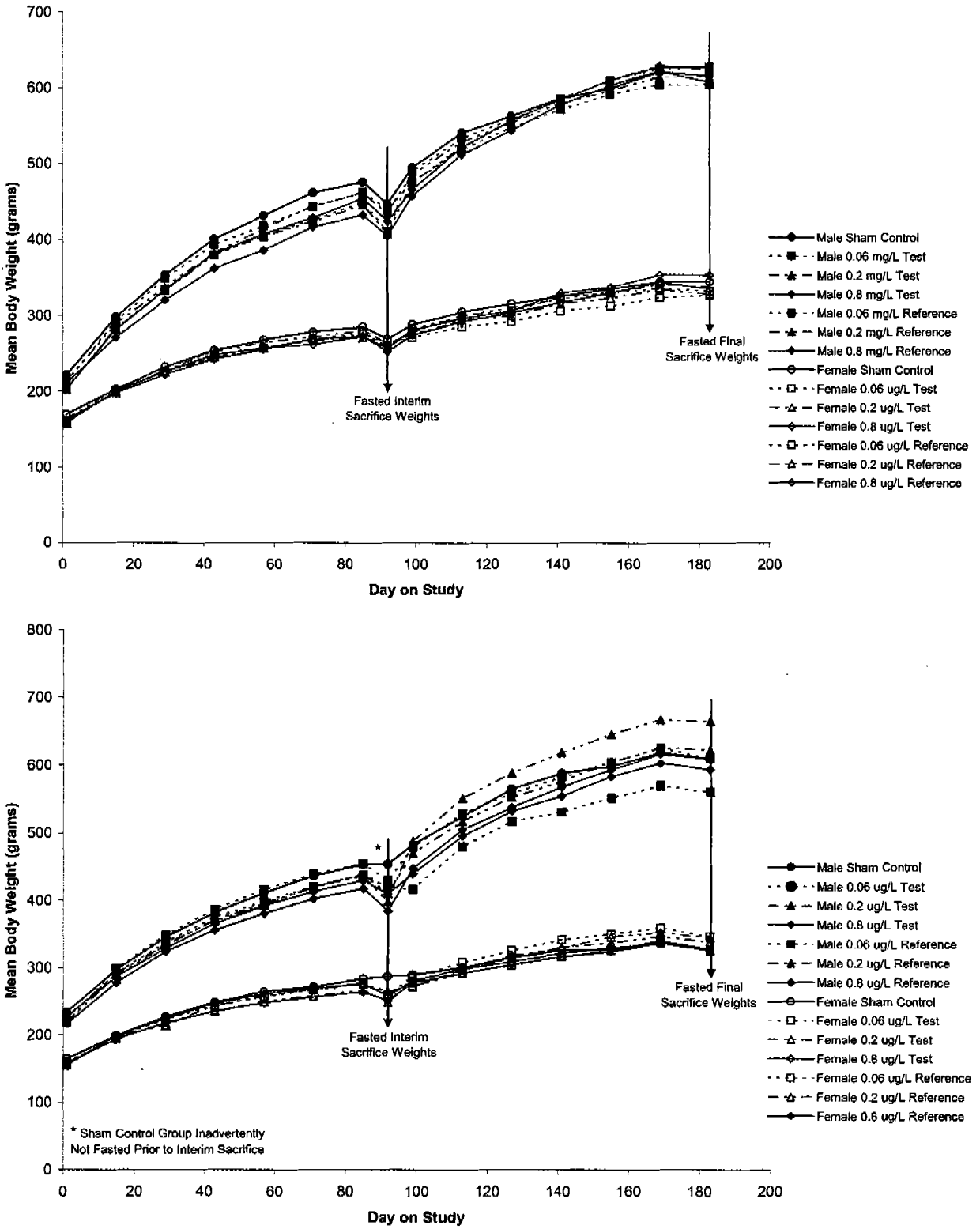


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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

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

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

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

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

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

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

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

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

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

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

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

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

Evaluation of Cell Proliferation Rates

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

REFERENCES

Ayres, P., Mosberg, A. T., and Coggins, C. R. 1990. Modernization of nose-only smoking machines for use in animal studies. *J. Am. Coll. Toxicol.* 9:441–446.

Ayres, P. H., Hayes, J. R., Higuchi, M. A., Mosberg, A. T., and Sagartz, J. W. 2001. Subchronic inhalation by rats of mainstream smoke from a cigarette that primarily heats tobacco compared to a cigarette that burns tobacco. *Inhal. Toxicol.* 13:149–186.

Baker, R. R., and Bishop, L. J. 2004. The pyrolysis of tobacco ingredients. *J. Anal. Appl. Pyrol.* 71:223–311.

Baker, R. R., Massey, E. H., and Smith, G. 2004. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chem. Toxicol.* 42:S53–S83.

Baumgartner, H., and Coggins, C. R. E. 1980. Description of a continuous-smoking inhalation machine for exposing small animals to tobacco smoke. *Beitr. Tabakforsch. Int.* 10:169–174.

Brecher, G., and Schneiderman, M. 1950. A time-saving device for the counting of reticulocytes. *Am. J. Clin. Pathol.* 20:1079.

Burger, G. T., Renne, R. A., Sagartz, J. W., Ayres, P. H., Coggins, C. R. E., Mosberg, A. T., and Hayes, A. W. 1989. Histologic changes in the respiratory tract induced by inhalation of xenobiotics: Physiologic adaptation or toxicity? *Toxicol. Appl. Pharmacol.* 101:521–542.

Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Food Chem. Toxicol.* 40:77–91.

Coggins, C. R. E., Fouillet, X. L., Lam, R., and Morgan, K. T. 1980. Cigarette smoke induced pathology of the rat respiratory tract. A comparison of the effects of the particulate and vapor phases. *Toxicology* 16:83–101.

Coggins, C. R. E., Duchosal, F., Musy, C., and Ventrone, R. 1981. The measurement of respiratory patterns in rodents, using whole body plethysmography and pneumotachography. *Lab. Anim.* 15:137–140.

Coggins, C. R. E., Ayres, P. H., Mosberg, A. T., and Burger, G. T. 1989. Comparative inhalation study in rats, using a second prototype of a cigarette that heats rather than burns tobacco. *Inhal. Toxicol.* 1:197–226.

Dalbey, W. E., Nettesheim, P., Griesemer, R., Caton, J. E., and Guerin, M. R. 1980. Chronic inhalation of cigarette smoke by F344 rats. *J. NCI.* 64:383–390.

Gaworski, C. L., Dozier, M. M., Gerhart, J. M., Rajendran, N., Brennecke, L. H., Aranyi, C., and Heck, J. D. 1997. 13-wk inhalation study of menthol cigarette smoke. *Food Chem. Toxicol.* 35:683–692.

Gaworski, C. L., Dozier, M. M., Heck, J. D., Gerhart, J. M., Rajendran, N., David, R. M., Brennecke, L. H., and Morrisey, R. 1998. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-wk inhalation exposures in rats. *Inhal. Toxicol.* 10:357–381.

Gopinath, C., Prentice, D. E., and Lewis, D. J. 1987. *Atlas of experimental toxicologic pathology*. Lancaster, PA: MTP Press.

Hill, M. A., Watson, C. R., and Moss, O. R. 1977. *NEWCAS—An interactive computer program for particle size analysis*. PNL-2405. Richland, WA: Battelle Pacific Northwest Laboratories.

Hoffman, D., and Hoffman, I. 1997. The changing cigarette, 1950–1995. *J. Toxicol. Environ. Health* 50:307–364.

Hoffman, D., and Hoffman, I. 2001. The changing cigarette: chemical studies and bioassays. In *National Cancer Institute (NCI) Monograph 13, Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine*, pp. 159–191. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Cancer Institute, Bethesda, MD, USA.

LaVoie, E. J., Hecht, S. S., Hoffman, D., and Wynder, E. L. 1980. The less harmful cigarettes and tobacco smoke flavours. In *Banbury Report 3, A Safe Cigarette?* eds. G. B. Gori and F. G. Back, pp. 251–260. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Lewis, D. J. 1980. Factors affecting the distribution of tobacco smoke-induced lesions in rodent larynx. *Toxicol. Lett.* 9:189–194.

Lewis, D. J. 1991. Morphologic assessment of pathological changes within the rat larynx. *Toxicol. Pathol*, 19:352–357.

National Academy of Sciences. 1996. *Guide for the care and use of laboratory animals*. Washington, DC: Institute of Laboratory Animal Resources, Commission on Life Sciences, National Reserch Council. National Academy Press.

Paschke, T., Scherer, G., and Heller, W. F. 2002. Effects of ingredients on cigarette smoke composition and biological activity: A literature review. *Beitr. Tabakforsch. Int./Contrib. Tobacco Res.* 20:107–247.

Renne, R. A., Gideon, K. M., Miller, R. A., Mellick, P. W., and Grumbein, S. L. 1992. Histologic methods and interspecies variations in the laryngeal histology of F344/N rats and B6C3F1 mice. *Toxicol. Pathol.* 20:44–51.

Rodgman, A. 2002a. Some studies of the effects of additives on cigarette mainstream smoke properties. I. Flavorants. *Beitr. Tabakforsch. Int.* 20:83–103.

Rodgman, A. 2002b. Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials. *Beitr. Tabakforsch. Int.* 20:279–299.

Rodgman, A., and Green, C. R. 2002. Toxic chemicals in cigarette mainstream smoke—Hazard and hoopla. *Beitr. Tabakforsch. Int.* 20:481–545.

Roemer, E., Tewes, F. J., Mesigen, T. J., Veltel, D. J., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. *Food Chem. Toxicol.* 40:105–111.

Rustemeier, K., Stabbert, R., Haussmann, H. J., Roemer, E., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food Chem. Toxicol.* 40:93–104.

Siegel, S. 1956. *Non-parametric statistics for the behavioral sciences*. New York: McGraw-Hill.

Vanscheeuwijck, P. M., Teredesai, A., Terpstra, P. M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food Chem. Toxicol.* 40:113–131.

Wehner, A. P., Renne, R. A., Greenspan, B. J., DeFord, H. S., Ragan, H. A., Westerberg, R. B., Wright, C. W., Buschbom, R. L., Burger, G. T., Hayes, A. W., Coggins, C. R. E., and Mosberg, A. T. 1990. Comparative subchronic inhalation bioassay in hamsters of a cigarette that only heats tobacco. *Inhal. Toxicol.* 2:255–284.

World Health Organization. 2001. *Advancing knowledge on regulating tobacco products*, pp. 40–46. Geneva: WHO.

Wynder, E. L., and Hoffman, D. 1967. *Tobacco and tobacco smoke. Studies in experimental carcinogenesis*, pp. 526–528. New York: Academic Press.

Young, J. T. 1981. Histopathologic examination of the rat nasal cavity. *Fundam. Appl. Toxicol.* 1:309–312.

European Food Safety Authority

The EFSA Journal (2008) 711, 1-50

Flavouring Group Evaluation 58 (FGE.58)

Consideration of phenol derivatives evaluated by JECFA (55th meeting) structurally related to ring substituted phenolic substances evaluated by EFSA in FGE.22 (2006)

(Commission Regulation (EC) No 1565/2000 of 18 July 2000)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission

(Question No EFSA-Q-2008-032J)

(Adopted on 3 July 2007)

PANEL MEMBERS

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

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular the Scientific Panel is requested to consider JECFA's 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 present consideration concerns 48 phenol and phenol derivatives evaluated by JECFA (55th meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 23 ring substituted phenolic substances evaluated in the Flavouring Group Evaluation 22 (FGE.22).

The Panel concluded that the 44 substances in the JECFA flavouring group of phenol derivatives are structurally related to the group of ring substituted phenolic substances evaluated by EFSA in FGE.22.

Further four substances were evaluated by the JECFA in this group, one is not in the Register (2-phenylphenol [JECFA-no: 735]), and phenol [FL-no: 04.041] and two phenyl esters, phenyl acetate and phenyl salicylate [FL-no: 09.688 and 09.689] will be considered together in a separate FGE.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 44 substances considered in this FGE.

For eight substances [FL-no: 04.037, 04.052, 04.053, 04.056, 07.046, 09.036, 09.102 and 09.288] the JECFA evaluation is only based on Maximised Survey-derived Daily Intake (MSDI) values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all 44 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 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity are available for 41 of the 44 JECFA evaluated substances. For one substance [FL-no: 07.046] information of the stereoisomeric composition is lacking and for two other substances [FL-no: 07.135 and 09.102] further information on the composition is requested.

Thus, for nine substances [FL-no: 04.037, 04.052, 04.053, 04.056, 07.046, 07.135, 09.036, 09.102 and 09.288] the Panel has reservations (only USA production volumes available and/or missing data on isomerism/composition). For the remaining 35 of the 44 JECFA evaluated phenol derivatives [FL-no: 04.005, 04.006, 04.007, 04.008, 04.009, 04.019, 04.022, 04.026, 04.027, 04.028, 04.031, 04.036, 04.042, 04.044, 04.045, 04.046, 04.047, 04.048, 04.049, 04.050, 04.051, 04.057, 04.064, 04.085, 07.005, 07.055, 07.124, 09.174, 09.228, 09.301, 09.429, 09.480, 09.518, 09.709 and 09.711] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEYWORDS

Phenol derivatives, JECFA, 55th meeting, phenyl, FGE.22

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2006/252/EC (EC, 2006). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000), 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 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 – 2006, during its 55th, 57th, 59th, 61st, 63rd and 65th meetings, the JECFA evaluated about 900 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, 2000). 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, 2000), 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 AFC Panel (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 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 Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. 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 Criterion 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 criterion 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 Panel evaluation could lead to a different opinion than that of the JECFA, e.g. Panel requests additional information on 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.

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 48 flavouring substances consisting of phenol and phenol derivatives. One of the JECFA evaluated substances, 2-phenylphenol [JECFA-no: 735], is not in the Register. Further three substances will not be dealt with in this FGE: phenol itself [FL-no: 04.041] and two phenyl esters, phenyl acetate and phenyl salicylate [FL-no: 09.688 and 09.689]. These three substances will be considered together in a separate FGE. This consideration will therefore only deal with 44 JECFA evaluated substances.

1.1.2. EFSA Considerations

The Panel concluded that all the 44 substances in the JECFA flavouring group of phenol derivatives are structurally related to the group of 23 ring-substituted phenolic substances evaluated by EFSA in the Flavouring Group Evaluation 22 (FGE.22).

1.2. Isomers

1.2.1. JECFA Status

None of the 44 Register substances in the group of the JECFA evaluated phenol derivatives have a chiral centre. One substance vanillylidene acetone [FL-no: 07.046] has a double bond corresponding to two possible geometric isomers.

1.2.2. EFSA Considerations

Information is lacking about the stereoisomerism for [FL-no: 07.046].

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 44 substances (JECFA, 2000d). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate except that information on isomerism is lacking for [FL-no: 07.046], see Section 1.2 and further information on the composition of [FL-no: 07.135 and 09.102] is requested.

2. Intake Estimations

2.1. JECFA Status

For 36 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the remaining eight substances production figures are only available for the USA

2.2. EFSA Considerations

As production figures are only available for the USA for eight substances, MSDI values for the EU cannot be calculated for these [FL-no: 04.037, 04.052, 04.053, 04.056, 07.046, 09.036, 09.102 and 09.288].

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken from JECFA (JECFA, 2001b)

In vitro

Negative results were reported in the standard assay for reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 incubated with *ortho*-cresol at up to 5000 µg/plate (Douglas et al., 1980; Florin et al., 1980; Nestmann et al., 1980; Pool & Lin, 1982; Haworth et al., 1983; Massey et al., 1994); *meta*-cresol and *para*-cresol at up to 5000 µg/plate (Douglas et al., 1980); Florin et al., 1980; (Nestmann et al., 1980; Pool & Lin, 1982; Haworth et al., 1983)); *para*-ethylphenol, 2,5-xylenol, 2,6-xylenol, and 3,4-xylenol at 367 µg/plate (Florin et al., 1980) 4-(1,1-dimethyl)ethyl phenol at up to 2000 µg/plate (Dean et al., 1985); thymol at up to 1000 µg/plate (Florin et al., 1980; Azizan & Blevins, 1995); resorcinol at up to 7700 µg/plate (Gocke et al., 1981; Haworth et al., 1983); guaiacol at up to 111 726 µg/plate (Douglas et al., 1980; Nestmann et al., 1980; Pool & Lin, 1982; Haworth et al., 1983; Aeschbacher et al., 1989)-dimethoxyphenol at up to 16 000 µg/plate (McMahon et al., 1979; Douglas et al., 1980; Florin et al., 1980; Pool & Lin, 1982) and 2-hydroxyacetophenone at 408 µg/plate (Florin et al., 1980), with and without metabolic activation. However, in an assay with a modified minimal ZLM medium for *Escherichia coli*, the results varied by bacterial strain (Gocke et al., 1981). Resorcinol was mutagenic at doses of 550-7700 µg/plate only in TA1535 without metabolic activation and in TA100 with metabolic activation. The same authors reported negative results in all five *S. typhimurium* strains with and without metabolic activation in the standard Vogel-Bonner medium, which contains a concentration of citrate and other ions that is two to four times higher (Gocke et al., 1981). Negative results with resorcinol at doses up to 3333 µg/plate were reported in another study when Vogel-Bonner medium

was used (Haworth et al., 1983). No mutagenicity was found in *E. coli* exposed to 2,6-dimethoxyphenol at concentrations up to 1000 µg/ml (McMahon et al., 1979).

Forward mutation was not induced in mouse lymphoma L5178YTk+/- cells by resorcinol at 125-2000 µg/ml without metabolic activation (McGregor et al., 1988a). Positive results in this assay were reported with 2-phenylphenol at doses of 0.32-60 µg/ml without metabolic activation and 0.32-5 µg/ml with activation, but these doses were cytotoxic (NTP, 1986e).

Sister chromatid exchange was not induced in human lymphocytes by *ortho*-cresol at concentrations up to 54 µg/ml, *meta*-cresol at up to 108.µg/ml, *para*-cresol at up to 54 µg/ml, *para*-ethyl phenol at up to 27 µg/ml, 2,6-xylenol at up to 31 µg/ml, resorcinol at up to 28 µg/ml, 2,6-dimethoxyphenol at up to 77 µg/ml (Jansson et al., 1986; Jansson et al., 1988) or *para*-vinylphenol at up to 12 µg/ml (Jansson et al., 1986). No evidence of sister chromatid exchange was found in human fibroblasts exposed to *ortho*-cresol at concentrations up to 433 µg/ml or to *meta*- or *para*-cresol at 865 µg/ml (Cheng & Kligerman, 1984), or in Chinese hamster ovary cells exposed to resorcinol at 0.6-2 µg/ml (Wild et al., 1981). Weakly positive results were reported with ortho-cresol at a concentration of 865 µg/ml (Cheng & Kligerman, 1984).

In vivo

The results of assays for genotoxicity *in vivo* were predominantly negative. The frequency of micronucleated polychromatic erythrocytes was not increased in mice after intraperitoneal injections of resorcinol of doses of 55-220 mg/kg bw (Gocke et al., 1981) [two doses of 220 mg/kg bw administered 24 h apart]. (Wild et al., 1981)).

The ability of ortho-cresol [FL-no: 04.027], meta-cresol [FL-no: 04.026], and para-cresol [FL-no: 04.028] to induce sister chromatid exchange was also studied *in vivo* (Cheng & Kligerman, 1984). Mouse bone-marrow cells, alveolar macrophages, and regenerating liver cells were examined after intraperitoneal administration of *ortho*- or *meta*-cresol at 200 mg/kg bw or *para*-cresol at 75 mg/kg bw. The results were negative.

Conclusion on genotoxicity

Overall, the 44 phenol derivatives in this group of flavouring substances are unlikely to be genotoxic *in vivo*.

For a summary of *in vitro* genotoxicity data considered by JECFA see Table 2.1.

3.2. Genotoxicity Studies - Text Taken from EFSA (EFSA, 2006h)

In vitro / in vivo

Data from *in vitro* tests are available for 12 candidate [FL-no: 04.020, 04.021, 04.065, 04.066, 04.070, 04.076, 04.077, 04.080, 04.095, 07.142, 07.164 and 07.243] and 18 supporting substances. Data from *in vivo* tests are available for one candidate [FL-no: 04.077] and six supporting substances. Most studies are of limited or insufficient quality or are inadequately reported, thus for some of the studies the validity of the results could not be evaluated.

Positive results were observed with three candidate substances [FL-no: 04.077, 04.080 and 07.142].

4-Methoxyphenol [FL-no: 04.077] did not induce gene mutations in bacteria (Haworth et al., 1983). In a gene mutation assay in mammalian cells (MLTK assay) a positive result was observed for 4-

methoxyphenol without metabolic activation and a negative result with metabolic activation using an S9 homogenate (Rogers-Back, 1986). In the test without metabolic activation an increase in the percentage of small colonies was noted indicating a potential for chromosomal aberrations. 4-Methoxyphenol induced chromosomal aberrations in CHO cells in the presence and absence of metabolic activation (Putman, 1986). 4-Methoxyphenol did not induce sister chromatid exchanges in human lymphocytes (Jansson et al., 1988), however, the study was of limited quality. Since 4-methoxyphenol did not induce chromosomal aberration *in vivo* in rat bone marrow cells after oral application (Esber, 1986) the results observed *in vitro* with 4-methoxyphenol were considered to be of no concern.

3,4-Methylenedioxyphenol [FL-no: 04.080] was reported to be negative in a bacterial mutagenicity assay in the presence and absence of metabolic activation while a positive result was reported in a gene mutation assay in mammalian cells (MLTK assay) both in the presence and absence of metabolic activation (Longfellow, 1985/1986). However, this information was only available as a very short abstract and the study reports were not available for evaluation. *In vivo* studies were not available for this candidate substance.

Acetovanillone [FL-no: 07.142] was positive in a yeast assay without metabolic activation (Nestmann & Lee, 1983). This result is not considered to preclude the substance to be evaluated through the Procedure. The substance was negative in bacterial mutagenicity assays in the presence and absence of metabolic activation (Nestmann et al., 1980; Xu et al., 1984). However, reporting of the bacterial assays and the quality of data were insufficient and the validity of the results could not be evaluated.

With the candidate substances 2-ethylphenol [FL-no: 04.070] and 2,4-dimethylphenol [FL-no: 04.066] negative results were observed in bacterial gene mutation assays (Zeiger et al., 1992; Mortelmans et al., 1986; Pool & Lin, 1982). All other results observed in several assays with these two and seven further candidate substances for which data were available were negative. However, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With supporting substances positive and negative results were obtained in *in vitro* tests.

2-Methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026], 4-methylphenol [FL-no: 04.028], 2-methoxyphenol [FL-no: 04.005], and 2,6-dimethoxyphenol [FL-no: 04.036] did not induce gene mutations in bacterial assays of acceptable quality (Haworth et al., 1983; Pool & Lin, 1982). The validity of a positive result observed with 2-methylphenol FL-no: 04.027] in bacteria (Claxton, 1985) cannot be evaluated.

2,6-Dimethylphenol [FL-no: 04.042] induced chromosomal aberrations in mammalian cells in the presence of S9 while the result was negative in the absence of metabolic activation (Völkner, 1994). The *in vitro* genotoxic potential of 2,6-dimethylphenol does not give rise to concern with respect to other alkylated phenols in this FGE, as they are alkyl substituted in either *m*- or *p*-positions. Phenols, substituted in *m*- or *p*-posititon are expected to be metabolised differently from 2,6-dimethylphenol.

2-Methoxyphenol [FL-no: 04.005], 2-methoxy-4-methylphenol [FL-no: 04.007], 2-methylphenol [FL-no: 04.027] and a mixture of 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] induced sister chromatid exchanges in human lymphocytes or CHO cells (Jansson et al., 1986) [FL-no: 04.005]; (Jansson et al., 1988) [FL-no: 04.007]; (Galloway

& Brusick, 1981) [FL-no: 04.027]; (Galloway & Brusick, 1980) [mixture]). In most cases the effects were observed in the presence and absence of metabolic activation.

The mixture of 2-methylphenol FL-no: 04.027], 3-methylphenol FL-no: 04.026] and 4-methylphenol FL-no: 04.028] resulted in an equivocal response in a UDS assay (Myhr & Brusick, 1980) while induction of UDS was observed with 4-methylphenol FL-no: 04.028] in another *in vitro* study (Crowley & Margard, 1978).

All other results observed in several *in vitro* assays with these and the remaining supporting substances were negative, however, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With the supporting substances 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] negative results were obtained in *in vivo* SCE assays (Cheng & Kligerman, 1984). However, these data were of limited quality. 3-Methylphenol [FL-no: 04.026] did not induce chromosomal aberrations in mice (Ivett et al., 1989). However, the validity of the result cannot be evaluated as the study is inadequately reported. 2-Methylphenol [FL-no: 04.027] and carvacrol [FL-no: 04.031] did not induce mutations in *Drosophila* (Sernau, 1989; Kono et al., 1995).

Conclusion on genotoxicity

Overall, the available genotoxicity data on the supporting substances would not preclude evaluation of the candidate substances through the Procedure. One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080] was reported to have genotoxic potential *in vitro*. *In vivo* studies were not available for this candidate substance. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Table 2.2 and 2.3.

3.3. EFSA Considerations

2,6-Dimethylphenol was not mutagenic in four strains of Salmonella typhimurium, when tested for gene mutations by base-pair changes or frame shifts. Neither did it induce gene mutations at the HPRT locus in V79 Chinese Hamster cells (Castle & Larsen, 1997).

2,6-Dimethylphenol induced structural chromosomal aberrations *in vitro* as determined by the chromosomal aberration test in the V79 Chinese hamster cell line. However, when tested *in vivo*, 2,6-Dimethylphenol did not induce chromosomal aberrations in bone marrow cells of male or female Sprague-Dawley rats (Castle & Larsen, 1997). See Table 2.4 for a summary of these studies.

The Panel concluded that the data available do not preclude evaluation of the 44 JECFA evaluated phenol derivatives through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 44 Phenol Derivatives by JECFA (JECFA, 2001a):

According to the JECFA all 44 substances belong to structural class I using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 43 phenol derivatives at step A3 in the JECFA Procedure – i.e. that the substances are expected to be metabolised to innocuous products (step 2) and that the intakes for the substances are below the threshold for structural class I (step A3). One substance [FL-no. 07.055], 4-(p-hydroxyphenyl)-2-butanone, does not occur endogenously in humans, therefore the evaluation proceeded to step A5, where it was considered as of no safety concern at the estimated level of intake based on a 13-week study in which a NOAEL of 280 mg/kg bw/day provides a margin of safety of more than 1000.

In conclusion the JECFA evaluated all 44 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 44 phenol derivatives are summarised in Table 3.1: Summary of Safety Evaluation of phenol derivatives (JECFA, 2001a).

4.2. Application of the Procedure to 23 Ring Substituted Phenolic Substances Evaluated by EFSA (EFSA, 2006h):

Twentythree candidate substances were evaluated in FGE.22. Nineteen substances are classified into structural class I and three into structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080], showed genotoxic potential *in vitro*. Therefore, the Panel concluded that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

The remaining 22 substances were concluded at step A3 – i.e. that the substances are expected to be metabolised to innocuous products (step 2) and that the estimated daily intakes are below the thresholds for the structural classes I and II (step A3).

In conclusion the Panel considered that the 22 substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

The stepwise evaluations of the 22 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.22) (see Table 3.2).

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for the 44 substances in the group of phenol derivatives.

5. Conclusion

The Panel concluded that the 44 substances in the JECFA flavouring group of phenol derivatives are structurally related to the group of ring substituted phenolic substances evaluated by EFSA in the Flavouring Group Evaluation 22 (FGE.22).

Further four substances were evaluated by the JECFA in this group, one is not in the Register (2-phenylphenol [JECFA-no: 735]), and phenol [FL-no: 04.041] and two phenyl esters, phenyl acetate and phenyl salicylate [FL-no: 09.688 and 09.689] will be considered together in a separate FGE.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 44 substances considered in this FGE.

For eight substances [FL-no: 04.037, 04.052, 04.053, 04.056, 07.046, 09.036, 09.102 and 09.288] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all 44 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 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity are available for 41 of the 44 JECFA evaluated substances. For one substance [FL-no: 07.046] information of the stereoisomeric composition is lacking and for two other substances [FL-no: 07.135 and 09.102] further information on the composition is requested.

Thus, for nine substances [FL-no: 04.037, 04.052, 04.053, 04.056, 07.046, 07.135, 09.036, 09.102 and 09.288] the Panel has reservations (only USA production volumes available and/or missing data on isomerism/composition). For the remaining 35 of the 44 JECFA evaluated phenol derivatives [FL-no: 04.005, 04.006, 04.007, 04.008, 04.009, 04.019, 04.022, 04.026, 04.027, 04.028, 04.031, 04.036, 04.042, 04.044, 04.045, 04.046, 04.047, 04.048, 04.049, 04.050, 04.051, 04.057, 04.064, 04.085, 07.005, 07.055, 07.124, 09.174, 09.228, 09.301, 09.429, 09.480, 09.518, 09.709 and 09.711] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

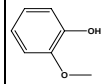
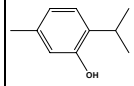
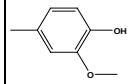
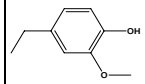
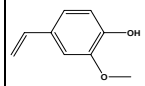
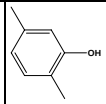
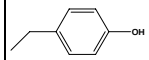
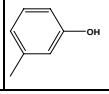
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
04.005 713	2-Methoxyphenol		2532 173 90-05-1	Solid $C_7H_8O_2$ 124.14	Slightly soluble Very soluble	203-206 28 IR 99 %	1.540-1.545 1.129-1.140	According to JECFA: Melting point is "28° (liquid which may crystallize)".
04.006 709	Thymol		3066 174 89-83-8	Solid $C_{10}H_{14}O$ 150.22	Slightly soluble Moderately soluble	232-233 48 IR 98 %	n.a. n.a.	According to JECFA: Melting point is "48° (minimum)".
04.007 715	2-Methoxy-4-methylphenol		2671 175 93-51-6	Liquid $C_8H_{10}O_2$ 138.17	Slightly soluble Miscible	220-222 IR 98 %	1.534-1.538 1.089-1.096	
04.008 716	4-Ethylguaiacol		2436 176 2785-89-9	Liquid $C_9H_{12}O_2$ 152.19	Slightly soluble Miscible	229-235 IR 98 %	1.524-1.534 1.056-1.066	
04.009 725	2-Methoxy-4-vinylphenol		2675 177 7786-61-0	Liquid $C_9H_{10}O_2$ 150.18	Insoluble Miscible	224 IR 96 %	1.534-1.538 1.090-1.096	
04.019 706	2,5-Dimethylphenol		3595 537 95-87-4	Solid $C_8H_{10}O$ 122.17	Slightly soluble Moderately soluble	211-212 70 IR 99 %	n.a. n.a.	According to JECFA: Melting point is "70° (minimum)".
04.022 694	4-Ethylphenol		3156 550 123-07-9	Solid $C_8H_{10}O$ 122.17	Slightly soluble Very soluble	218-219 47-48 IR 99 %	n.a. n.a.	
04.026 692	3-Methylphenol		3530 617 108-39-4	Liquid C_7H_8O 108.14	Slightly soluble Miscible	201 IR 98 %	1.537-1.543 1.028-1.033	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

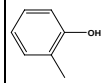
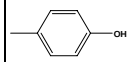
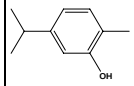
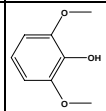
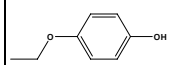
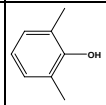
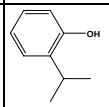
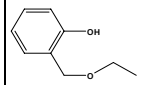
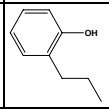
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
04.027 691	2-Methylphenol		3480 618 95-48-7	Solid C_7H_8O 108.14	Soluble Very soluble	191 31-32 IR 98 %	1.544-1.548 1.041-1.046	According to JECFA: Melting point is "31-32° (liquid which may crystallize below 30°)".
04.028 693	4-Methylphenol		2337 619 106-44-5	Solid C_7H_8O 108.14	Slightly soluble Very soluble	201-202 32-36 IR 99 %	n.a. n.a.	
04.031 710	Carvacrol		2245 2055 499-75-2	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Miscible	236-238 IR 98 %	1.521-1.528 0.974-0.979	
04.036 721	2,6-Dimethoxyphenol		3137 2233 91-10-1	Solid $C_8H_{10}O_3$ 154.17	Slightly soluble Moderately soluble	261-262 53 IR 98 %	n.a. n.a.	According to JECFA: Melting point is "53° (minimum)".
04.037 720	4-Ethoxyphenol		3695 2258 622-62-8	Solid $C_8H_{10}O_2$ 138.17	Slightly soluble Moderately soluble	246-247 64 IR 95 %	n.a. n.a.	According to JECFA: Melting point is "64° (minimum)".
04.042 707	2,6-Dimethylphenol		3249 11261 576-26-1	Solid $C_8H_{10}O$ 122.17	Very soluble	212 45-49 IR 99 %	n.a. n.a.	SW 8).
04.044 697	2-Isopropylphenol		3461 11234 88-69-7	Liquid $C_9H_{12}O$ 136.19	Slightly soluble Miscible	213-214 IR 98 %	1.525-1.530 0.989-0.999	
04.045 714	2-(Ethoxymethyl)phenol		3485 11905 20920-83-6	Liquid $C_9H_{12}O_2$ 152.19	Slightly soluble Miscible	111-113 (26hPa) MS 99 %	1.517-1.523 1.047-1.052	
04.046 695	2-Propylphenol		3522 11908 644-35-9	Liquid $C_9H_{12}O$ 136.19	Slightly soluble Miscible	224 IR 96 %	1.524-1.528 0.988-0.996	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

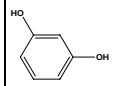
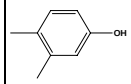
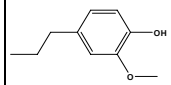
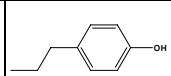
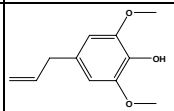
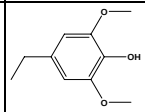
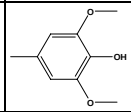
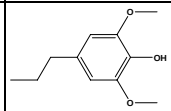
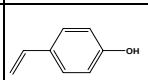
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
04.047 712	Benzene-1,3-diol		3589 11250 108-46-3	Solid $C_6H_6O_2$ 110.11	Soluble Moderately soluble	277-281 109 IR 98 %	n.a. n.a.	
04.048 708	3,4-Dimethylphenol		3596 11262 95-65-8	Solid $C_8H_{10}O$ 122.17	Slightly soluble Moderately soluble	225 62-68 IR 98 %	n.a. n.a.	
04.049 717	2-Methoxy-4-propylphenol		3598 2785-87-7	Liquid $C_{10}H_{14}O_2$ 166.22	Slightly soluble Miscible	250 IR 98 %	1.520-1.525 1.034-1.040	
04.050 696	4-Propylphenol		3649 645-56-7	Liquid $C_9H_{12}O$ 136.19	Insoluble Miscible	232 IR 98 %	1.523-1.527 0.980-0.986	
04.051 726	4-Allyl-2,6-dimethoxyphenol		3655 11214 6627-88-9	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	168 (14 hPa) IR 98 %	1.548-1.550 1.089-1.095	
04.052 723	4-Ethyl-2,6-dimethoxyphenol		3671 11231 14059-92-8	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	106 (0.3 hPa) MS 98 %	1.536-1.537 1.075-1.080	
04.053 722	4-Methyl-2,6-dimethoxyphenol		3704 6638-05-7	Solid $C_9H_{12}O_3$ 168.19	Insoluble Moderately soluble	145-146 (16hPa) 37-42 IR 97 %	n.a. n.a.	
04.056 724	2,6-Dimethoxy-4-propylphenol		3729 6766-82-1	Liquid $C_{11}H_{16}O_3$ 196.25	Insoluble Miscible	115 (0.5 hPa) IR 98 %	1.529-1.530 1.071-1.076	
04.057 711	4-Vinylphenol		3739 11257 2628-17-3	Solid C_8H_8O 120.15	Soluble Moderately soluble	189 68 MS 99 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

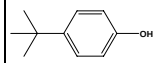
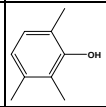
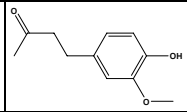
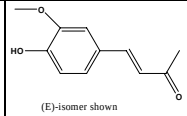
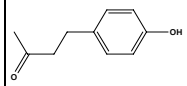
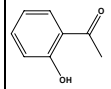
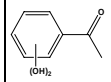
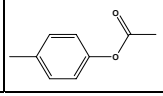
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
04.064 733	4-(1,1-Dimethylethyl)phenol		3918 98-54-4	Solid $C_{10}H_{14}O$ 150.22		236 98-101 IR 98 %	n.a. n.a.	SE 7), SW 8).
04.085 737	2,3,6-Trimethylphenol		3963 2416-94-6	Solid $C_9H_{12}O$ 136.10	Insoluble 50% Soluble in ethyl alcohol	228 63-64 IR 99 %	n.a. n.a.	
07.005 730	Vanillyl acetone		3124 139 122-48-5	Solid $C_{11}H_{14}O_3$ 194.23	Slightly soluble Moderately soluble	187-188 (18hPa) 40-41 IR 95 %	n.a. n.a.	
07.046 732	Vanillylidene acetone 6)	 (E)-isomer shown	3738 691 1080-12-2	Solid $C_{11}H_{12}O_3$ 192.21	Slightly soluble Moderately soluble	 129-130 IR 97 %	n.a. n.a.	CASrn does not specify stereoisomer.
07.055 728	4-(p-Hydroxyphenyl)butan-2-one		2588 755 5471-51-2	Solid $C_{10}H_{12}O_2$ 164.20	Insoluble Moderately soluble	 80 IR 96 %	n.a. n.a.	According to JECFA: Melting point is "80° (minimum)".
07.124 727	2-Hydroxyacetophenone		3548 11784 118-93-4	Liquid $C_8H_8O_2$ 136.15	Slightly soluble Miscible	215-220 IR 95 %	1.556-1.560 1.127-1.133	
07.135 729	2,4-Dihydroxyacetophenone 9)		3662 11884 28631-86-9	Solid $C_8H_8O_3$ 152.15	Insoluble to slightly soluble Moderately soluble	 90 IR 96 %	n.a. n.a.	CASrn does not specify position of hydroxy groups, incompletely defined substance. According to JECFA: Melting point is "90° (minimum)".
09.036 699	p-Tolyl acetate		3073 226 140-39-6	Liquid $C_9H_{10}O_2$ 150.18	Slightly soluble Miscible	208-212 IR 98 %	1.499-1.503 1.044-1.052	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

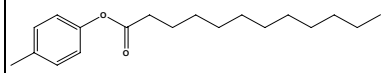
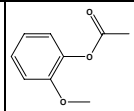
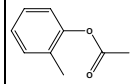
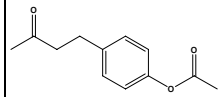
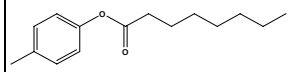
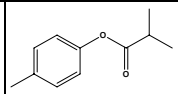
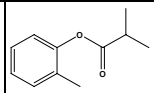
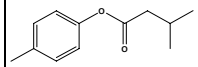
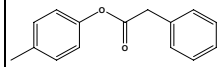
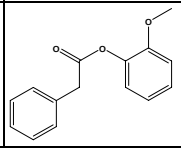
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
09.102 704	p-Tolyl dodecanoate 9)		3076 378 10024-57-4	Liquid $C_{19}H_{30}O_2$ 290.45	Insoluble	208-210 (13hPa) NMR 90 %	1.494-1.500 0.946-0.952	According to JECFA: Min. assay value is "90" and secondary components "p-Tolyl tetradecanoate, p-Tolyl decanoate, p-Tolyl hexadecanoate".
09.174 718	2-Methoxyphenyl acetate		3687 552 613-70-7	Liquid $C_9H_{10}O_3$ 166.18	Insoluble to slightly soluble Miscible	240-241 IR 98 %	1.507-1.513 1.127-1.134	
09.228 698	o-Tolyl acetate		3072 2078 533-18-6	Liquid $C_9H_{10}O_2$ 150.18	Insoluble Miscible	208 IR 99 %	1.497-1.503 1.046-1.053	
09.288 731	4-(4-Acetoxyphenyl)butan-2-one		3652 3572-06-3	Liquid $C_{12}H_{14}O_3$ 206.24	Insoluble Miscible	155 (3 hPa) IR 93 %	1.506-1.512 1.096-1.100	According to JECFA: Min. assay value is "93 (min. 95% combined o- and p- isomers)" and "contains 2-5% ortho isomer".
09.301 703	p-Tolyl octanoate		3733 59558-23-5	Liquid $C_{15}H_{22}O_2$ 234.34	Insoluble Miscible	265 MS 96 %	1.478-1.488 0.952-0.960	
09.429 701	p-Tolyl isobutyrate		3075 304 103-93-5	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	237 IR 95 %	1.484-1.490 0.990-0.997	
09.480 700	o-Tolyl isobutyrate		3753 681 36438-54-7	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	107 (10 hPa) IR 95 %	1.482-1.488 1.000-1.007	
09.518 702	4-Methylphenyl isovalerate		3387 10545 55066-56-3	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble Miscible	103 (3 hPa) IR 98 %	1.485-1.489 0.977-0.987	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Phenol Derivatives

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
09.709 705	p-Tolyl phenylacetate		3077 236 101-94-0	Solid $C_{15}H_{14}O_2$ 226.27	Insoluble Moderately soluble	310 71 IR 97 %	n.a. n.a.	According to JECFA: Melting point is "71° (minimum)".
09.711 719	Guaiacyl phenylacetate		2535 238 4112-89-4	Solid $C_{15}H_{14}O_3$ 242.27	Insoluble Very soluble	201 (3 hPa) 40-43 IR 97 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) SE: Missing data on solubility in ethanol.

8) SW: Missing data on solubility.

9) Composition of mixture not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro*) for 44 Phenol Derivatives (JECFA, 2001b)

Table 2.1: Summary of Genotoxicity Data for 44 Phenol Derivatives (JECFA, 2001b)

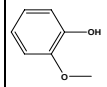
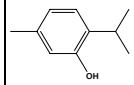
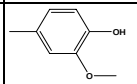
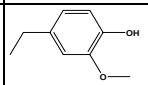
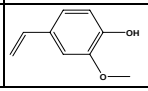
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
04.005 713	2-Methoxyphenol		Reverse mutation	S. typhimurium TA98, TA100, TA102	1-111 726 µg/plate[a,b]	Negative	(Aeschbacher et al., 1989)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	16 000 µg/plate[a,b]	Negative	(Douglas et al., 1980)
			Reverse mutation	S. typhimurium TA1535, TA1537, TA98, TA100	33-10 000 µg/plate[a,b]	Negative	(Haworth et al., 1983)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	16 000 µg/plate[a,b]	Negative	(Nestmann et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5000 µg/plate[a,b]	Negative	(Pool & Lin, 1982)
			Sister chromatid exchange	Human lymphocytes	≤ 31 µg/ml	Positive	(Jansson et al., 1988)
04.006 709	Thymol		Reverse mutation	S. typhimurium TA97, TA98, TA100	1000 µg/ml[a,b]	Negative	(Azizan & Blevins, 1995)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA7537, TA1538	451 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Sister chromatid	Syrian hamster embryo cells	0.3-30 µg/ml	Positive	(Fukuda, 1987)
			Unscheduled DNA synthesis	Syrian hamster embryo cells	0.3-10 µg/ml[a] 1-10 µg/ml[b]	Negative Positive	(Fukuda, 1987)
04.007 715	2-Methoxy-4-methylphenol		Sister chromatid exchange	Human lymphocytes	≤ 138 µg/ml	Positive	(Jansson et al., 1988)
04.008 716	4-Ethylguaiacol		Sister chromatid exchange	Human lymphocytes	0-152 µg/ml	Negative	(Jansson et al., 1988)
04.009 725	2-Methoxy-4-vinylphenol		Sister chromatid exchange	Human lymphocytes	≤ 75 µg/ml	Positive	(Jansson et al., 1988)

Table 2.1: Summary of Genotoxicity Data for 44 Phenol Derivatives (JECFA, 2001b)

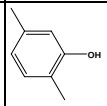
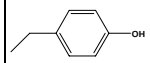
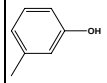
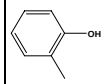
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
04.019 706	2,5-Dimethylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	367 µg/plate[a,b]	Negative	(Florin et al., 1980)
04.022 694	4-Ethylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	367 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Sister chromatid exchange	Human lymphocytes	0-27 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-2.7 µ/ml	Negative	(Jansson et al., 1988)
04.026 692	3-Methylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	2000 µg/plate[a,b]	Negative	(Douglas et al., 1980)
			Reverse mutation	S. typhimurium TA98, A100, TA1535, TA1537, TA1538	324 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Reverse mutation	S. typhimurium TA 1535, TA1537, TA98, TA100	3.3-333 µg/plate[a,b]	Negative[d]	(Haworth et al., 1983)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537 TA1538	2000 µg/plate[a,b]	Negative[d]	(Nestmann et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5000 µg/plate[a,b]	Negative	(Pool & Lin, 1982)
			Sister chromatid exchange	Human fibroblasts	86.5-865 µg/ml	Negative	(Cheng & Kligerman, 1984)
			Sister chromatid exchange	Human lymphocytes	0-108 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-108 µg/ml	Negative	(Jansson et al., 1988)
04.027 691	2-Methylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	2.5 µl/plate[a,b]	Negative	(Douglas et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	324 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Reverse mutation	S. typhimurium TA 1535, TA1537, TA98, TA100	1-100 µg/plate[a,b]	Negative	(Smith et al., 1996)
			Reverse mutation	S. typhimurium TA98, TA100	5 µg/plate[a,b]	Negative	(Massey et al., 1994)
			Reverse mutation	S. typhimurium TA98, TA100, TA7535, TA1537 TA1538	2600 µg/plate[a,b]	Negative[b]	(Nestmann et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5000 µg/plate[a,b]	Negative	(Pool & Lin, 1982)
			Sister chromatid exchange	Human fibroblasts	86.5-433 µg/ml 865 µg/ml	Negative Positive	(Cheng & Kligerman, 1984)
			Sister chromatid exchange	Human lymphocytes	0-54 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-54 µg/ml	Negative	(Jansson et al., 1988)

Table 2.1: Summary of Genotoxicity Data for 44 Phenol Derivatives (JECFA, 2001b)

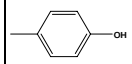
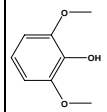
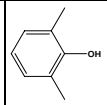
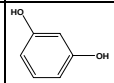
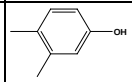
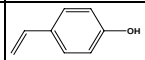
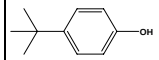
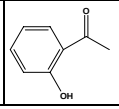
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
04.028 693	4-Methylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate[a,b]	Negative	(Douglas et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	324 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Reverse mutation	S. typhimurium TA 1535, TA1537, TA98, TA100	3.3-333 µg/plate[a,b]	Negative	(Haworth et al., 1983)
			Reverse mutation	S. typhimurium TA98, TA 100	5 µg/plate[a,b]	Negative	(Massey et al., 1994)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate[a,b]	Negative[d]	(Nestmann et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5000 µg/plate[a,b]	Negative	(Pool & Lin, 1982)
			Sister chromatid exchange	Human fibroblasts	86.5-865 µg/ml	Negative	(Cheng & Kligerman, 1984)
			Sister chromatid exchange	Human lymphocytes	0-54 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-54 µg/ml	Negative	(Jansson et al., 1988)
04.036 721	2,6-Dimethoxyphenol		Reverse mutation	S. typhimurium TA98, TA700, TA1535, TA1537, TA1538	16 000 µg/plate[a,b]	Negative	(Douglas et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	463 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537	0.1-1000 µg/ml[a,b]	Negative	(McMahon et al., 1979)
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5000 µg/plate[a,b]	Negative	(Pool & Lin, 1982)
			Reverse mutation	E. coli	1-1000 µg/ml[a,b]	Negative	(McMahon et al., 1979)
			Sister chromatid exchange	Human lymphocytes	0-77 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-77 µg/ml	Negative	(Jansson et al., 1988)
04.042 707	2,6-Dimethylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	367 µg/plate[a,b]	Negative	(Florin et al., 1980)
			Sister chromatid exchange	Human lymphocytes	0-31 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-31 µg/ml	Negative	(Jansson et al., 1988)
04.047 712	Benzene-1,3-diol		Reverse mutation[c]	S. typhimurium TA1535	550-7700 µg/plate[a] 0-7700 µg/plate[b]	Positive Negative	(Gocke et al., 1981)
			Reverse mutation[c]	S. typhimurium TA100	550-7700 µg/plate[b] 0-7700 µg/plate[a]	Positive Negative	(Gocke et al., 1981)
			Reverse mutations	S. typhimurium TA98, TA1537, TA1538	0-7700 µg/plate[a,b]	Negative	(Gocke et al., 1981)

Table 2.1: Summary of Genotoxicity Data for 44 Phenol Derivatives (JECFA, 2001b)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	0-7700 µg/plate[a,b]	Negative	(Gocke et al., 1981)
			Reverse mutation	S. typhimurium TA 1535, TA1537, TA98, TA100	33-3333 µg/plate[a,b]	Negative	(Haworth et al., 1983)
			Forward mutation	Mouse lymphoma cells	125-2000 µg/ml[a]	Positive	(McGregor et al., 1988b)
			Sister chromatid exchange	Human lymphocytes	0-28 µg/ml	Negative	(Jansson et al., 1986)
			Sister chromatid exchange	Human lymphocytes	0-28 µg/ml	Negative	(Jansson et al., 1988)
			Sister chromatid exchange	Chinese hamster embryo cells	0.6-2.2 µg/ml	Negative	(Wild et al., 1981)
04.048 708	3,4-Dimethylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	367 µg/plate[a,b]	Negative	(Florin et al., 1980)
04.057 711	4-Vinylphenol		Sister chromatid exchange	Human lymphocytes	0-12 µg/ml	Negative	(Jansson et al., 1988)
04.064 733	4-(1,1-Dimethylethyl)phenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.2-2000 µg/plate[a,b]	Negative	(Dean et al., 1985)
			Reverse mutation	E. coli. WP_2 and WP_2 uvrA	0.2-2000 µg/plate[a,b]	Negative	(Dean et al., 1985)
			Mitotic gene conversion	S. cerevisiae JD1	0.2-2000 µg/plate[a,b]	Negative	(Dean et al., 1985)
			Chromosomal aberration	Rat liver cell lines RL_1, RL_2	Not specified[e]	Negative	(Dean et al., 1985)
07.124 727	2-Hydroxyacetophenone		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	408 µg/plate[a,b]	Negative	(Florin et al., 1980)

[a] *Without metabolic activation.*

[b] *With metabolic activation.*

[c] *ZLM medium used in place of Vogel-Bonner medium.*

[d] *Presumably non-mutagenic, but solubility did not allow testing in amounts that result in lethality.*

[e] *Concentrations selected corresponded to 0.5, 0.25, and 0.125 of the concentration that caused 50% growth, inhibition (not specified) as determined in an assay for cytotoxicity.*

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.22

Substances listed in brackets are JECFA evaluated substances in FGE.22

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Methylphenol [04.027])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2.5 µl/plate (26,200µg/plate)	Negative[1, 2]	(Douglas et al., 1980)	17
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17 Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 1 to 100 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17 Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	17
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2600 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17 Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	4 concentrations from 5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17 Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	17
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	500 µg/plate	Negative[1, 2]	(Nuodex Inc., 1980a)	17
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[4] Positive[5]	(Claxton, 1985)	17 Result cannot be evaluated since it was reported only as a very short summary in table format. The paper was on methodological aspects of the assay and not specifically on this compound.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mM (54 µg/ml)	Negative	(Jansson et al., 1986; Jansson et al., 1988)	17 Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	86.5 - 865 µg/ml without S9	Equivocal	(Cheng & Kligerman, 1984)	17 Limited quality. Only the highest concentration resulted in a result statistically significantly different from control (1.2-fold increase only). A second experiment was not performed.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange	Chinese Hamster ovary cells	4 concentrations from 12.5 to 75 nl/ml (78.6 µg/ml) without S9, 11 concentrations from 1.56 to 700 nl/ml (733 µg/ml) with S9	Positive[1] Positive[2]	(Galloway & Brusick, 1981)	17 Acceptable quality. Statistically significant dose-related increase (up to two-fold).
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17 Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (52.4 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	17 Limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.
	DNA Repair assay	*E. coli* W3110	5000 µg/ml	Negative[1, 2]	(Pepper Hamilton and Scheetz, 1980)	17 Test substance included 60 % *o*-cresol.
	DNA repair assay	*E. coli*	5000 µg/ml	Negative[1, 2]	(Nuodex Inc., 1980b)	17.
(3-Methylphenol [04.026])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1, 2]	(Canter, 1981)	17.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (108 µg/ml)	Negative	(Jansson et al., 1986); (Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	17
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	10 µg/ml	Negative	(Cifone, 1988a)	17.
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.7 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	17. Limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(4-Methylphenol [04.028])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1 µl/plate (1030 µg/plate)	Negative[1, 2]	(Crowley & Margard, 1978)	17.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (54 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	17.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (2.7 µg/ml)	Negative	(Daugherty & Franks, 1986)	17. Not relevant since only an inhibition of UV-induced UDS was measured.. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative control was not included.
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.5 µg/ml)	Equivocal	(Myhr & Brusick, 1980)	17. Limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.
	Unscheduled DNA synthesis	WI-38 human embryonic lung fibroblast cells	Not unambiguously reported	Positive	(Crowley & Margard, 1978)	17. Unpublished study report of limited quality because concentrations were not unambiguously reported and only 3 concentrations have been tested. However, the result was reproducible. Liquid scintillation counting.
2-Ethylphenol [04.070]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535	5 doses from 0.01 to 10 mg/plate	Negative[1, 2]	(Zeiger et al., 1992)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (367 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
3-Ethylphenol [04.021]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-Ethylphenol [04.022])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17. Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (27 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-(1,1-Dimethyl)ethyl phenol [04.064])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mutation assay	*E. coli* WP2 and WP2 *uvrA*	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mutation assay	*S. cerevisiae* JD1	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Chromosomal aberration assay	Rat liver cell RL_1, RL_2	Not specifically indicated[7]	Negative	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
	Chromosome aberration assay	Chinese hamster lung cells	Not reported	Negative[8]	(Kusakabe et al., 2002)	17.
	Mouse lymphoma assay	L5178Y *tk* +/- mouse lymphoma cells	80 µg/ml	Negative	(Honma et al., 1999b)	17.
2,3-Dimethylphenol [04.065]	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (61 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
2,4-Dimethylphenol [04.066]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0, 0.33, 1, 3.3, 10, 33 µg/plate	Negative[1, 2]	(Mortelmans et al., 1986)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[9] (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate[10]	Negative[1, 2]	(Pool & Lin, 1982)	Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
(2,5-Dimethylphenol [04.019])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17. Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(2,6-Dimethylphenol [04.042])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium*	5 mg/plate	Negative[1, 2]	(Schechtman et al., 1980)	17.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	(preincubation method)	TA98; TA100; TA1535; TA1537; TA1538	(5000 µg/plate)			
	Chromosome aberration assay	Chinese hamster V79 cells	3 concentrations from 10 to 100 µg/ml (without S9) and 5 concentrations from 30 to 600 µg/ml (with S9)	Negative[1] Positive[2]	(Völkner, 1994)	17. Acceptable quality. This GLP-study was in accordance with OECD guideline 473 (1983). A final report was not available and the draft was not signed. However, the results and conclusions available as draft report are considered valid.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (31 µg/ml)	Negative	(Jansson et al., 1986)	17 . Limited quality (selection of maximum concentration not justified and experiment not repeated).
(3,4-Dimethylphenol [04.048])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17 Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
3,5-Dimethylphenol [04.020]	Ames assay (plate incorporation, preincubation, spot test, and treat-and-plate methods)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; *E. coli* WP2; WP2*uvrA*	6 concentrations from 125 to 4000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mitotic gene conversion assay	*S. cerevisiae* JD1	Not reported	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Chromosome aberration assay	Rat liver cells RL_4	3 concentrations from 0.125 to 0.5 of GI_{50} (50% growth inhibition). Values in µg/ml or µmol/ml not reported.	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
2,4,6-Trimethylphenol [04.095]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[10] (409 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(Thymol [04.006])	Ames assay	*S. typhimurium* TA97; TA98; TA100	1000 µg/ml	Negative[1, 2]	(Azizan & Blevins, 1995)	17.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	451 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100	Not reported	Negative[1, 2]	(Azizan & Blevins, 1995)	17.
	Sister chromatid exchange	SHE cells	5 concentrations from 0.3 to 30 µg/ml	Equivocal	(Fukuda, 1987)	17. Validity cannot be evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
	Unscheduled DNA synthesis	SHE cells	4 concentrations from 0.3 to 10 µg/ml	Equivocal	(Fukuda, 1987)	17. Validity cannot be fully evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
(Carvacrol [04.031])	Ames assay	*S. typhimurium* TA98; TA100	2 concentrations (8 and 16 ppm)	Negative[1, 2]	(Kono et al., 1995)	17. Not in accordance with OECD guideline 471 (only two strains used and only two concentrations tested). In Japanese with a short summary in English.
	Ames assay (plate incorporation assay)	*S. typhimurium* TA98; TA100	3 concentrations from 0.6 to 2.5 µmol/plate	Negative[1, 2]	(Stammati et al., 1999)	17. This study was not in accordance with OECD guideline 471 (only two strains used and only 3 concentrations tested).
	Bacterial DNA repair test	*E. coli* WP2 *trpE65*; CM8781 *trpE65; uvrA155, recA56, lexA*	4 concentrations from 2.5 to 6 µmol/paper disk	Positive	(Stammati et al., 1999)	17. Effects were measured as inhibition zones. This assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
	SOS Chromotest	*E. coli* PQ37	4 concentrations (not unambiguously reported)	Negative	(Stammati et al., 1999)	17. Concentrations not unambiguously reported, only without S9 tested. This assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
(4-Vinylphenol [04.057])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Methoxyphenol [04.005])	Ames assay	*S. typhimurium* TA98; TA100; TA102	111,726 µg/plate	Negative[1, 2]	(Aeschbacher et al., 1989)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 333 to 11,740 µg/plate in one experiment and 5 concentrations from 33 to 3333 µg/plate in two further experiments performed in another laboratory	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Three experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 16,000 µg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 conentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentration up to 0.5 mmol/L (62 µg/ml)	Positive	(Jansson et al., 1988)	17. Acceptable quality. Only the highest concentration resulted in a statistically significant increase. The effect was very waek but reproducible.
3-Methoxyphenol [04.076]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (124 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Methoxyphenol [04.077]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 167 µg/plate in the first experiment and 5 concentrations from 100 to 5000 µg/plate in the second experiment performed in another laboratory	Negative[2,]	(Haworth et al., 1983)	Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (plate incorporation method)	*S. typhimurium* TA100; TA1530	Up to 4 µmol/plate	Negative[1, 2, 12]	(Bartsch et al., 1980)	As only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation Validity cannot be evaluated as details of the

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						result were not reported.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	27 to 2000 µg/ml (without S9) 1.3 to 100 µg/ml (with S9)	Positive[1] Negative[2]	(Rogers-Back, 1986)	The validity of this unpublished report cannot fully be evaluated since all pages in table format are lacking.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.05 mmol/L (6.2 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Chromosome aberration assay	Chinese hamster ovary cells	954, 1269, and 1692 µg/ml (each in the presence and absence of S9)	Positive[1, 2]	(Putman, 1986)	The validity of this unpublished report cannot fully be evaluated since all pages in table format are lacking.
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (3.1 µg/ml)	Equivocal	(Daugherty & Franks, 1986)	Not relevant since only an inhibition of UV-induced UDS was measured. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative control was not included.
(2-Methoxy-4-methylphenol [04.007])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (138 µg/ml)	Positive	(Jansson et al., 1988)	17. Acceptable quality. The effect was weak (twofold increase) but dose-related and statistically significant.
(4-Ethylguaiacol [04.008])	Sister chromatid exchange	Human lymphocytes	5 concentration up to 1 mmol/L (152 µg/ml)	Negative	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
(2,6-Dimethoxyphenol [04.036])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	463 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Mutation assay	*E. coli*	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	17.
	Sister chromatid exchange	Human lymphocytes	4 concentrations up to 0.5 mmol/L (77 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Hydroxy-3,5-dimethoxyacetophenone [07.164]	Ames assay (plate incorporation assay)	*S. typhimurium* TA97; TA98; TA100; TA102	6 concentrations from 10 to 4000 µg/plate	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality. Strain TA 1535 was not used although recommended by OECD 471 (1983 and 1997) which may be acceptable but the test

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[1]	(Nestmann & Lee, 1983)	Insufficient quality. Details of concentrations and results not reported.
	Sister chromatid exchange	Human peripheral lymphocytes	4 concentrations from 3.3 to 100 µg/ml	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality as the test was not repeated in an independent experiment. Otherwise in accordance with OECD 479 (1986).
(2-Methoxy-4-vinylphenol [04.009])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (75 µg/ml)	Equivocal	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated). Weak effect (only the highest concentration resulted in a twofold increase of SCE frequency which was statistically significant but was not repeated in a second experiment).
3,4- Methylenedioxyphenol [04.080]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	4 concentrations from 1 to 10 µM/plate (1381 µg/plate)	(Not applicable)[13]	(Kaur & Saini, 2000)	Limited relevance. Antimutagenic activity was investigated only. The substance was tested only in combination with mutagens.
	Ames Assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	33 - 3333 µg/plate	Negative[1, 2, 14]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the Chemical Carcinogenesis Research Information System database. Details of methods and results were not available.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	25 - 215 µg/ml	Positive[1, 2]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the CCRIS database. Details of methods and results were not available.
(2-Hydroxyacetophenone [07.124])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	408 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
4- Hydroxy acetophenone [07.243]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (4085 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
Acetovanillone [07.142]	Ames assay (preincubation and plate incorporation methods)	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Xu et al., 1984)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains used. Concentration range not reported. Details of results not reported.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; *S. cerevisiae* XV185-14C	6 concentrations from 100 to 1000 µg/ml	Negative[15] Positive[15]	(Nestmann & Lee, 1983)	Tested only without S9, however the positive results reported seem to be reliable.
(Vanillyl acetone [07.005])	Ames assay	*S. typhimurium*	1000 µg/plate	Negative[2, 16]	(Mikulasova & Bohovicova,	17.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	(plate incorporation method)	TA98; TA100			2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	17.

GI = Growth inhibition.

IP = Intraperitoneal.

1) Without metabolic activation.

2) With metabolic activation.

3) Presumably non-mutagenic but solubility did not allow testing in amounts that result in lethality.

4) Negative results in TA100, with and without S9 metabolic activation.

5) Positive results in TA98, with and without metabolic activation.

6) The use of metabolic activation was not reported.

7) The concentrations selected for this assay corresponded to 0.5, 0.25, and 0.125 of the concentration causing 50 % growth inhibition (this concentration was not specified) as determined from a cytotoxicity assay.

8) Test substance was negative in a short-term assay without S9 metabolic activation and in a long-term assay (48 hrs.) with and without S9 metabolic activation. The test substance gave positive results in the short-term assay with S9 metabolic activation.

9) Tested quantitatively with TA100. Substance was cytotoxic at 30 µmol/plate.

10) 5000 µg/plate resulted in cytotoxicity which was defined as a thinning of the background lawn.

11) Tested quantitatively with TA98. Substance was cytotoxic at 30 µmol/plate.

12) The presentation of the result in the publication obviously led the petitioner to the interpretion that the substance was positive in TA1530 but this is not correct. From the footnotes of the publication it becomes clear that the substance was tested in TA100 and TA1530 and that the result was negative. However, as only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation.

13) Antimutagenicity study. Sesamol greatly reduced the mutagenic effects of t-*BOOH.*

14) Test with both rat and mouse S-9 metabolic activation.

15) Negative response for gene conversion (strain D7) and a positive response for reversion (strain XV185-14C).

16) Dose level was the highest non-toxic dose level examined. At 2500 µg/ml cytotoxicity was observed.

17) Summarised by JECFA, 55th meeting (JECFA, 2001b).

Table 2.3: Genotoxicity (*in vivo*) EFSA / FGE.22

Substances listed in brackets are JECFA evaluated substances in FGE.22

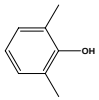
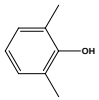
Table 2.3: Summary of Genotoxicity Data (*in vivo*) EFSA / FGE.22

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylphenol [04.027])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	1 Limited quality since only two animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Sex- linked recessive lethal test	*D. melanogaster*	Oral	0, 100, 500, 1000 µg/ml	Negative	(Sernau, 1989)	1 Acceptable quality. GLP study generally in accordance with OECD 477 (1984).
(3-Methylphenol [04.026])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	1 Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Chromosome aberration assay	Mouse bone marrow	Oral (gavage)	0, 96, 320, 960 mg/kg	Negative	(Ivett et al., 1989)	1 GLP study in accordance with OECD guideline 475 (1984). However, the validity of the result cannot be evaluated as all pages with results in table format are lacking.
(4-Methylphenol [04.028])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 75 mg/kg	Negative	(Cheng & Kligerman, 1984)	1 Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
(Carvacrol [04.031])	*In vivo* Spot test	*D. melanogaster* BINSC; Oregon-R		1.40 ppm; 0.35 ppm	Negative	(Kono et al., 1995)	1 Validity cannot be evaluated. Publication is in Japanese with a short summary in English. Results reported only for two doses in table format. Not clear if control groups were treated concomitantly.
4-Methoxyphenol [04.077]	*In vivo* Chromosome aberration assay	Rat	Oral (gavage)	0, 100, 333, 1000 mg/kg bw	Negative	(Esber, 1986)	The study design was in accordance with OECD guideline 475 (1984). The study was incompletely reported, however, the study report contained sufficient details to conclude that the outcome of the study is negative.

1) Summarised by JECFA, 55th meeting (JECFA, 2001b).

Table 2.4: Additional Genotoxicity Studies (*in vitro / in vivo*) not Included in JECFA Evaluation

Table 2.4: Additional Genotoxicity Studies (*in vitro/ in vivo*) not Included in JECFA Evaluation

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
04.042 707	2,6-Dimethylphenol		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537	10, 33.3, 100, 333, 1000, 2500, 5000 µg/plate[a]	Negative	(Poth, 1994b)
			Chromosomal aberration	V79 Chinese hamster cells	30, 100, 300 µg/ml (18 hours)[b], 600 µg/ml (28 hours)[b]	Positive	(Völkner, 1994)
			Chromosomal aberration	V79 Chinese hamster cells	10, 30, 100 µg/ml (18 hours)[c], 100 µg/ml (28 hours)[c]	Negative	(Völkner, 1994)
			HPRT assay	V79 Chinese hamster cells	30, 300, 350, 400 µg/ml[c] 30, 100, 200, 300, 600 µg/ml[b]	Negative	(Poth, 1994a)
In vivo							
04.042 707	2,6-Dimethylphenol		Chromosomal aberration	Rat bone marrow	350, 700, 1400 mg/kg bw[d] 300, 600, 1200 mg/kg bw[e]	Negative	(Gudi & Putman, 1966)

a With and without metabolic activation.

b With metabolic activation.

c Without metabolic activation.

d Male rats.

e Female rats.

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of 44 Phenol Derivatives (JECFA, 2001a)

Table 3.1: Summary of Safety Evaluation of 44 JECFA Evaluated Phenol Derivatives (JECFA, 2001a)

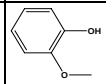
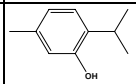
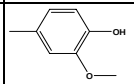
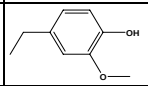
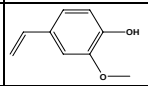
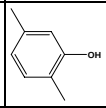
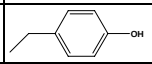
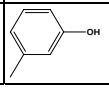
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
04.005 713	2-Methoxyphenol		44 16	Class I A3: Intake below threshold	4)	6)	6)
04.006 709	Thymol		51 160	Class I A3: Intake below threshold	4)	6)	6)
04.007 715	2-Methoxy-4-methylphenol		31 3	Class I A3: Intake below threshold	4)	6)	6)
04.008 716	4-Ethylguaiacol		6.9 0.4	Class I A3: Intake below threshold	4)	6)	6)
04.009 725	2-Methoxy-4-vinylphenol		2.6 1	Class I A3: Intake below threshold	4)	6)	6)
04.019 706	2,5-Dimethylphenol		0.49 0.03	Class I A3: Intake below threshold	4)	6)	6)
04.022 694	4-Ethylphenol		3.5 0.1	Class I A3: Intake below threshold	4)	6)	6)
04.026 692	3-Methylphenol		0.12 0.1	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of Safety Evaluation of 44 JECFA Evaluated Phenol Derivatives (JECFA, 2001a)

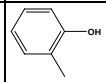
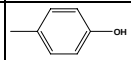
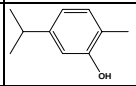
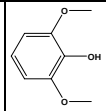
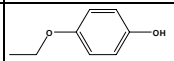
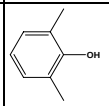
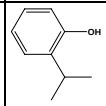
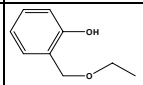
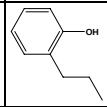
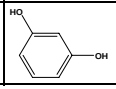
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
04.027 691	2-Methylphenol		250 0.1	Class I A3: Intake below threshold	4)	6)	6)
04.028 693	4-Methylphenol		0.97 1	Class I A3: Intake below threshold	4)	6)	6)
04.031 710	Carvacrol		14 0.3	Class I A3: Intake below threshold	4)	6)	6)
04.036 721	2,6-Dimethoxyphenol		5.4 12	Class I A3: Intake below threshold	4)	6)	6)
04.037 720	4-Ethoxyphenol		ND 0.4	Class I A3: Intake below threshold	4)	7)	7)
04.042 707	2,6-Dimethylphenol		1.7 1	Class I A3: Intake below threshold	4)	6)	6)
04.044 697	2-Isopropylphenol		14 0.3	Class I A3: Intake below threshold	4)	6)	6)
04.045 714	2-(Ethoxymethyl)phenol		1.5 0.01	Class I A3: Intake below threshold	4)	6)	6)
04.046 695	2-Propylphenol		0.12 1	Class I A3: Intake below threshold	4)	6)	6)
04.047 712	Benzene-1,3-diol		1.2 0.3	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of Safety Evaluation of 44 JECFA Evaluated Phenol Derivatives (JECFA, 2001a)

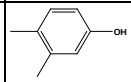
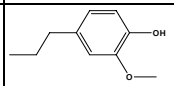
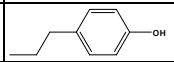
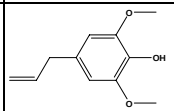
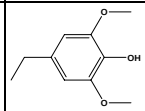
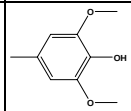
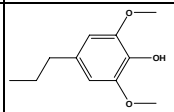
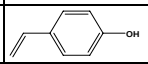
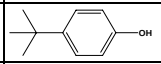
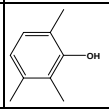
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
04.048 708	3,4-Dimethylphenol		5.7 1	Class I A3: Intake below threshold	4)	6)	6)
04.049 717	2-Methoxy-4-propylphenol		180 0.1	Class I A3: Intake below threshold	4)	6)	6)
04.050 696	4-Propylphenol		0.049 0.1	Class I A3: Intake below threshold	4)	6)	6)
04.051 726	4-Allyl-2,6-dimethoxyphenol		0.012 6	Class I A3: Intake below threshold	4)	6)	6)
04.052 723	4-Ethyl-2,6-dimethoxyphenol		ND 1	Class I A3: Intake below threshold	4)	7)	7)
04.053 722	4-Methyl-2,6-dimethoxyphenol		ND 0.04	Class I A3: Intake below threshold	4)	7)	7)
04.056 724	2,6-Dimethoxy-4-propylphenol		ND 0.1	Class I A3: Intake below threshold	4)	7)	7)
04.057 711	4-Vinylphenol		0.12 6	Class I A3: Intake below threshold	4)	6)	6)
04.064 733	4-(1,1-Dimethylethyl)phenol		0.012 0.01	Class I A3: Intake below threshold	4)	6)	6)
04.085 737	2,3,6-Trimethylphenol		0.24 0.3	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of Safety Evaluation of 44 JECFA Evaluated Phenol Derivatives (JECFA, 2001a)

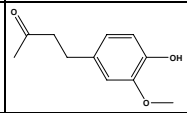
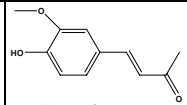
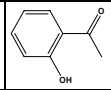
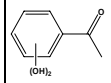
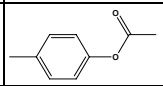
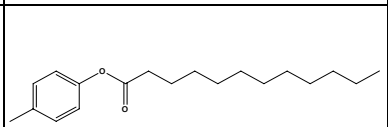
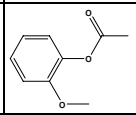
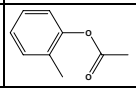
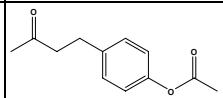
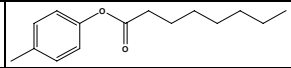
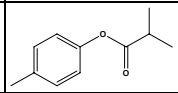
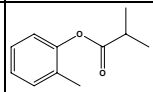
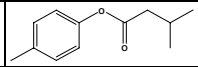
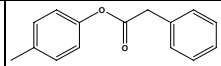
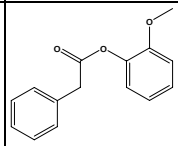
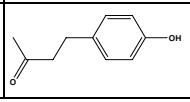
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.005 730	Vanillyl acetone		34 83	Class I A3: Intake below threshold	4)	6)	6)
07.046 732	Vanillylidene acetone	 (E)-isomer shown	ND 0.1	Class I A3: Intake below threshold	4)	7)	7) CASrn does not specify stereoisomers. Stereoisomeric composition to be specified.
07.124 727	2-Hydroxyacetophenone		0.12 0.01	Class I A3: Intake below threshold	4)	6)	6)
07.135 729	2,4-Dihydroxyacetophenone		0.012 0.1	Class I A3: Intake below threshold	4)	6)	CASrn does not specify position of hydroxy groups, incompletely defined substance. Composition of mixture to be specified
09.036 699	p-Tolyl acetate		ND 70	Class I A3: Intake below threshold	4)	7)	7)
09.102 704	p-Tolyl dodecanoate		ND 0.3	Class I A3: Intake below threshold	4)	7)	7) According to JECFA: Min. assay value is "90" and secondary components "p-Tolyl tetradecanoate, p-Tolyl decanoate, p-Tolyl hexadecanoate". Composition of mixture to be specified.
09.174 718	2-Methoxyphenyl acetate		0.012 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.228 698	o-Tolyl acetate		0.12 40	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of Safety Evaluation of 44 JECFA Evaluated Phenol Derivatives (JECFA, 2001a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.288 731	4-(4-Acetoxyphenyl)butan-2-one		ND 0.1	Class I A3: Intake below threshold	4)	7)	7)
09.301 703	p-Tolyl octanoate		0.024 1	Class I A3: Intake below threshold	4)	6)	6)
09.429 701	p-Tolyl isobutyrate		0.037 0.01	Class I A3: Intake below threshold	4)	6)	6)
09.480 700	o-Tolyl isobutyrate		0.024 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.518 702	4-Methylphenyl isovalerate		0.37 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.709 705	p-Tolyl phenylacetate		0.61 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.711 719	Guaiacyl phenylacetate		0.37 2	Class I A3: Intake below threshold	4)	6)	6)
07.055 728	4-(p-Hydroxyphenyl)butan-2-one		2400 3800	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6)	6)

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

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

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

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

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

6) No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

7) MSDI based on USA production figure.

ND: not determined

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.22)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

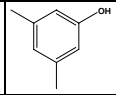
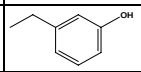
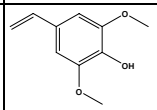
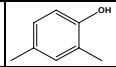
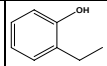
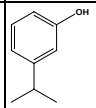
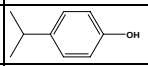
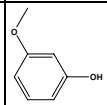
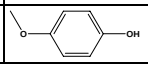
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.020	3,5-Dimethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.021	3-Ethylphenol		0.073	Class I A3: Intake below threshold	4)	6)	
04.061	2,6-Dimethoxy-4-vinylphenol		1.2	Class I A3: Intake below threshold	4)	6)	
04.065	2,3-Dimethylphenol		0.013	Class I A3: Intake below threshold	4)	6)	
04.066	2,4-Dimethylphenol		0.011	Class I A3: Intake below threshold	4)	6)	
04.070	2-Ethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.072	3-Isopropylphenol		0.0012	Class I A3: Intake below threshold	4)	6)	
04.073	4-Isopropylphenol		0.24	Class I A3: Intake below threshold	4)	6)	
04.076	3-Methoxyphenol		0.011	Class I A3: Intake below threshold	4)	6)	
04.077	4-Methoxyphenol		0.12	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

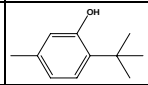
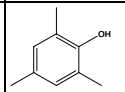
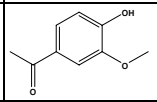
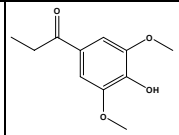
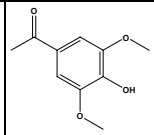
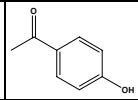
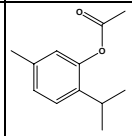
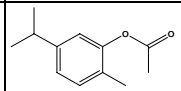
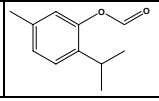
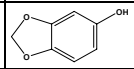
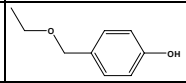
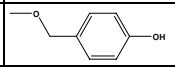
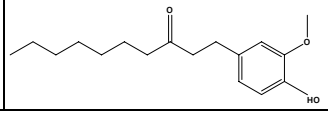
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.078	5-Methyl-2-(tert-butyl)phenol		0.061	Class I A3: Intake below threshold	4)	6)	
04.095	2,4,6-Trimethylphenol		0.0097	Class I A3: Intake below threshold	4)	6)	
07.142	Acetovanillone		2.2	Class I A3: Intake below threshold	4)	6)	
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one		0.026	Class I A3: Intake below threshold	4)	6)	
07.164	4-Hydroxy-3,5-dimethoxyacetophenone		0.24	Class I A3: Intake below threshold	4)	6)	
07.243	4-Hydroxyacetophenone		0.016	Class I A3: Intake below threshold	4)	6)	
09.253	2-Isopropyl-5-methylphenyl acetate		1.1	Class I A3: Intake below threshold	4)	6)	
09.337	Carvacryl acetate		0.61	Class I A3: Intake below threshold	4)	6)	
09.893	2-Isopropyl-5-methylphenyl formate		0.52	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.080	3,4-Methylenedioxyphenol		1.7	Class I No evaluation			a)
04.091	Ethyl 4-hydroxybenzyl ether		0.0012	Class II A3: Intake below threshold	4)	6)	
04.092	4-Hydroxybenzyl methyl ether		0.61	Class II A3: Intake below threshold	4)	6)	
07.234	5-Paradol		0.012	Class II A3: Intake below threshold	4)	6)	

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

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

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

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

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

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

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

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

a) Evaluation deferred pending further genotoxicity data

REFERENCES:

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

Azizan, A., Blevins, R.D., 1995. Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the ames salmonella/microsomal assay. Arch. Environ. Contam. Toxicol. 28, 248-258.

Bartsch, H., Malaveille, C., Camus, A.M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C., Montesano, R., 1980. Validation and comparative studies on 180 chemicals with S. typhimurium strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76, 1-50.

Canter, D.A., 1981. Letter from Dept of Health and Human services to U S EPA regarding Salmonella assays performed on cresols, with attachments. EPA Doc. 40-8160101, microfiche no. OTS0517549. Date 2/10/81. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Castle, L., Larsen, J.C., 1997, Food Contact Material Constituent Summary Safety Data Sheet for 2,6-Dimethylphenol, EU-SCF WG-FCM 72nd Meeting, 17-19 November 1997.

Cheng, M., Kligerman, A.D., 1984. Evaluation of the genotoxicity of cresols using sisterchromatid exchange (SCE). Mutat. Res. 137(1), 51-55.

Cifone, M.A., 1988a. Mutagenicity test on meta-cresol in a rat primary hepatocyte unscheduled DNA Synthesis assay with cover letter dated 07/06/88. Chemical Manufacturers Association. EPA Doc. 40-8860250, microfiche no. OTS0517692. Date 6/28/88. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Claxton, L.D., 1985. Assessment of bacterial mutagenicity methods for volatile and semivolatile compounds and mixtures. Environ. Int. 11, 375-383.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

Crowley, J.P., Margard, W., 1978. Determination of mutagenic/carcinogenic and cytotoxic potential of four chemical compounds (summary report). Sherwin Williams Co. EPA Doc. 40-7860090, microfiche no. OTS0517540. Date 10/31/78. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Daugherty, J.P., Franks, H., 1986. Effect of monocyclic derivatives on DNA repair in human lymphocytes. Res. Commun. Chem. Pathol. Pharmacol. 54,133-136.

Dean, B.J., Brooks, T.M., Hodson-Walker, G., Hutson, D.H., 1985. Genetic toxicology testing of 41 industrial chemicals. Mutat. Res. 153, 57-77.

Douglas, G.R., Nestmann, E.R., Betts, J.L., Mueller, J.C., Lee, E.G.H., Stich, H.F.,San, H.C., Brouzes, R.J.P., Chmelauskas, A.L., Paavila, H.D., Walden, C.C., 1980. Mutagenic activity in pulp mill effluents. In: Jolley, R. L., Brungs, W.A., Cumming, R.B., Jacobs, V.A., (Eds.). Water Chlorination: Environmental Impact and Health Effects. vol. 3. Ann Arbor Science Publishers Inc., Ann Arbor, MI, pp. 865-880.

EC, 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) no. 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

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

EFSA, 2006h. Opinion of the Scientific Panel AFC related to Flavouring Group Evaluation 22 (FGE.22): Ring-substituted phenolic substances from chemical groups 21 and 25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000).Adopted on 27 September 2006. EFSA-Q-2003-165.

Epler, J.L., Rao, T.K., Guerin, M.R., 1979. Evaluation of feasibility of mutagenic testing of shale oil products and effluents. Environ. Health Perspect. 30, 179-184.

Esber, H.J., 1986. In vivo cytogenetics study in rats - compound W1188.01 with cover letter dated 08/17/92. Proctor & Gamble Co. EPA Doc. 88-920007233, microfiche no. OTS0545546. Date 7/09/86. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

Fukuda, S., 1987. Assessment of the carcinogenic hazard of 6 substances used in dental practices. I.Morphological transformations, DNA damage and SCE in cultured Syrian hamster embryo cells induced by camphor, eugenol, thymol, EDTA, benzalkonium chloride and benzethonium chloride. Shigaku 74(6), 1365-1383. (In Japanese)

Galloway, S.M., Brusick, D.J., 1980. Mutagenicity evaluation of sample containing 33 1/3% each ortho-, meta-, and para-cresol in sister chromatid exchange assay with Chinese hamster ovary (CHO) cells. Final report. Cresol Task Force. EPA Doc. FYI-OTS-0780-0079, microfiche no. OTS0000079-0. Date 060180. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Galloway, S.M., Brusick, D.J., 1981. Sister chromatid exchange assay, Ames assay, mouse lymphoma forward mutation assay, and cell transformation on o-cresol. Pepper, Hamilton & Scheetz. EPA Doc. 40-8160079, microfiche no. OTS0517531. Date 5/01/81. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Gocke, E., King, M.-T., Eckhardt, K., Wild, D., 1981. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat. Res. 90, 91-109.

Gudi, R., Putman, D.L., 1966. In vivo rat bone marrow cytogenetic assay with 2,6-dimethylphenol, Microbiological Associates, Inc., 5221 River Road, Bethesda, Maryland 20826 and 9900 Blackwell Road, Rockville, Maryland 20850, Report: G95CA85.105, February 22.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E., 1983. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutag. Suppl. 1, 3-142.

Honma, M., Zhang, L.-S., Sakamoto, H., Ozaki, M., Takeshita, K., Momose, M., Hayashi, M., Sufuni, T., 1999. The need for long-term treatment in the mouse lymphoma assay. Mutagenesis 14(1), 23-29.

Ivett, J.L., Brown, B.M., Rodgers, C., Anderson, B.E., Resmick, M.A., Zeiger, E., 1989. Chromosomal aberrations and sister chromatid exchange tests in chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Environ. Mol. Mutag. 14, 165-187.

Jansson, T., Curvall, M., Hedin, A., Enzell, C., 1986. In vitro studies of biological effects of cigarette smoke condensate. II. Induction of sister-chromatid in human lymphocytes by weakly acidic, semivolatile constituents. Mutat. Res. 169, 129-139.

Jansson, T., Curvall, M., Hedin, A., Enzell, C., 1988. In vitro studies of the biological effects of cigarette smoke condensate. III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke. Mutat. Res. 206, 17-24.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2000d. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Commitee of Food Additives. 55th meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.

JECFA, 2001a. Evaluation of certain food addtives and contaminants. Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 901. Geneva, 6-15 June 2000.

JECFA, 2001b. Safety evaluation of certain food additives and contaminants. Fifty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 46. IPCS, WHO, Geneva.

JECFA, 2006c. Joint FAO/WHO Expert Committee on Food Additives. Sixty-seventh meeting Rome, 20-29 June 2006, Summary and Conclusions. issued 7 July 2006

Kaur, I.P., Saini, A., 2000. Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity. Mutat. Res. 470(1), 71-76.

Kono, M., Yoshida, Y., Itaya, Y., Shimobo, K., Yoshikawa, K., Terashita, T., Shishiyama, J., 1995. Antimicrobial activity and mutagenicity of allyl isothiocyanates and several essential oils from spices. Mem. Fac. Agri. Kinki Univ. 28, 11-19. (In Japanese)

Kusakabe, H., Yamakage, K., Wakuri, S., Sasaki, K., Nakagawa, Y., Watanabe, M., Hayashi, M., Sofuni, T., Ono, H., Tanaka, N., 2002. Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on the testing results of 98 high production volume industrial chemicals. Mutat. Res. 517, 187-198.

Longfellow, D., 1985/1986. Mutagenicity studies. Sesamol. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. As cited in Chemical Carcinogenesis Research Information System (CCRIS), a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov) on July 1, 2004.

Massey, I.J., Aitken, M.D., Ball, L.M., Heck, P.E., 1994. Mutagenicity screening of reaction products from the enzyme-catalyzed oxidation of phenolic pollutants. Environ. Toxicol. Chem. 13(11), 1743-1752.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., Caspary, W.J., 1988a. Responses of the L5178Y tk+/tk- mouse lymphona cell forward mutation assay: III. 72 coded chemicals. Environ. Mol. Mutag. 12, 85-153.

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

McMahon, R.E., Cline, J.C., Thompson, C.Z., 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the ames test for bacterial mutagens. Cancer Res. 39, 682-693.

Mikulasova, M., Bohovicova, I., 2000. Genotoxic effect of vanillin derivatives. Biologia (Bratislava) 55(3), 229-234.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E., 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environ. Mol. Mutag. 8(Suppl. 7), 1-119.

Myhr, B.C., Brusick, D.J., 1980. Evaluation of ortho-,meta-,and para-cresol 33 1/3% each in primary rat hepatocyte unscheduled DNA synthesis assay, draft report. Cresol Task Force. EPA Doc. FYI-OTS-0980-0079, microfiche no. OTS0000079-0. Date 080180. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nestmann, E.R., Lee, E.G.H., 1983. Mutagenicity of constituents of pulp and paper mill effluent in growing cells of Saccharomyces cerevisiae. Mutat. Res. 119, 273-280.

Nestmann, E.R., Lee, E.G., Matula, T.I., Douglas, G.R., Mueller, J.C., 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. Mutat. Res. 79, 203-212.

NTP, 1986e. NTP technical report on the toxicology and carcinogenesis studies of ortho-phenylphenol (CAS no. 90-43-7) alone and with 7,12-dimethylbenz(a)anthracene (CAS no. 57-97-6) in in swiss CD-1 mice (dermal studies). NTP-TR 301. NIH Publication no. 86-2557.

Nuodex Inc., 1980a. Mutagenicity studies ON R-1044. EPA Doc. 878211352, microfiche no. OTS0206261. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nuodex Inc., 1980b. Evaluation of R-1044 in the E. coli DNA repair - suspension assay. EPA Doc. 878211353, microfiche no. OTS0206261. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Pepper Hamilton and Scheetz, 1980. Evaluation of R-1044 In the E. coli DNA repair suspension assay with cover letter to EPA dated 10/11/83. EPA Doc. 40-8360166, microfiche no. OTS0507480. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Pfuhler, S., Stehrer-Schmid, P., Dorsch, W., Wagner, H., Wolf, H.U., 1995. Investigation of genotoxic effects of the anti-asthmatic and anti-inflammatory drugs apocyninand acetosyringenin in the Salmonella typhimurium mutagenicity assay and the SCE-test with human lymphocytes. Phytomedicine 1(4), 319-322.

Pool, B.L., Lin, P.Z., 1982. Mutagenicity testing in the Salmonella typhimurium assay of phenolic compounds and phenolic fractions obtained from smokehouse condensates. Food Chem. Toxicol. 20, 383-391.

Poth, A., 1994a. Gene Mutation Assay in Chinese Hamster V79 Cells in vitro (V79/HPRT) with 2,6-dimethylphenol, Cytotest Research Gmbh & Co. KG, D-64380 Rossdorf, F.R.G., CCR/RCC report: 441802/360966, July 07.

Poth, A., 1994b. Salmonella typhimurium Reverse Mutation Assay with 2,6-dimethylphenol, Cytotest Cell Research Gmbh & Co. KG, D-64380 Rossdorf, F.R.G., CCR/RCC report: 441801/360955, July 07.

Putman, D.L., 1986. Initial submission: In vitro cytogenicity study with 4-methoxyphenol in Chinese Hamster Ovary (CHO) Cells (final report) with cover letter dated 08/17/92. Proctor & Gamble Co. EPA Doc. 88-920007112, microfiche no. OTS0545451. Date 8/06/86. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Rogers-Back, A., 1986. Initial submission: L5178Y: Mouse lymphoma assay with cover letter dated 08/17/92. Proctor & Gamble Company. EPA Doc. 88-920007210, microfiche no. OTS0545512. Date 8/13/86. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

SCF, 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Schechtman, L.M., Curren, R.D., Parmar, A.S., Sinsky, P.M., 1980. Activity of T1570 in the Salmonella/microsomal assay for bacterial mutagenicity with attachments and cover letter dated 11/21/91. EPA Doc. 86-920000183, microfiche no. OTS0534388. Date 5/13/80. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sernau, D., 1989. Mutagenicity tests on ortho- and para-cresol: drosophila melanogaster sex-linked recessive lethal test (final report) with attachments and cover letter dated 03/21/89. Chemical Manufacturers Association. EPA Doc. 40-8960320, microfiche no. OTS0529221. Date 2/22/89. pp. 1-33. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Smith, R.L., Newberne, P., Adams, T.B., Ford, R.A., Hallagan, J.B., FEMA Expert Panel, 1996. 17. GRAS Substances. Food Technol. 50(10), 72-78, 80-81. (With correction table for use levels for sodium (±)2-(4-methoxyphenoxy)propanoate. Food Technol. 51(2), 32).

Stammati, A., Bonsi, P., Zucco, F., Moezelaar, R., Alakomi, H.L., von Wright, A., 1999. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. Food Chem. Toxicol. 37(8), 813-823.

Völkner, W., 1994. Support: Letter from General Elec Co. to US EPA re: Chromosome aberration assay in Chinese hamster V79 cells In vitro with 2, 6-dimethylphenol with attachments and cover letter dated 06/01/94. General Elec Co. EPA Doc. 8EHQ-0694-1027, microfiche no. OTS0527745-2. Date 06/01/94. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Wild, D., King, M.T., Eckhardt, K., Gocke, E., 1981. Mutagenic activity of aminophenols, and diphenols, and relations with chemical structure. Mutat. Res. 85(6), 456.

Xu, J., Whong, W.-Z., Ong, T.-M., 1984. Validation of the Salmonella (SV50)-arabinoseresistant forward mutation assay system with 26 compounds. Mutat. Res. 130(2), 79-86.

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

SCIENTIFIC PANEL MEMBERS

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

ACKNOWLEDGEMENT

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Jørn Gry, Vibe Beltoft, Frederikke Bentzen, Karin Nørby and Trine Klein Reffstrup for their contribution to the draft opinion

WHO Technical Report Series
901

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Fifty-fifth report of the
Joint FAO/WHO Expert Committee on
Food Additives

World Health Organization
Geneva 2001

WHO Library Cataloguing-in-Publication Data

Joint FAO/WHO Expert Committee on Food Additives (2000 : Geneva, Switzerland)
Evaluation of certain food additives and contaminants : fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives.

(WHO technical report series ; 901)

1.Food additives — toxicity 2.Food additives — analysis 3.Flavoring agents — analysis 4.Food contamination I.Title II.Series

ISBN 92 4 120901 1 (NLM classification: WA 712)
ISSN 0512-3054

Typeset in Hong Kong
Printed in Switzerland
2000/13521 — Best-set/Schuler — 6000

Contents

Joint FAO/WHO Expert Committee on Food Additives

Geneva, 6–15 June 2000

Members

Ms J. Baines, Senior Nutritionist, Australia New Zealand Food Authority, Canberra, ACT, Australia

Professor J.R. Bend, Department of Pharmacology and Toxicology, Faculty of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada

Dr Junshi Chen, Director, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing, China

Professor S.M. Dagher, Department of Biology, American University of Beirut, Beirut, Lebanon

Dr C.E. Fisher, Consultant, Hatfield, Herts, England

Dr D.G. Hattan, Director, Division of Health Effects Evaluation, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*Joint Rapporteur*)

Dr Y. Kawamura, Section Chief, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan

Dr A.G.A.C. Knaap, Centre for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands

Dr P.M. Kuznesof, Leader, Chemistry and Exposure Assessment Team, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*Vice-Chairman*)

Dr J.C. Larsen, Head, Department of Biochemical and Molecular Toxicology, Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark

Mrs I. Meyland, Senior Scientific Adviser, Institute of Food Research and Nutrition, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark (*Joint Rapporteur*)

Dr G. Pascal, Scientific Director, Human Nutrition and Food Safety, National Institute for Agricultural Research, Paris, France

Dr A. Pintér, Director, National Institute of Environmental Health, Budapest, Hungary

Professor R. Walker, Emeritus Professor of Food Science, School of Biological Sciences, University of Surrey, Guildford, Surrey, England (*Chairman*)

Secretariat

Dr P.J. Abbott, Australia New Zealand Food Authority, Canberra, ACT, Australia (*WHO Temporary Adviser*)

Dr L.M. Barraj, Novigen Sciences Inc., Washington, DC, USA (*WHO Temporary Adviser*)

Dr D.C. Bellinger, Associate Professor of Neurology, Harvard Medical School, Children's Hospital Neuroepidemiology Unit, Boston, MA, USA (*WHO Temporary Adviser*)

Dr M. Bolger, Division of Risk Assessment, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*WHO Temporary Adviser*)

Ms M.L. Costarrica, Food Quality Liaison Group, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Joint Secretary*)

Dr M. DiNovi, Division of Product Manufacture and Use, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*WHO Temporary Adviser*)

Dr R.L. Ellis, Food Quality Liaison Group, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy

Dr R. Goyer, Chapel Hill, NC, USA (*WHO Temporary Adviser*)

Dr J. Greig, Food Standards Agency, London, England (*WHO Temporary Adviser*)

Mr E.F.F. Hecker, Chairman, Codex Committee on Food Additives and Contaminants, Department of Veterinary and Food Policy and Environmental Affairs, Ministry of Agriculture, Nature Management and Fisheries, The Hague, Netherlands (*WHO Temporary Adviser*)

Dr J.L. Herrman, Scientist, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*WHO Joint Secretary*)

Professor J.H. Hotchkiss, Department of Food Science, Cornell University, Ithaca, NY, USA (*FAO Consultant*)

Dr F. Kayama, Division of Environmental Immunology and Toxicology, Department of Health Science, Jichi Medical School, Tochigi, Japan (*WHO Temporary Adviser*)

Dr A. Mattia, Division of Product Policy, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*WHO Temporary Adviser*)

Dr G. Moy, Food Safety, WHO, Geneva, Switzerland

Dr I.C. Munro, President, CanTox Health Sciences International, Mississauga, Ontario, Canada (*WHO Temporary Adviser*)

Dr A. Nishikawa, Division of Pathology, National Institute of Health Sciences, Tokyo, Japan (*WHO Temporary Adviser*)

Dr J.A. Pennington, Research Nutritionist, Division of Nutrition Research Coordination, National Institutes of Health, Bethesda, MD, USA (*FAO Consultant*)

Dr M.V. Rao, Head, Chemistry Unit, Food and Environment Laboratory, Dubai Municipality, Dubai, United Arab Emirates (*FAO Consultant*)

Professor A.G. Renwick, Head, Clinical Pharmacology Group, University of Southampton, Southampton, England (*WHO Temporary Adviser*)

Professor S. Resnik, Food Technology, Department of Industry, Faculty of Exact and Natural Science, University Campus, Buenos Aires, Argentina (*FAO Consultant*)

Dr H. Sakurai, Occupational Health Research and Development Centre, Japan Industrial Safety and Health Association, Tokyo, Japan (*WHO Temporary Adviser*)

Professor I.G. Sipes, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ, USA (*WHO Temporary Adviser*)

Dr G.J.A. Speijers, Head, Section on Public Health, Centre for Substances and Risk Assessment, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (*WHO Temporary Adviser*)

Ms E. Vavasour, Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

Dr P.J.P. Verger, Scientific Directorate on Human Nutrition and Food Safety, National Institute for Agricultural Research, Paris, France (*FAO Consultant*)

Mrs H. Wallin, Senior Food Control Officer, National Food Administration, Helsinki, Finland (*FAO Consultant*)

Dr D.B. Whitehouse, Consultant, Bowdon, Cheshire, England (*FAO Consultant*)

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

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.

Specifications are issued separately by FAO under the title:

Compendium of food additives specifications, Addendum 8. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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

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

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 6 to 15 June 2000. The meeting was opened by Mrs P.K. Singh, Executive Director, Sustainable Development and Healthy Environments, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mrs Singh noted that Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), had been used by the Committee as a basis for consistent, credible evaluations during the past 13 years. However, in view of the tremendous scientific advances that had been made during that time and the increasing complexity and scope of the evaluations, FAO and WHO were considering the possibility of updating and consolidating the principles for the risk assessment of food additives and contaminants and of veterinary drug residues used by the Joint FAO/WHO Expert Committee on Food Additives and of pesticide residues used by the Joint FAO/WHO Meeting on Pesticide Residues.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been fifty-four previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the fifty-third meeting (Annex 1, reference *143*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
— to undertake toxicological evaluations of certain food additives, flavouring agents and contaminants (sections 3–5 and Annex 2);
— to assess the intake of specific food additives (section 6); and
— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 7 and Annex 2).

2.1 Modification of the agenda

Cross-linked sodium carboxymethyl cellulose and lycopene were removed from the agenda because no data were submitted. Paprika oleoresin was added to the agenda at the request of the Thirty-second Session of the Codex Committee on Food Additives and Contaminants (*2*), which had asked for clarification of the previous

evaluation. Microcrystalline wax was also added to the agenda for the review of the specifications.

Caustic sulfite caramel was evaluated under the name "caramel colour II", and cochineal extract, carmine and carminic acid were evaluated under the names "cochineal extract" and "carmines".

2.2 Principles governing the toxicological evaluation of compounds on the agenda

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

2.2.1 *Principles governing the safety evaluation of flavouring agents*

α,β-Unsaturated compounds

Five α,β-unsaturated flavouring agents were considered by the Committee at its forty-ninth meeting (Annex 1, reference *131*), but their safety was not assessed at that time. Additional data on metabolism were considered necessary for the assessment of four α,β-unsaturated lactones (Nos 245, 246, 276 and 438), and the evaluation of *cis*-3- and *trans*-2-hexenyl propionate (No. 147) was postponed, pending consideration of other α,β-unsaturated carbonyl compounds. At its present meeting, the Committee considered data on furfural, cinnamaldehyde, structural analogues of cinnamaldehyde, pulegone and esters of the corresponding alcohols, which are predicted to be metabolized by formation of α,β-unsaturated carbonyls. The available data on the toxicity of these compounds showed a number of adverse effects at high doses in experimental animals, and no-observed-effect levels (NOELs) for these effects were identified. The presence of protective cellular processes, such as conjugation with glutathione, provides adequate detoxification capacity at the low doses associated with use of such compounds as flavouring agents. In

consequence, the Committee concluded that the presence of an α,β-unsaturated carbonyl group in a flavouring agent, or its formation during metabolism, would not preclude evaluation of that substance by the Procedure for the Safety Evaluation of Flavouring Agents.

Grouping of flavouring agents

The Committee recognized that a single flavouring agent could be a member of more than one group of such agents: for example, allyl 2-furoate was considered as a member of the group of allyl esters evaluated at the forty-sixth meeting (Annex 1, reference *122*), whereas five other esters of furoic acid were considered as members of the group of furfuryl alcohol and related agents evaluated at the present meeting. The Committee recognized that evaluations of combined intakes of a group of flavouring agents should take into account all relevant, structurally related agents, irrespective of the group in which they were evaluated.

Estimating intake of flavouring agents

The Committee considered use of the "per capita × 10"[1] method for estimating the intake of flavouring agents, as well as alternative procedures, such as the theoretical added maximum dietary intake and stochastic modelling methods, which are based on dietary surveys. The Committee concluded that its use of "per capita × 10" is appropriate currently. However, it recognized the potential usefulness of the other methods and noted that use of the "per capita × 10" method may, in some cases, result in an underestimate of the intake of persons with high levels of consumption of specific foods. The Committee was aware of research on methods to predict the intake of flavouring agents and concluded that data from such studies could be used to investigate the use of the "per capita × 10" method and alternative methods of intake assessment at future meetings of the Committee.

Correction factor for "poundage" data for flavouring agents

The Committee relies on "poundage" data provided by manufacturers and food producers to estimate intake of flavouring agents. Such data reflect the amounts of the substances that are used in foods. At previous meetings, the Committee used data that had been "corrected" for under-reporting by application of a factor of 0.6 (see Annex 1, reference *137*, page 50), an estimate of the fraction of the total amount of the material that was reported. The Committee

[1] This method is based on the assumption that 10% of the population consumes all of the flavouring agents used in foods. In the calculation, the reported annual poundage of a flavouring agent is converted to micrograms and then divided by 10% of the population (of either Europe or the USA) and by 365 days to arrive at a daily intake.

was aware that the poundage data reported from the USA for the flavouring agents evaluated at the present meeting had been corrected by a factor of 0.8. This factor was used because the investigators had found that approximately 87% of the poundage data on all flavouring agents had been reported in the most recent survey, completed in 1995 (*3*). A factor of 0.6 would continue to be applied to the reported data from Europe.

2.2.2 *Need for data*

In response to a request by the Thirty-first Session of the Codex Committee on Food Additives and Contaminants (*4*), three food additives were placed on the agenda of the present meeting of the Committee for consideration of their uses in the draft General Standard for Food Additives. The Committee was asked to consider the use of benzoyl peroxide in milk products, the use of nitrous oxide as a packaging gas, and the use of stearyl tartrate both as an emulsifier and as a flour treatment agent at a higher concentration than previously specified by the Committee. Although the specifications for these three substances were updated, no evidence for the use of stearyl tartrate could be found, and none of the substances could be evaluated toxicologically because no relevant information was provided.

While the Committee wishes to be responsive to the requests of the Codex Committee, it emphasized that it can evaluate substances only if relevant data on toxicology, intake and specifications are provided. The Committee therefore requested the Codex Committee on Food Additives and Contaminants to ensure that the necessary data are available before referring a substance for its consideration.

2.2.3 *Recommendations of the World Health Assembly*

The Committee noted that the Fifty-third World Health Assembly had requested the Director-General inter alia "to strengthen the expert advisory bodies that provide scientific guidance on food safety issues related to chemicals, and to maintain an updated databank of this scientific evidence to support Member States in making health-related decisions in these matters" (*5*). The Committee welcomed this recognition of the significance of its activities in assisting Member States to maintain and improve the safety of food supplies.

The Committee also noted the Assembly's request to the Director-General "to ensure that the procedures for designating experts and preparing scientific opinions are such as to guarantee

the transparency, excellence and independence of the opinions delivered" (*5*). The Committee will continue to strive to achieve the highest standards of excellence and independence that have enhanced the general acceptance of its opinions to date. Furthermore, the Committee recognized the importance of transparency in the selection of experts, its modus operandi, and descriptions of its evaluations.

2.3 Principles governing the establishment and revision of specifications

2.3.1 *General specifications for enzyme preparations*

At its fifty-third meeting (Annex 1, reference *143*), the Committee revised Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) to indicate that only non-toxicogenic and non-pathogenic strains may be used as source organisms in the production of enzyme preparations for use in food. The text has been published in FAO Food and Nutrition Paper, No. 52, Add. 7 (Annex 1, reference *145*).

The specifications for a number of enzyme preparations were considered at the present meeting, and specific wording was included in each case to require that only non-toxicogenic and non-pathogenic strains be used as source organisms. As this wording precludes, by definition, the production of toxins, specifications incorporating this wording will usually not require that specific limits be placed on potentially toxic by-products.

2.3.2 *Determination of low concentrations of metals*

The Committee continues to pay attention to improving its specifications for limits and methods of analysis for metals. At its present meeting, the Committee recognized that where concentrations of lead of 2 mg/kg or less have been specified in existing monographs, the recommended sample preparations and instrumental methods are not wholly satisfactory. The Committee therefore decided to discontinue use of the phrase "Prepare a sample solution as directed for organic compounds in the limit test and determine by atomic absorption spectroscopy", and to use instead the wording "Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in "Instrumental methods" of FAO Food and Nutrition Paper, No. 5, Rev. 2" (Annex 1, reference *100*).

3. Specific food additives

The Committee evaluated two food additives for the first time and re-evaluated nine food additives already considered at previous meetings. In addition, the Committee evaluated a large number of flavouring agents using the Procedure for the Safety Assessment of Flavouring Agents (see section 4). Information on the evaluations and on specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Flavouring agents

3.1.1 *Furfural*

Furfural was evaluated previously by the Committee at its thirty-ninth and fifty-first meetings (Annex 1, references *101* and *137*). An Acceptable Daily Intake (ADI) was not established at either meeting because of concern about the finding of tumours in male mice given furfural in corn oil by gavage and the fact that no NOEL was identified for hepatotoxicity in male rats. In a study in mice, the combined incidence of adenomas and carcinomas was increased in males at the highest dose tested (175 mg/kg of body weight per day).

In order to address the concern regarding the formation of liver tumours in mice, the Committee at its fifty-first meeting requested the results of studies of DNA binding or adduct formation in vivo to clarify whether furfural interacts with DNA in the liver of mice (Annex 1, reference *137*). While no specific studies of DNA binding were submitted, the results of an assay for unscheduled DNA synthesis in mice in vivo was reviewed by the Committee at its present meeting. This study, in which single doses of up to 350 mg/kg of body weight were given, was particularly relevant since it addressed potential DNA repair in the cells in which tumours arose, namely hepatocytes. The negative results obtained in this assay were considered by the Committee to provide evidence that the liver tumours observed in the long-term study in mice were unlikely to have occurred through a genotoxic mechanism. The Committee considered that the concerns raised previously with respect to the liver tumours in mice were adequately addressed by this study and that a study of DNA binding was unnecessary.

At its fifty-first meeting, the Committee also requested the results of a 90-day toxicity study in rats to identify a NOEL for hepatotoxicity. At its present meeting, the Committee reviewed the results of a 13-week study in rats in which microencapsulated furfural was administered in the diet. It noted that, in a complementary study, microen-

capsulated furfural was rapidly and completely released in an aqueous environment and therefore considered that this formulation was suitable for a feeding study. In the 13-week dietary study, minor hepatocellular alterations were observed in males, but not in females, at doses of 82 and 160 mg/kg of body weight per day. While these changes might be judged not to be adverse, the Committee took a conservative view, and considered the NOEL to be 53 mg/kg of body weight per day, at which dose there was no evidence of hepatic alterations. This result contrasts with those of the previous studies in rats, in which furfural was administered in corn oil by gavage. The Committee considered the NOEL obtained in the 13-week study to be valid because: (i) dietary administration is more appropriate than administration by gavage for compounds normally consumed in the diet; (ii) the peak tissue concentrations observed after administration of bolus doses by gavage are much higher than those seen after administration in the diet; (iii) microencapsulation prevents loss due to volatilization of compounds such as furfural; (iv) microencapsulated furfural is rapidly released in an aqueous environment; and (v) corn oil is known to produce morphological changes in the livers of mice and rats when administered by gavage over a long period.

The estimated daily intake of furfural from its use as a flavouring agent was determined from "poundage" data provided by the flavour industry (see page 22). On the basis of these data, the estimated daily intake was 9 μg/kg of body weight in Europe and 8 μg/kg of body weight in the USA. The Committee was aware that intake of furfural from its use as a flavouring agent accounts for only a minor fraction of the total dietary intake of this substance.

The metabolism of furfuryl alcohol, furfural and 10 derivatives of furfuryl alcohol, furfural and furoic acid was considered by the Committee at its present meeting as part of its evaluation of this group of flavouring agents (see section 4.2). The derivatives considered were furfuryl acetate, furfuryl propionate, furfuryl pentanoate, furfuryl octanoate, furfuryl 3-methylbutanoate, methyl 2-furoate, propyl 2-furoate, amyl 2-furoate, hexyl 2-furoate and octyl 2-furoate. Furfuryl alcohol, furfural and these derivatives are all metabolized to the same metabolite, furoic acid. Accordingly, the Committee decided to establish a group ADI of 0–0.5 mg/kg of body weight for furfural, furfuryl alcohol and these derivatives, based on the NOEL of 53 mg/kg of body weight per day in the 13-week study on furfural in rats and a safety factor of 100.

An addendum to the toxicological monograph was prepared. The specifications for furfural were revised (see section 7.2.3).

3.1.2 *Paprika oleoresin*

Paprika oleoresin was evaluated by the Committee at its fourteenth meeting (Annex 1, reference *22*), when no ADI was established because it was recognized that use of this material as a spice was self-limiting for technological and organoleptic reasons. At its Thirty-second Session (*2*), the Codex Committee on Food Additives and Contaminants requested clarification of this evaluation from the Committee. At its present meeting, the Committee did not evaluate the available data on paprika oleoresin, but reviewed the previous evaluation of this substance, which stated that "oleoresins of paprika ... are derived from a widely consumed natural foodstuff, and there were no data indicative of a toxic hazard. The use of the oleoresins as a spice was self-limiting and obviates the need for an ADI." At its present meeting, the Committee interpreted this statement to mean that the use of paprika oleoresin as a spice is acceptable. It was aware, however, that paprika oleoresin is also used as a food colour and drew attention to the fact that it has not been evaluated for this use.

A toxicological monograph was not prepared. The existing specifications were maintained.

3.2 Food colours

3.2.1 *Caramel colour II*

Caramel colour II (caustic sulfite caramel) differs from the other three classes of caramel colour (caramel colour I — plain caramel or caustic caramel; caramel colour III — ammonia caramel; caramel colour IV — sulfite ammonia caramel) in that it is manufactured using sulfite compounds rather than ammonium compounds. The processes used in the production of each of the four classes of caramel colour and their synonyms are described in the toxicological monograph prepared by the Committee at its twenty-ninth meeting (Annex 1, reference *72*).

The Committee was informed that caramel colour II is manufactured only in France and the USA. The volume of production of this substance represents less than 1% of the total volume of production of caramel colours. Caramel colour II is used mainly in distilled spirits (e.g. rum, whisky and brandy) and dietary intake of this compound depends primarily on the level of consumption of these alcoholic beverages. Caramel colour II may also be used in herbal infusions, extracts of meat and fish, coffee and vanilla, salted meats, sauces, bouillons, soups and tea beverages. The estimated dietary intake of caramel colour II in the USA, based on production data, is approximately 0.034 mg/kg of body weight per day. If it is assumed

that all the caramel colour II produced in the USA is consumed in distilled spirits, the intake would be 2.2 mg/kg of body weight per day for adult consumers of distilled spirits. The estimated dietary intake of caramel colour II in Australia, based on the average consumption of distilled spirits recorded in the 1995 National Nutrition Survey and on maximum levels of use of this colour, is 0.28 mg/kg of body weight per day. For consumers of distilled spirits at the 95th percentile of consumption, the estimated dietary intake is 0.91 mg/kg of body weight per day.

Caramel colours were evaluated by the Committee at its thirteenth, fifteenth and twenty-ninth meetings (Annex 1, references *19*, *26* and *70*). When evaluating the safety of caramel colours produced by the ammonium sulfite process at its twenty-fourth meeting (Annex 1, reference *53*), the Committee noted that no ADI had been allocated to caustic sulfite caramel colour (subsequently named "caramel colour II" at the twenty-ninth meeting). At its twenty-ninth meeting, the Committee concluded that caramel colour II is sufficiently different from the other classes of caramel colours to warrant a separate evaluation but that there were insufficient data to do so. No ADI was established.

The data summarized below were included in the toxicological monograph prepared by the Committee at its twenty-ninth meeting (Annex 1, reference *72*). The existing database consists of the results of a 90-day study in Fischer 344 rats given caramel colour II in their drinking-water to deliver doses of 4–16 g/kg of body weight per day and the results of several assays for genotoxicity. The principal observations in the toxicity study were: a dose-related decrease in body weight and reduced food consumption and fluid intake, mainly affecting the groups that received doses of 12 and 16 g/kg of body weight per day; slight, dose-related increases in absolute and relative kidney weights and full and empty caecum weights, with no evidence of significant histopathological changes; dose-related staining of the gastrointestinal tract and mesenteric lymph nodes, with deposits of yellow pigment observed microscopically in the caecal mucosa and mesenteric lymph nodes (only the highest-dose groups were examined). Negative results were obtained with caramel colour II in two assays for reverse mutation in *Salmonella typhimurium*, in an assay for chromosomal aberration in vitro, and in an assay for DNA repair in vitro. No new studies on caramel colour II were available for review by the Committee at its present meeting.

In reviewing the database on caramel colour II, the Committee concluded that the decreased body-weight gain and renal hypertrophy observed in rats in the 90-day study were the consequence of

the reduced consumption of food and fluids, which probably resulted from the treated drinking-water being unpalatable. The Committee considered that the pigmentation of mesenteric lymph nodes and enlargement of the caecum were not of toxicological significance and concluded that the NOEL was the highest dose tested, 16 g/kg of body weight per day.

The Committee established an ADI of 0–160 mg/kg of body weight per day for caramel colour II, based on the NOEL of 16 g/kg of body weight per day in the 90-day study in rats and a safety factor of 100.

A toxicological monograph was not prepared. A monograph summarizing the available data on intake was prepared. Caramel colour II is included in the existing specifications for caramel colours, which were revised with minor changes.

3.2.2 *Cochineal extract and carmines*

Cochineal extract is obtained from the dried bodies of female *Dactylopius coccus* Costa insects (cochineal). The extract is used directly in food and is also processed further to carmines. Specifications exist for cochineal extract and carmines, both of which contain carminic acid as the colouring principle. The Committee evaluated cochineal extract at its eighteenth and twenty-first meetings, but did not allocate an ADI at either meeting (Annex 1, references *35* and *44*). It evaluated carmines at its twenty-first, twenty-fifth and twenty-sixth meetings (Annex 1, references *44*, *56* and *59*). At its twenty-sixth meeting, the Committee allocated an ADI of 0–5 mg/kg of body weight for carmines, as ammonium carmine or the equivalent of calcium, potassium or sodium salts. At its present meeting, the Committee considered the potential allergenicity of cochineal extract, carmine and carminic acid (collectively referred to here as "cochineal colours") in response to a request from the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*4*). The ADI for carmines was not reconsidered and was retained at its present value.

At its fifty-third meeting (Annex 1, reference *143*), the Expert Committee considered the report of an ad hoc Panel on Food Allergens that had been convened to consider issues relating to the allergenicity of foodstuffs. The Panel had identified three criteria for adding foodstuffs to the list of common allergenic foods developed by the Codex Committee on Food Labelling, if found to be necessary. The Expert Committee, at its fifty-third meeting, concluded that these criteria form a suitable basis for addressing the allergenicity of food and food products. These criteria are as follows:

(i) the existence of a credible cause-and-effect relationship, based on a positive reaction to a double-blind placebo-controlled food challenge or unequivocal reports of a reaction with the typical features of a severe allergic or intolerance reaction;
(ii) the existence of reports of systemic reactions after exposure to the foodstuff; and
(iii) data on the prevalence of food allergies in children and adults, supported by appropriate clinical studies, in the general population of several countries; alternatively, data on the comparative prevalence of a specific food allergy in groups of patients in several countries could be used.

Adverse reactions to cochineal colours after occupational exposure, dermal contact or consumption of coloured food and drinks have been the subject of case reports. The reported effects were the consequence of allergic reactions, and the involvement of an immunologically mediated mechanism has been demonstrated. The nature of the adverse reactions, e.g. urticaria, rhinitis, diarrhoea and anaphylaxis, provides clear evidence that systemic reactions can follow exposure of a sensitized individual to cochineal colours. Some of the adverse reactions were severe and required emergency treatment. The weight of evidence suggests that proteins in the food colours are the allergenic species; however, the structures of the proteins and the role of protein-bound carminic acid in the allergic reaction are unknown. The Committee considered that the available data satisfied the first two criteria for the addition of a foodstuff to the Codex Committee's list of common allergenic foods.

The available data on allergic reactions to food and drinks containing cochineal colours are derived predominantly from case reports. Although tests on control groups of patients have been reported, in general these studies were not designed so as to allow estimation of the incidence or prevalence of allergy to cochineal colours in the general population. Additional data suggest that sensitization to cochineal colours is rare, but they did not allow estimation of even comparative prevalence rates between countries. The third criterion for the addition of a foodstuff to the Codex Committee's list of common allergenic foods was not therefore considered to be satisfied.

Cochineal colours are present in many foods and drinks. The quantity of the cochineal colours that provoked an adverse reaction in an individual was estimated in only one study. Because the occurrence and severity of an allergic reaction after ingestion of a specific amount of cochineal colours depends on the sensitivity of each atopic individual, the Committee concluded that estimates of the long-term

intake of these colours in a population were irrelevant to its deliberations.

The Committee concluded that cochineal extract, carmines, and, possibly, carminic acid in foods and beverages may initiate or provoke allergic reactions in some individuals. Because some of the adverse reactions are severe, it considered that appropriate information, for example noting the presence of cochineal colours in foods and beverages, should be provided to alert individuals who are allergic to these compounds.

An addendum to the toxicological monograph was prepared.

In response to a request from the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*4*), the Committee revised the specifications for cochineal extract to include a limit for residual ethanol. The Committee also reduced the limit on lead to 2 mg/kg, in line with its policy on lead and heavy metals. The Committee also revised the specifications for carmines by replacing the current limits for arsenic and heavy metals by a single limit for lead of 2 mg/kg.

3.3 Sweetening agents

3.3.1 *Aspartame–acesulfame salt*

Aspartame–acesulfame salt is intended for use as a replacement for aspartame and acesulfame potassium in applications where use of both substances is permitted. During manufacture, the potassium moiety of acesulfame potassium is replaced by aspartame to produce a salt composed of equimolar amounts of aspartame and acesulfame, in a 2:1 ratio by weight. The salt is more stable to decomposition under storage conditions or in powdered forms than is a simple mixture of aspartame and acesulfame potassium.

Aspartame and acesulfame potassium have both been evaluated previously by the Committee. Aspartame was evaluated at the twenty-fourth meeting (Annex 1, reference *53*), when the Committee allocated an ADI of 0–40 mg/kg of body weight. Acesulfame potassium was evaluated at the twenty-fifth, twenty-seventh and thirty-seventh meetings (Annex 1, references *56*, *62* and *94*). At the thirty-seventh meeting, the Committee established an ADI of 0–15 mg/kg of body weight, on the basis of a 2-year study in rats.

Data on the production of aspartame–acesulfame salt and on the properties that are relevant to an assessment of its safety were available. Aspartame–acesulfame salt dissociates rapidly and completely to its components in aqueous media or on contact with saliva or

gastric fluid, indicating that no new issues would be introduced into the evaluations of the safety of aspartame or acesulfame potassium. Consequently, the Committee concluded that the aspartame and acesulfame moieties of the salt would be covered by the ADIs for aspartame (0–40 mg/kg of body weight) and acesulfame potassium (0–15 mg/kg of body weight).

A toxicological monograph was not prepared. New specifications were prepared.

3.3.2 *D-Tagatose*

D-Tagatose is an epimer of D-fructose and is produced from D-galactose by isomerization under alkaline conditions in the presence of calcium. It is used as a sweetener, texturizer, stabilizer, humectant and formulation aid. D-Tagatose has not been evaluated previously by the Committee.

In a study in rats adapted to consumption of D-tagatose, 80–90% of an oral dose was absorbed.

The predicted daily intake of D-tagatose was determined on the basis of data on food consumption in the USA and the assumption that all foods in the categories being considered contain the additive at the maximum technological level. For the population of the USA, intake of this sugar from all proposed uses (except chewing-gum, dietary supplements and meal replacements) was predicted to be 9 g/day for consumers with mean intakes and 18 g/day for those with intakes at the 90th percentile. Intake from chewing-gum was predicted to be 4 g/day for consumers with mean intakes and 8 g/day for those with intakes at the 90th percentile. Similar results were obtained for the predicted intakes of young people aged 3–5 years, 6–12 years and 13–19 years. The estimated intakes of D-tagatose from dietary supplements and meal replacements were 3 g and 5 g per eating occasion, respectively. An analysis based on the same assumptions, combined with available data on food consumption from Australia and the European Union, showed that the predicted intake of D-tagatose would be similar in these regions.

D-Tagatose was tested in Sprague-Dawley rats in a series of short-term toxicity studies. The observed increases in liver weights and liver hypertrophy were found to be due, at least in part, to glycogen accumulation. The hepatic changes were partially reversed after exclusion of D-tagatose from the diet. Recovery from the induced liver hypertrophy took longer than recovery from glycogen accumulation. Data from short-term studies of the mechanism of glycogen accumulation suggest that the hepatic changes are due to

physiological changes in Sprague-Dawley rats and that Wistar rats are less sensitive to expression of these effects.

The precise metabolic pathway of D-tagatose that leads to gluconeogenesis has not been established. D-Tagatose is metabolized more slowly than fructose. A similar biochemical effect characterized by glycogen accumulation occurs in patients with hereditary fructose intolerance, and this reaction can increase the rates of purine breakdown and accumulation of uric acid. D-Tagatose is more effective than fructose in increasing the concentration of uric acid in serum.

In two studies of developmental toxicity in Sprague-Dawley rats, minimal effects were observed in dams, including reduced food consumption at doses greater than 12 g/kg of body weight per day and initial depression of weight gain, which returned to normal later in the study. A dose-related, statistically significant increase in liver weight was found, but histological examination of the livers revealed no abnormalities. No effects were found in either study on reproductive or developmental parameters.

The results of tests for genotoxicity in vitro and in vivo were consistently negative.

A number of studies of gastrointestinal effects have been conducted in healthy human volunteers and in patients with type 2 diabetes. Nausea and adverse gastrointestinal effects were reported in healthy adults given D-tagatose at high doses. Studies in which baseline serum concentrations of insulin and glucose were investigated showed no effect following administration of single or multiple doses, but a decreased glycaemic response was observed when D-tagatose was given before a glucose tolerance test.

Elevated serum uric acid concentrations were reported in three out of six studies in which this parameter was measured; in two of these studies, the values exceeded the normal range. In the three studies in which parameters indicative of liver function or hepatic changes were measured, no effects were observed.

On the basis of the available data, the Committee concluded that D-tagatose is not genotoxic, embryotoxic or teratogenic. The Committee noted that the increased liver weights and hepatocellular hypertrophy seen in Sprague-Dawley rats occurred concurrently with increased glycogen deposition; however, the reversal of increased glycogen storage after removal of D-tagatose from the feed occurred more rapidly than regression of the liver hypertrophy.

Although the gastrointestinal symptoms seen in adult humans with the expected daily intake of D-tagatose were minor, the Committee was concerned about the increased serum uric acid concentrations observed in a number of studies in humans following administration of either single or repeated doses of D-tagatose. Similar increases are seen with other sugars, such as fructose, but D-tagatose appears to be a more potent inducer of this effect. The Committee noted that the effect of D-tagatose has not been studied in people prone to high serum uric acid concentrations.

The Committee concluded that an ADI could not be allocated to D-tagatose because of concern about its potential to induce liver glycogen deposition and hypertrophy and to increase serum uric acid concentrations. Two studies in Sprague-Dawley and Wistar rats were submitted that might help to resolve the relevance of the induction of liver glycogen deposition and hypertrophy, but the reports were received in draft form and were not suitable for consideration at the present meeting. Before reviewing the compound again, the Committee would wish to evaluate the final reports of these studies and data to clarify the extent, mechanism and toxicological consequences of the increased serum uric acid concentrations observed in humans exposed to D-tagatose.

A toxicological monograph and new specifications were prepared.

3.4 Miscellaneous substances

3.4.1 *Benzoyl peroxide*

Benzoyl peroxide was evaluated for use as a bleaching agent in flour by the Committee at its seventh meeting (Annex 1, reference *7*), when it concluded that treatment of flour at concentrations of up to 40 mg/kg was acceptable. The Codex Committee's draft General Standard for Food Additives proposes a maximum concentration of 300 mg/kg for treatment of flours and starches, 1000 mg/kg for total ripened cheese and concentrations consistent with good manufacturing practice for whey and whey products. The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*4*) for the Expert Committee to evaluate the higher levels of use of benzoyl peroxide recommended for flour and starches and its additional uses in milk products.

At its seventh meeting, the Committee noted that when benzoyl peroxide is used as a bleaching agent in flour, it reacts with the oxidizable substances that are present and is converted to benzoic acid; any remaining traces of benzoyl peroxide are reduced still

further during baking due to reduction to benzoic acid. On this basis, the issues requiring consideration were the acceptability of small amounts of benzoic acid in bread, the possible effects of oxidative treatment on the nutritional value of flour, and the possible formation of harmful substances or anti-metabolites. A group ADI of 0–5 mg/kg of body weight for benzoic acid and its calcium, potassium and sodium salts, benzyl acetate, benzyl alcohol, benzaldehyde and benzyl benzoate was maintained at the forty-sixth meeting of the Committee (Annex 1, reference *122*).

The intake of benzoic acid derived from benzoyl peroxide was estimated on the basis of the maximum levels of use of benzoyl peroxide proposed in the draft General Standard for Food Additives and data on consumption of flours, starches and cheese products in the WHO Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets, derived from food balance sheets. The mean intake of benzoic acid arising from the use of benzoyl peroxide was estimated to range from 20 to 120 mg/day. These values are likely to be overestimates.

The Committee noted that the intake of benzoic acid from foodstuffs treated with benzoyl peroxide should be considered together with intake from other dietary sources of benzoates in the group ADI of 0–5 mg/kg of body weight. When the Committee evaluated the intake of benzoates at its fifty-first meeting (Annex 1, reference *137*), it noted that the intake of some consumers may exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intakes above the ADI.

The Committee was informed that the Codex Committee on Food Additives and Contaminants had revised the maximum levels of benzoates proposed in the draft General Standard for Food Additives on the basis of the Expert Committee's evaluation of these substances at its fifty-first meeting. The Committee noted that the intake of benzoates derived from benzoyl peroxide should be included in future assessments of benzoate intake, although foodstuffs treated with benzoyl peroxide would not be labelled to indicate the presence of benzoate residues. In addition, the Committee noted that ingestion of benzoic acid has been associated with intolerance reactions.

At its present meeting, the Committee noted the importance of assessing the nutritional and toxicological implications of treatment of foods with benzoyl peroxide with respect to potential effects on

proteins, vitamins, antioxidants and physiologically important lipids. No information was available to the Committee that would assist such an assessment.

The Committee concluded that, in the absence of information about the nutritional and toxicological consequences of the proposed food uses of benzoyl peroxide and information on total benzoic acid intake in the context of all food additive uses, no conclusion could be drawn about the acceptability of the proposed uses.

A toxicological monograph was not prepared. The existing specifications for benzoyl peroxide were revised.

3.4.2 *Nitrous oxide*

Nitrous oxide was considered by the Committee at its twenty-second and twenty-ninth meetings (Annex 1, references *47* and *70*) for use as a propellant for food in aerosol containers. At its twenty-ninth meeting, the Committee concluded that use of nitrous oxide as a propellant for food was acceptable. An ADI was not established.

The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*4*) for the Expert Committee to evaluate the safety of the additional use of nitrous oxide as a packaging gas in modified atmospheric packaging.

No information on intake of nitrous oxide from its use as a packaging gas was available, although intake from this use is likely to be low. The Committee concluded that the use of nitrous oxide as a packaging gas could not be evaluated until such information became available.

A toxicological monograph was not prepared. The existing specifications were revised.

3.4.3 *Stearyl tartrate*

Stearyl tartrate was evaluated by the Committee at its ninth meeting (Annex 1, reference *11*) for use in strengthening dough before bread baking. At that time, the Committee considered that use of stearyl tartrate at concentrations of up to 500 mg/kg of flour was acceptable on the basis of data on its hydrolysis, metabolism and toxicity in experimental animals.

As higher levels of use and new uses were included in the draft General Standard for Food Additives, the Expert Committee was asked by the Codex Committee on Food Additives and Contaminants

(*4*) to re-evaluate stearyl tartrate at its present meeting. As no new data were submitted or were found in an extensive search of the literature, the Committee referred to the data reviewed at its ninth meeting (Annex 1, reference *11*). However, it was noted that the references on which the corresponding monograph was based were no longer available. The Committee concluded that either the original toxicological and metabolic studies should be made available or that new studies demonstrating hydrolysis in vivo should be submitted before the substance could be re-evaluated. The Committee also requires data on the intake of stearyl tartrate from all existing and proposed uses. The Committee noted that an ADI of 0–30 mg/kg of body weight had previously been established for L(+)-tartaric acid at its seventeenth meeting (Annex 1, reference *32*) and that the intake of stearyl tartrate relative to this ADI should be considered.

A toxicological monograph was not prepared. The existing specifications were revised.

3.4.4 *Trehalose*

Trehalose is a disaccharide that occurs naturally in insects, plants, fungi and bacteria. The major dietary source of naturally occurring trehalose is mushrooms. The commercial product is the dihydrate and is produced from liquefied starch by a multistep enzymatic process. Trehalose is used as a texturizer, stabilizer, humectant and sweetener in bakery goods, beverages, confectionery, fruit jam, breakfast cereals, rice and noodles. Trehalose has not previously been considered by the Committee.

The daily intake of trehalose was predicted on the basis of conservative assumptions, by combining the highest proposed levels of use. For adults in the USA, the mean predicted intake from all proposed uses, except chewing-gum, was 7 g/day, and that of consumers with intakes at the 90th percentile was 16 g/day. The mean intake per eating occasion ranged from 4 g to 10 g, while intake at the 90th percentile ranged from 8 g to 19 g per eating occasion. Consumers with mean intakes of chewing-gum and those with intakes at the 90th percentile would ingest 0.4 g/day and 0.8 g/day of trehalose, respectively. For adults in Australia, the predicted mean intake of trehalose (including from chewing-gum) ranged from 6 g/day to 10 g/day. However, the data from both Australia and the USA were based on short-term dietary recall, which tends to result in overestimates of habitual intake.

Trehalose is hydrolysed to glucose by the enzyme trehalase in the intestinal mucosa, and the small amount of intact trehalose that may

be absorbed is hydrolysed by trehalase in the plasma, liver or kidney. Trehalase deficiency has been identified in some individuals, but its prevalence appears to be very low in most populations, with the possible exception of that of Greenland, where an 8% prevalence has been recorded.

Studies in which trehalose was administered in the diet have been performed in mice and dogs. In a 3-month study in mice, slight, sporadic changes in clinical biochemistry were seen in males at the highest dose tested, 7.3 g/kg of body weight per day, but there was no evidence of pathological alterations. In a 14-day study in dogs, no clinical or morphological evidence of toxicity was seen at 5 g/kg of body weight per day, which was the highest dose tested.

In a two-generation study in rats, no evidence was found of an effect on reproduction. Similarly, in studies of developmental toxicity in rats and rabbits, there was no evidence of teratogenicity. The results of assays for genotoxicity were negative. No long-term studies were available, but these were considered unnecessary since trehalose is rapidly metabolized to glucose at the levels of intake predicted from the proposed uses. The toxicological data available on the enzymes used in the preparation of trehalose, some of which have been evaluated by the Committee previously, did not raise any concern.

Studies in humans indicate that trehalose is well tolerated. Increased frequencies of malabsorption and gastrointestinal symptoms were noted in individuals consuming single doses of 20 g or more. In the limited data on individuals with known or suspected trehalase deficiency, the only effects seen were the gastrointestinal effects expected of an undigested disaccharide.

On the basis of the available information, the Committee established an ADI "not specified"[1] for trehalose.

A toxicological monograph and new specifications were prepared.

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

4. Flavouring agents evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

Four groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in Fig. 1 (Annex 1, references *116*, *122*, *131* and *137*).

The Committee noted that, in applying the Procedure, a flavouring agent is first assigned to a structural class, as identified at the forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

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

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

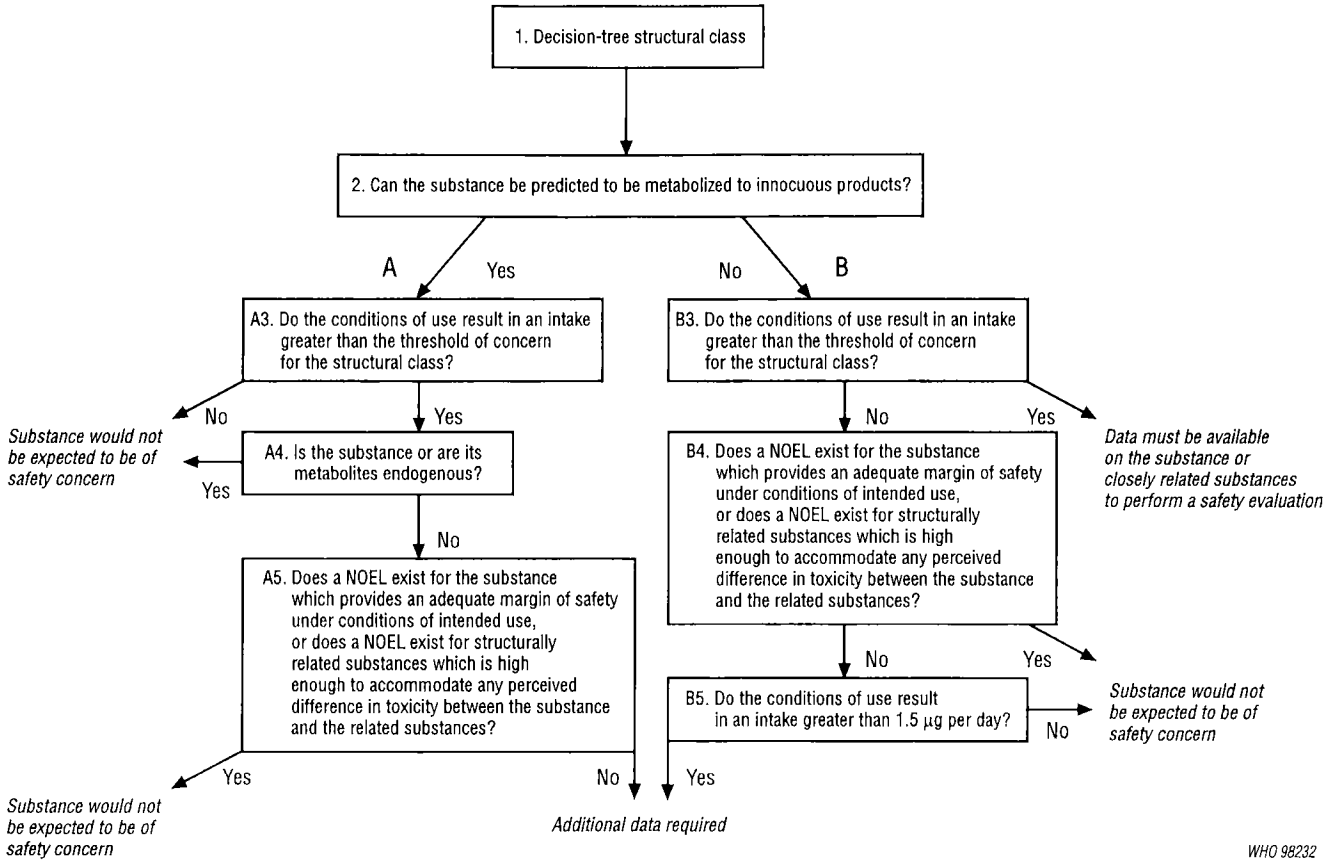
Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

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

Intake data

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. In Europe,

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in the European Union during the previous year. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products. In the USA, a series of surveys was conducted between 1970 and 1987 by the National Research Council of the National Academy of Sciences (under contract to the Food and Drug Administration) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added to a number of broad food categories.

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

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

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

4.1 Cinnamyl alcohol and related flavouring agents

The Committee evaluated a group of flavouring agents that included cinnamyl alcohol (No. 647), cinnamaldehyde (No. 656), cinnamic acid (No. 657) and 52 structurally related substances (Table 1) using the Procedure for the Safety Assessment of Flavouring Agents (see Fig. 1).

One member of this group, allyl cinnamate (No. 19), had been evaluated previously by the Committee at its forty-sixth meeting in a separate group of allyl ester flavouring agents examined by the Procedure (Annex 1, reference *122*).

Cinnamaldehyde (No. 656) was evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), when it established a conditional ADI of 0–1.25 mg/kg of body weight. At its twenty-third meeting, the Committee converted the previous conditional ADI to a temporary ADI of 0–0.7 mg/kg of body weight (Annex 1, reference *50*), which was extended at its twenty-fifth and twenty-eighth meetings (Annex 1, references *56* and *66*). At its thirty-fifth meeting,

Table 1

Summary of the results of safety evaluations of cinnamyl alcohol and 54 related flavouring agents[a]

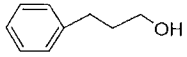
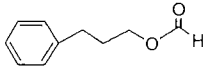
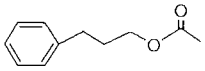
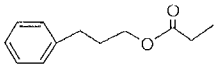
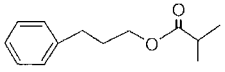
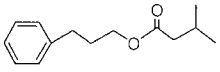
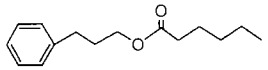
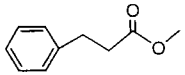
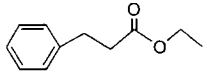
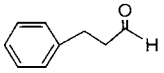
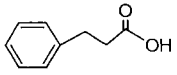
Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
3-Phenyl-1-propanol (3-phenylpropanol)	636	122-97-4 	No Europe: 60 USA: 31	NR	NR	See note 1	No safety concern
3-Phenylpropyl formate (benzenepropanol formate)	637	104-64-3 	No Europe: ND USA: 0.8	NR	NR	See note 2	
3-Phenylpropyl acetate (benzenepropanol acetate)	638	122-72-5 	No Europe: 41 USA: 9	NR	NR	See note 2	
3-Phenylpropyl propionate (benzenepropanol propionate)	639	122-74-7 	No Europe: 0.2 USA: 0.3	NR	NR	See note 2	
3-Phenylpropyl isobutyrate	640	103-58-2 	No Europe: 4 USA: 16	NR	NR	See note 2	
3-Phenylpropyl isovalerate	641	5452-07-3 	No Europe: 0.01 USA: 0.1	NR	NR	See note 2	

Table 1 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
3-Phenylpropyl hexanoate	642	6281-40-9 	No Europe: ND USA: 0.4	NR	NR	See note 2	No safety concern
Methyl 3-phenylpropionate	643	103-25-3 	No Europe: ND USA: 3	NR	NR	See note 2	
Ethyl 3-phenylpropionate	644	2021-28-5 	No Europe: 1 USA: 0.07	NR	NR	See note 2	
3-Phenylpropionaldehyde (benzenepropanal)	645	104-53-0 	No Europe: 19 USA: 19	NR	NR	See note 1	
3-Phenylpropionic acid (benzenepropanoic acid)	646	501-52-0 	No Europe: 23 USA: 0.5	NR	NR	See note 3	

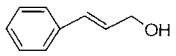
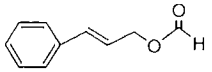
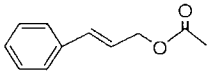
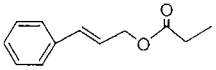
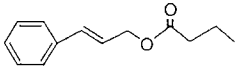
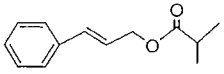
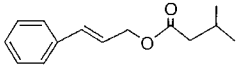
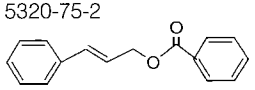
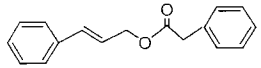
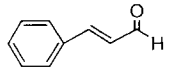
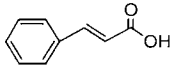
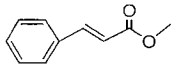
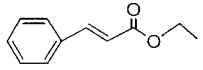
Cinnamyl alcohol	647	104-54-1 	Yes Europe: 1800 USA: 1900	No	Yes[d]	See note 4	No safety concern
Cinnamyl formate	649	104-65-4 	No Europe: 2 USA: 17	NR	NR	See note 5	
Cinnamyl acetate	650	103-54-8 	No Europe: 210 USA: 300	NR	NR	See note 5	
Cinnamyl propionate	651	103-56-0 	No Europe: 4 USA: 25	NR	NR	See note 5	
Cinnamyl butyrate	652	103-61-7 	No Europe: 3 USA: 2	NR	NR	See note 5	
Cinnamyl isobutyrate	653	103-59-3 	No Europe: 13 USA: 22	NR	NR	See note 5	
Cinnamyl isovalerate	654	140-27-2 	No Europe: 5 USA: 8	NR	NR	See note 5	

Table 1 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Cinnamyl benzoate	760	5320-75-2 	No Europe: ND USA: 1	NR	NR	See note 6	No safety concern
Cinnamyl phenylacetate	655	7492-65-1 	No Europe: 0.003 USA: 1	NR	NR	See note 7	
Cinnamaldehyde (3-phenyl-2-propenal)	656	104-55-2 	Yes Europe: 2500 USA: 59 000	No	Yes[e]	See note 4	
Cinnamic acid (3-phenyl-2-propenoic acid)	657	621-82-9 	No Europe: 32 USA: 44	NR	NR	See note 8	
Methyl cinnamate	658	103-26-4 	Yes Europe: 2800 USA: 830	No	Yes[f]	See note 9	
Ethyl cinnamate	659	103-36-6 	No Europe: 100 USA: 70	NR	NR	See note 9	

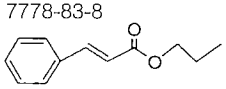
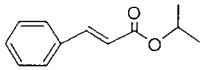
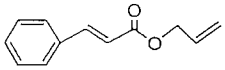
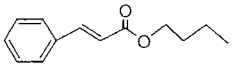
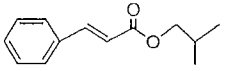
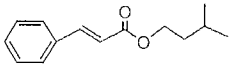
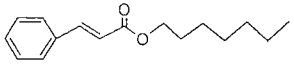
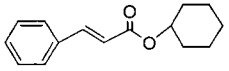
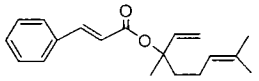
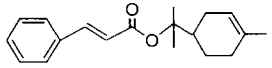
Propyl cinnamate	660	7778-83-8 	No Europe: 0.4 USA: 4	NR	NR	See note 9	No safety concern
Isopropyl cinnamate	661	7780-06-5 	No Europe: 19 USA: 3	NR	NR	See note 9	
Allyl cinnamate (3-propenyl 3-phenyl-2-propenoate)	19	1866-31-5 	No Europe: 5 USA: 0.3	NR	NR	See note 9	
Butyl cinnamate	663	538-65-8 	No Europe: 0.4 USA: 0.2	NR	NR	See note 9	
Isobutyl cinnamate	664	122-67-8 	No Europe: 1 USA: 3	NR	NR	See note 9	
Isoamyl cinnamate (isopentyl cinnamate)	665	7779-65-9 	No Europe: 8 USA: 6	NR	NR	See note 9	
Heptyl cinnamate	666	10032-08-3 	No Europe: 2 USA: 52	NR	NR	See note 9	

Table 1 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Cyclohexyl cinnamate	667	7779-17-1 	No Europe: 0.4 USA: 0.04	NR	NR	Cyclohexyl cinnamate is hydrolysed to cinnamic acid (see note 8) and cyclohexanol. Cyclohexanol is mainly conjugated with glucuronic acid and excreted	No safety concern
Linalyl cinnamate	668	78-37-5 	No Europe: 7 USA: 3	NR	NR	Linalyl cinnamate is hydrolysed to cinnamic acid (see note 8) and linalool. Linalool undergoes ω- and ω-1-oxidation to yield polar metabolites which are excreted	
Terpinyl cinnamate ((*Z*)-1-methyl-1-(4-methyl-3-cyclohexen-1-yl)ethyl cinnamate)	669	10024-56-3 	No Europe: 0.01 USA: 0.5	NR	NR	Terpinyl cinnamate is hydrolysed to cinnamic acid (see note 8) and terpineol. Terpineol undergoes ω- and ω-1-oxidation to yield polar metabolites which are excreted	

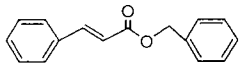
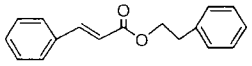
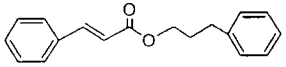
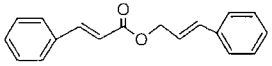
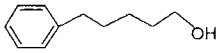
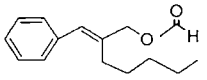
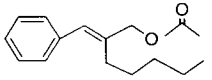
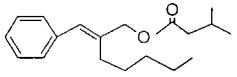
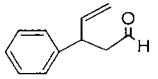
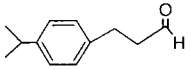
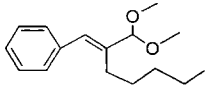
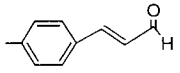
Benzyl cinnamate	670	103-41-3 	No Europe: 44 USA: 69	NR	NR	Benzyl cinnamate is hydrolysed to cinnamic acid (see note 8) and benzyl alcohol. Benzyl alcohol is oxidized to benzoic acid and excreted as hippuric acid	No safety concern
Phenethyl cinnamate	671	103-53-7 	No Europe: 6 USA: 50	NR	NR	Phenethyl cinnamate is hydrolysed to cinnamic acid (see note 8) and phenethyl alcohol. Phenethyl alcohol is oxidized to phenylacetic acid and excreted as the glucuronic acid conjugate	
3-Phenylpropyl cinnamate	672	122-68-9 	No Europe: 0.6 USA: 37	NR	NR	See notes 1 and 8	
Cinnamyl cinnamate	673	122-69-0 	No Europe: 2 USA: 36	NR	NR	See notes 4 and 8	
5-Phenylpentanol (benzenepentan-1-ol)	675	10521-91-2 	No Europe: ND USA: 0.1	NR	NR	See note 1	
α-Amylcinnamyl formate (2-(phenylmethylene)heptyl formate)	676	7493-79-0 	No Europe: 1.4 USA: 0.5	NR	NR	See note 10	

Table 1 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
α-Amylcinnamyl acetate (2-(phenylmethylene)heptyl acetate)	677	7493-78-9 	No Europe: 3 USA: 260	NR	NR	See note 10	No safety concern
α-Amylcinnamyl isovalerate (2-(phenylmethylene)heptyl isovalerate)	678	7493-80-3 	No Europe: 0.01 USA: 0.5	NR	NR	See note 10	
3-Phenyl-4-pentenal (3-phenylpent-4-enal)	679	939-21-9 	No Europe: 1 USA: 2	NR	NR	See note 11	
3-(*p*-Isopropylphenyl) propionaldehyde (3-(*p*-cumenyl)propion-aldehyde)	680	7775-00-0 	No Europe: ND USA: 0.1	NR	NR	See note 1	
α-Amylcinnamaldehyde dimethyl acetal ((2-(dimethoxymethyl)-1-heptenyl)benzene)	681	91-87-2 	No Europe: 0.01 USA: 0.007	NR	NR	See note 10	
p-Methylcinnamaldehyde (3-(4-methylphenyl)-2-propenal)	682	1504-75-2 	No Europe: 0.01 USA: 0.9	NR	NR	See note 4	

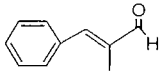
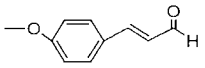
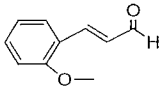
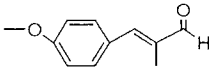
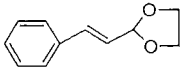
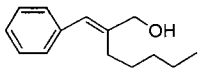
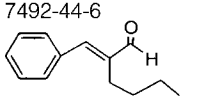
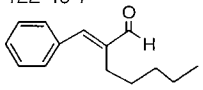
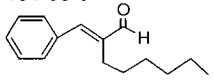
α-Methylcinnamaldehyde	683	101-39-3 	No Europe: 3 USA: 390	NR	NR	See note 4	No safety concern
p-Methoxycinnamaldehyde	687	1963-36-6 	No Europe: 0.04 USA: 0.01	NR	NR	See note 4	
o-Methoxycinnamaldehyde	688	1504-74-1 	No Europe: 0.6 USA: 71	NR	NR	*o*-Methoxycinnamaldehyde is oxidized to the corresponding acid, conjugated with glycine, and excreted. Alternatively, the acid may undergo β-oxidation to yield the β-hydroxycarboxylic acid derivative, which is also excreted	
p-Methoxy-α-methylcinnamaldehyde (4′-methoxy-2-methylcinnamaldehyde)	689	65405-67-6 	No Europe: 0.3 USA: 0.05	NR	NR	See note 4	
Structural class II							
Cinnamaldehyde ethylene glycol acetal (2-styryl-1,3-dioxolane)	648	5660-60-6 	Yes Europe: 690 USA: 0.007	No	Yes[9]	Hydrolysed to the corresponding alcohol and aldehyde	

Table 1 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
α-Amylcinnamyl alcohol (2-pentyl-3-phenylprop-2-en-1-ol)	674	101-85-9 	No Europe: 4 USA: 1	NR	NR	α-Amylcinnamyl alcohol is oxidized to the corresponding aldehyde, which is further oxidized to α-amylcinnamic acid and excreted	No safety concern
α-Butylcinnamaldehyde	684	7492-44-6 	No Europe: 0.01 USA: 0.07	NR	NR	See note 11	
α-Amylcinnamaldehyde (α-pentylcinnamaldehyde)	685	122-40-7 	No Europe: 25 USA: 23	NR	NR	See note 11	
α-Hexylcinnamaldehyde	686	101-86-0 	No Europe: 87 USA: 11	NR	NR	See note 11	

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

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

[b] The names of the flavouring agents are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where flavouring agents were evaluated under their trivial name, the systematic name is given in parentheses.

[c] The thresholds for human intake for structural classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

[d] The NOEL of 54 mg/kg of body weight per day in a 4-month toxicity study in rats is >1000 times the estimated intake of cinnamyl alcohol when used as a flavouring agent.

[e] The NOEL of 620 mg/kg of body weight per day in a 13-week toxicity study in rats is >600 times the estimated intake of cinnamaldehyde when used as a flavouring agent.

[f] The NOELs of 54 mg/kg of body weight per day for related substance No. 647 and 80 mg/kg of body weight per day for related substance No. 659 are >1000 times the estimated intake of methyl cinnamate when used as a flavouring agent.

[g] The NOEL of 620 mg/kg of body weight per day for related substance No. 656 is >10 000 times the estimated intake of cinnamaldehyde ethylene glycol acetal when used as a flavouring agent.

Notes to Table 1

1. Oxidized to yield the corresponding acid, which undergoes further β-oxidation of the side-chains and cleavage to yield the benzoic acid derivative. It then conjugates with glycine and/or glucuronic acid, and is excreted in the urine.
2. Hydrolysed to the corresponding acid and alcohol. The acid is completely oxidized and the alcohol, 3-phenyl-1-propanol, is further metabolized and excreted (see note 1).
3. Undergoes β-oxidation of the side-chains and cleavage to yield the corresponding benzoic acid derivative. It then conjugates with glycine and/or glucuronic acid and is excreted in the urine.
4. Oxidized to cinnamic acid (or its corresponding derivative), which is further oxidized to benzoic acid (or its corresponding derivative). The latter substance is excreted as hippuric acid (or its corresponding derivative).
5. Hydrolysed to cinnamyl alcohol and the corresponding carboxylic acid. Cinnamyl alcohol is oxidized and excreted (see note 4); the carboxylic acid is either completely oxidized or conjugated and excreted primarily in the urine.
6. Hydrolysed to cinnamyl alcohol and benzoic acid. Cinnamyl alcohol is oxidized to cinnamic acid, which is further oxidized to benzoic acid (see note 4).
7. Hydrolysed to cinnamyl alcohol and phenylacetic acid. Cinnamyl alcohol is oxidized to cinnamic acid, which is further oxidized to benzoic acid (see note 4). Phenylacetic acid is excreted as the glucuronic acid conjugate.
8. Undergoes β-oxidation and is excreted as hippuric acid.
9. Rapidly hydrolysed to cinnamic acid (see note 8) and the corresponding alcohol. The corresponding alcohol is completely oxidized.
10. Hydrolysed to α-amylcinnamyl alcohol (No. 674) and the corresponding acid, which is excreted. α-Amylcinnamyl alcohol is oxidized to α-amylcinnamaldehyde, which is further oxidized to α-amylcinnamic acid and excreted.
11. Oxidized to the corresponding acid and excreted.

the Committee did not extend the temporary ADI (Annex 1, reference *88*) because the data that were required were not available.

At its twenty-fifth meeting, the Committee concluded that cinnamyl anthranilate should not be used as a food additive (Annex 1, reference *56*). This substance is structurally related to the group of flavouring agents considered here, but differs in that it is hydrolysed to cinnamyl alcohol and anthranilic acid only slowly, resulting in systemic intake of the intact ester. In contrast, cinnamyl alcohol and related flavouring agents undergo rapid hydrolysis.

Twenty-two of the 55 flavouring agents in this group are natural components of foods. Concentrations of cinnamaldehyde of up to 750 g/kg have been detected in oils from natural sources, such as the inner bark and leaves of *Cinnamomum* trees that are used to make cinnamon. However, intake of these 22 agents is primarily via food additives rather than from natural sources.

4.1.1 *Estimated daily per capita intake*

The total annual volume of production of the 55 cinnamyl compounds in this group destined for use as flavouring agents is approximately 60 tonnes in Europe and 480 tonnes in the USA. Approximately 30% of the total annual volume of production in Europe and over 93% of that in the USA is accounted for by cinnamaldehyde (No. 656), while 54% of the total annual volume of production in Europe is accounted for by cinnamyl alcohol (No. 647) and methyl cinnamate (No. 658). The estimated daily per capita intakes in Europe are 2.5 mg of cinnamaldehyde (No. 656), 1.8 mg of cinnamyl alcohol (No. 647) and 2.8 mg of methyl cinnamate (No. 658). The estimated daily per capita intakes in the USA are 59 mg of cinnamaldehyde (No. 656), 1.9 mg of cinnamyl alcohol (No. 647) and 0.83 mg of methyl cinnamate (No. 658). The estimated daily per capita intakes of all the other flavouring agents in the group are in the range 0.003–690 µg, with most of the values being at the low end of this range. The daily per capita intake of each substance in Europe and the USA is reported in Table 1.

4.1.2 *Absorption, metabolism and elimination*

Cinnamyl alcohol (No. 647), cinnamaldehyde (No. 656) and its *p*- and *o*-methoxy derivatives (Nos 687 and 688), cinnamic acid (No. 657) and its corresponding methyl ester (No. 658), and the saturated analogue 3-phenylpropionic acid (No. 646) have all been shown to be rapidly absorbed from the gut, metabolized and excreted primarily in the urine and to a minor extent in the faeces.

Esters of cinnamic acid and structurally related aromatic esters have been shown to be hydrolysed rapidly to the component acid and alcohol. The aromatic primary alcohols and aldehydes in this group and those formed by the hydrolysis of esters and acetals are readily oxidized to cinnamic acid or one of its structurally related carboxylic acids. In animals, most carboxylic acids, including cinnamic acid, are converted to acyl coenzyme A esters. Cinnamoyl coenzyme A undergoes either conjugation with glycine or β-oxidation, eventually leading to the formation of benzoyl coenzyme A. This in turn is either conjugated with glycine, yielding hippuric acid, or hydrolysed to yield free benzoic acid, which is then excreted.

Cinnamyl derivatives containing α-methyl substituents, such as α-methylcinnamaldehyde (No. 683), are extensively metabolized by β-oxidation and cleavage to yield mainly the corresponding hippuric acid derivative. Because *o*-oxygenated ring substituents (e.g. *o*-methoxycinnamaldehyde, No. 688) selectively inhibit oxidation of coenzyme A esters of β-hydroxycarboxylic acid derivatives via the β-oxidation pathway, these derivatives are excreted as glycine conjugates. In contrast, *p*-oxygenated ring substituents (e.g. *p*-methoxycinnamaldehyde, No. 687) are oxidized via the β-oxidation pathway, eventually yielding hippuric acid derivatives.

4.1.3 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

Step 1

In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the above-mentioned substances, the Committee assigned 50 of the 55 substances to structural class I. These are the simple aromatic compounds with a saturated propyl or unsaturated propenyl side-chain containing a primary oxygenated functional group, which have little toxic potential. The remaining five substances, which are those containing a heterocyclic ring (No. 648) or aromatic rings bearing substituents other than 1–5 carbon aliphatic groups (Nos 674, 684–686), were assigned to structural class II.

Step 2

All the substances in this group are predicted to be metabolized to innocuous products (see page 20). The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Step A3

The estimated daily per capita intakes of 47 of the 50 substances in structural class I and of four of the five substances in structural class II were below the thresholds for substances in these classes (1800μg

and 540μg, respectively). According to the Procedure, the safety of these 51 flavouring agents raises no concern when they are used at their currently estimated levels of intake.

The estimated daily per capita intake of cinnamyl alcohol (No. 647) is 1800μg in Europe and 1900μg in the USA. The estimated daily per capita intake of cinnamaldehyde (No. 656) is 2500μg in Europe and 59000μg in the USA. The estimated daily per capita intake of methyl cinnamate (No. 658) is 2800μg in Europe and 830μg in the USA. The estimated daily per capita intake of cinnamaldehyde ethylene glycol acetal (No. 648) is 690μg in Europe and 0.007μg in the USA. The estimated daily per capita intakes of these four flavouring agents therefore exceed the thresholds for their respective structural classes (i.e. 1800μg for Nos 647, 656 and 658, and 540μg for No. 648). Accordingly, the evaluation of these substances proceeded to step A4.

Step A4

None of these four flavouring agents is endogenous. Accordingly, the evaluation of these agents proceeded to step A5.

Step A5

The NOEL of 54mg/kg of body weight per day for cinnamyl alcohol (No. 647) in a 4-month toxicity study in rats is >1000 times the estimated intake of this substance from its use as a flavouring agent in Europe (30μg/kg of body weight) and the USA (32μg/kg of body weight). The NOEL of 620mg/kg of body weight per day for cinnamaldehyde (No. 656) in a 13-week toxicity study in rats is >10000 times the estimated intake of this substance from its use as a flavouring agent in Europe (42μg/kg of body weight) and >600 times that in the USA (990μg/kg of body weight).

The Committee considered the NOEL of 54mg/kg of body weight per day for cinnamyl alcohol (No. 647) appropriate for evaluating the safety of methyl cinnamate (No. 658), because cinnamyl alcohol is oxidized to cinnamic acid, which is a product of hydrolysis of methyl cinnamate. In addition, the Committee noted that a NOEL of 80mg/kg of body weight per day had been identified for a closely related ester, ethyl cinnamate (No. 659), in a 4-month toxicity study in rats. Both of these NOELs are >1000 times the estimated intake of methyl cinnamate (No. 658) from its use as a flavouring agent in Europe and the USA. Cinnamaldehyde ethylene glycol acetal (No. 648) is rapidly hydrolysed to cinnamaldehyde (No. 656); the NOEL of 620mg/kg of body weight per day for cinnamaldehyde is >10000 times the estimated intake of cinnamaldehyde ethylene glycol acetal from its use as a flavouring agent in Europe and the USA.

The Committee therefore concluded that cinnamyl alcohol (No. 647), cinnamaldehyde (No. 656), methyl cinnamate (No. 658) and cinnamaldehyde ethylene glycol acetal (No. 648) would not be expected to be of safety concern.

Table 1 summarizes the evaluation of cinnamyl alcohol and 54 related substances used as flavouring agents.

4.1.4 *Consideration of combined intakes*

In the unlikely event that all foods containing all 50 substances in structural class I were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for class I. In the unlikely event that all foods containing all five substances in structural class II were consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for class II. However, all 55 substances in this group are expected to be efficiently metabolized and would not saturate the metabolic pathways. Overall evaluation of the data indicates that combined intake would not present a safety concern.

4.1.5 *Conclusions*

The Committee concluded that the substances in this group would not present safety concerns at the current estimated levels of intake. In using the Procedure, the Committee noted that where toxicity data were available, they were consistent with the results of the safety evaluation.

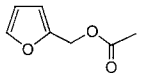
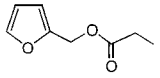
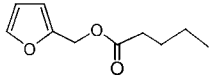
A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.2 Furfuryl alcohol and related flavouring agents

The Committee evaluated a group of flavouring agents that included furfuryl alcohol (No. 451), furfural (No. 450), five esters formed from furfuryl alcohol and simple aliphatic carboxylic acids (Nos 739–743), five esters formed from simple aliphatic alcohols and furoic acid (Nos 746–750), and three structurally related furfuryl derivatives, namely 5-methylfurfural (No. 745), 2-benzofurancarboxaldehyde (No. 751) and 2-phenyl-3-carbethoxyfuran (No. 752) (Table 2) using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). These flavouring agents were grouped on the basis of the criterion that all are hydrolysed and/or metabolized to furoic acid or a substituted furoic acid.

The Committee has evaluated only one member of this group previously, namely furfural (No. 450). Furfural was considered by the

Table 2
Summary of the results of the safety evaluation of furfuryl alcohol and 14 related flavouring agents[a]

Flavouring agent[b]	No.	CAS no. and structure	*Step B3*[c] Does intake exceed the threshold for human intake?	*Step B4* NOEL for substance or structurally related substance?	Conclusion based on current intake
Structural class II					
Furfuryl alcohol[d] (2-hydroxymethylfuran)	451	98-00-0 	No Europe: 210 USA: 24	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10 000 times the estimated intake of furfuryl alcohol when used as a flavouring agent	No safety concern
Furfuryl acetate[d] (2-furanmethanol acetate)	739	623-17-6 	No Europe: 18 USA: 21	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >100 000 times the estimated intake of furfuryl acetate when used as a flavouring agent	
Furfuryl propionate[d] (2-furanmethanol propionate)	740	623-19-8 	No Europe: 2 USA: 5	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >100 000 times the estimated intake of furfuryl propionate when used as a flavouring agent	
Furfuryl pentanoate[d] (2-furanylmethyl pentanoate)	741	36701-01-6 	No Europe: 0.3 USA: 14	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >100 000 times the estimated intake of furfuryl pentanoate when used as a flavouring agent	

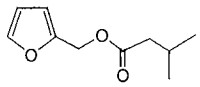
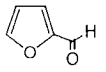
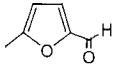
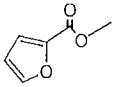
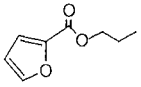
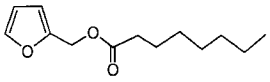
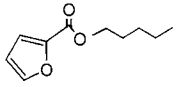
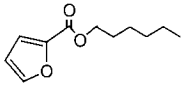
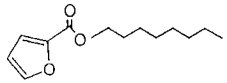
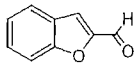
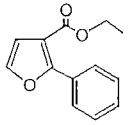
Furfuryl 3-methylbutanoate[d] (2-furanylmethyl-3-methylbutanoate)	743	13678-60-9 	No Europe: 0.03 USA: 1	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >1 million times the estimated intake of furfuryl 3-methylbutanoate when used as a flavouring agent	No safety concern
Furfural[d] (2-furfuraldehyde)	450	98-01-1 	No Europe: 520 USA: 460	Yes The NOEL of 53 mg/kg of body weight per day in a 13-week toxicity study in rats is >1000 times the estimated intake of furfural when used as a flavouring agent	
5-Methylfurfural (5-methyl-2-furfuraldehyde)	745	620-02-0 	No Europe: 160 USA: 25	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10000 times the estimated intake of 5-methylfurfural when used as a flavouring agent	
Methyl 2-furoate[d]	746	611-13-2 	No Europe: 35 USA: 37	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10000 times the estimated intake of methyl 2-furoate when used as a flavouring agent	
Propyl 2-furoate[d]	747	615-10-1 	No Europe: ND USA: 0.1	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10 million times the estimated intake of propyl 2-furoate when used as a flavouring agent	

Table 2 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step B3*[c] Does intake exceed the threshold for human intake?	*Step B4* NOEL for substance or structurally related substance?	Conclusion based on current intake
Structural class III					
Furfuryl octanoate[d] (2-furanylmethyl octanoate)	742	39252-03-4 	No Europe: 0.01 USA: 6	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >100 000 times the estimated intake of furfuryl octanoate when used as a flavouring agent	No safety concern
Amyl 2-furoate[d] (pentyl 2-furoate)	748	1334-82-3 	No Europe: ND USA: 0.1	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10 million times the estimated intake of amyl 2-furoate when used as a flavouring agent	
Hexyl 2-furoate[d]	749	39251-86-0 	No Europe: ND USA: 0.1	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >10 million times the estimated intake of hexyl 2-furoate when used as a flavouring agent	

Octyl 2-furoate[d]	750	39251-88-2 	No Europe: 3 USA: 0.1	Yes The NOEL of 53 mg/kg of body weight per day for related substance No. 450 is >1 million times the estimated intake of octyl 2-furoate when used as a flavouring agent	No safety concern
2-Benzofuran-carboxaldehyde	751	4265-16-1 	No Europe: ND USA: 0.01	Yes The NOEL of 25 mg/kg of body weight per day in a 90-day toxicity study in rats is >100 million times the estimated intake of 2-benzofuran-carboxaldehyde when used as a flavouring agent	
2-Phenyl-3-carbethoxyfuran (ethyl 2-phenyl-3-furoate)	752	50626-02-3 	No Europe: 0.01 USA: 2	Yes The NOEL of 13 mg/kg of body weight per day in a 90-day toxicity study in rats is >400 times the estimated intake of 2-phenyl-3-carbethoxyfuran when used as a flavouring agent	

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

[a] *Step 2*: None of the substances in this group is expected to be metabolized to innocuous products.

[b] The names of the flavouring agents are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where flavouring agents were evaluated under their trivial name, the systematic name is given in parentheses.

[c] The thresholds for human intake for structural classes II and III are 540 µg per day and 90 µg per day, respectively. All intake values are expressed in µg per day.

[d] An ADI for furfural of 0–0.5 mg/kg of body weight was established at the present meeting. Furfuryl alcohol and a number of derivatives of furfuryl alcohol and furoic acid are metabolized to the same metabolite (furoic acid) as furfural. The ADI for furfural should be considered a group ADI for furfural, furfuryl alcohol and these derivatives, which include furfuryl acetate, furfuryl propionate, furfuryl pentanoate, furfuryl octanoate, furfuryl 3-methylbutanoate, methyl 2-furoate, propyl 2-furoate, amyl 2-furoate, hexyl 2-furoate and octyl 2-furoate.

Committee at its thirty-ninth and fifty-first meetings, but no ADI was established (Annex 1, references *101* and *137*). At its present meeting, the Committee established a group ADI of 0–0.5 mg/kg of body weight for furfural (No. 450), furfuryl alcohol (No. 451), and 10 derivatives of furfuryl alcohol and furoic acid on the basis of a NOEL of 53 mg/kg of body weight per day in a 13-week study on furfural in rats and a safety factor of 100 (see section 3.1.1).

Seven of the 15 substances in this group have been detected as natural components of foods, including roasted coffee, beer, milk, roasted almonds, white bread and whisky.

4.2.1 *Estimated daily per capita intake*

The total annual volume of production of the 15 substances in this group is 6600 kg in Europe and 4500 kg in the USA. These values are equivalent to total daily per capita intakes of 940 µg in Europe and 590 µg in the USA. Furfural (No. 450) accounted for approximately 55% of the total daily per capita intake in Europe (520 µg) and 77% of that in the USA (460 µg).

4.2.2 *Absorption, metabolism and elimination*

Furfuryl esters are hydrolysed to furfuryl alcohol (No. 451) and the corresponding carboxylic acid. Furfuryl alcohol (No. 451) is subsequently oxidized to furfural (No. 450), which is then oxidized to 2-furoic acid. Furoate esters (Nos 746–750) are or are predicted to be hydrolysed directly to 2-furoic acid and the corresponding alcohol. Furoic acid forms a coenzyme A thioester, which may be either metabolized to a glycine conjugate that is excreted in urine or condensed with acetyl coenzyme A to form 2-furanacryloyl coenzyme A, which is converted to a glycine conjugate and excreted in urine. The three remaining furfuryl derivatives (5-methylfurfural (No. 745), 2-benzofurancarboxaldehyde (No. 751) and 2-phenyl-3-carbethoxyfuran (No. 752)) are expected to follow similar metabolic pathways, i.e. hydrolysis of the ester, oxidation and conjugation with glycine, followed by side-chain oxidation (No. 745) or aromatic oxidation (Nos 751 and 752). In rodents, a minor pathway has been identified which involves oxidation of the furan ring to produce carbon dioxide and as yet unidentified metabolites.

4.2.3 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

Step 1

In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the above-mentioned substances, the Com-

mittee assigned nine of the 15 substances (Nos 450, 451, 739–741, 743 and 745–747) to structural class II. The remaining six substances (Nos 742 and 748–752) were assigned to structural class III.

Step 2

The available data on the metabolism of individual members of the group were sufficient to draw conclusions about the probable metabolic fate of all members of the group. Most (Nos 450, 451, 739–743 and 746–750) are predicted to be metabolized to 2-furoic acid or a 2-furoic acid derivative, which is either conjugated with glycine and excreted in the urine, or condensed with acetyl coenzyme A and conjugated with glycine before excretion in the urine. Because of concern about the results of the toxicological studies on furfural in rodents, these substances cannot be predicted to be metabolized to innocuous products. The evaluation of all substances in this group therefore proceeded via the right-hand side of the decision-tree.

Step B3

The estimated daily per capita intakes of all nine substances in structural class II and all six substances in structural class III are below the thresholds of concern for these classes (540 μg and 90 μg, respectively). Accordingly, the evaluation of all 15 substances proceeded to step B4.

Step B4

For furfural (No. 450), the NOEL of 53 mg/kg of body weight per day in a 13-week feeding study in rats provides an adequate margin of safety (>1000 times) in relation to the estimated intake of this substance in Europe and the USA. This NOEL is also appropriate for evaluating the safety of furfuryl alcohol (No. 451) and the structurally related substances furfuryl acetate (No. 739), furfuryl propionate (No. 740), furfuryl pentanoate (No. 741), furfuryl octanoate (No. 742) and furfuryl 3-methylbutanoate (No. 743), because all of these esters would be hydrolysed to furfuryl alcohol and then oxidized to furfural. The NOEL for furfural is also appropriate for evaluating the safety of the esters of furoic acid, namely methyl 2-furoate (No. 746), propyl 2-furoate (No. 747), amyl 2-furoate (No. 748), hexyl 2-furoate (No. 749) and octyl 2-furoate (No. 750), which would be hydrolysed to furoic acid (the major metabolite of furfural). This NOEL is also appropriate for assessing the safety of 5-methylfurfural (No. 745), which would participate in the same metabolic pathways as the furoic acid esters and also undergoes alkyl oxidation. For 2-benzofurancarboxaldehyde (No. 751), the NOEL of 25 mg/kg of body weight per day in a 90-day

feeding study in rats provides an adequate margin of safety (>100 million) in relation to the estimated intake of this substance in the USA. For 2-phenyl-3-carbethoxyfuran (No. 752), the NOEL of 13 mg/kg of body weight per day in a 90-day feeding study in rats provides an adequate margin of safety (>400) in relation to the estimated intake of this substance in Europe and the USA.

Table 2 summarizes the evaluation of furfuryl alcohol and 14 related substances used as flavouring agents.

4.2.4 *Consideration of combined intakes*

In the unlikely event that all foods containing all nine substances in structural class II (together with allyl 2-furoate evaluated previously by the Committee) were consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for class II. In the unlikely event that all foods containing all six substances in structural class III were consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for class III. However, on the basis of the wide margin of safety between the levels of estimated intake and the NOEL for furfural and the fact that the available detoxification pathways (glycine conjugation or condensation followed by glycine conjugation) would not be saturated at the current estimated levels of intake, the Committee concluded that the combined intake would not be of safety concern.

4.2.5 *Conclusions*

On the basis of the predicted metabolism of these substances and data on their toxicity, the Committee concluded that consumption of furfuryl alcohol and the 14 related substances in this group would not give rise to safety concerns at the current estimated levels of intake. In applying the Procedure, the Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluation.

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

4.3 Phenol and phenol derivatives

The Committee evaluated a group of 48 flavouring agents (Table 3) using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). The group included phenol (No. 690), two esters of phenol (Nos 734 and 736) and resorcinol (No. 712); alkyl-, alkenyl- or

Table 3
Summary of the results of safety evaluations of phenol and 47 phenol derivatives used as flavouring agents[a]

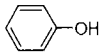
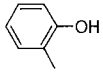
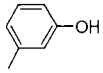
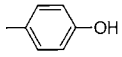
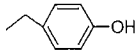
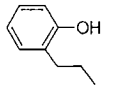
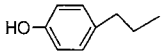
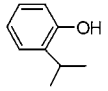
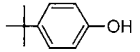
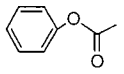
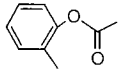
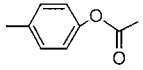
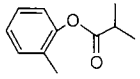
Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
Phenol	690	108-95-2 	No Europe: 6 USA: 1	NR	NR	See note 1	No safety concern
o-Cresol	691	95-48-7 	No Europe: 290 USA: 0.1	NR	NR	See note 1	
m-Cresol	692	108-39-4 	No Europe: 0.1 USA: 0.1	NR	NR	See note 1	
p-Cresol	693	106-44-5 	No Europe: 1 USA: 1	NR	NR	See note 1	
p-Ethylphenol (4-ethylphenol)	694	123-07-9 	No Europe: 4 USA: 0.1	NR	NR	See note 1	
o-Propylphenol (2-propylphenol)	695	644-35-9 	No Europe: 0.1 USA: 1	NR	NR	See note 1	

Table 3 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
p-Propylphenol (4-propylphenol)	696	645-56-7 	No Europe: 0.1 USA: 0.1	NR	NR	See note 1	No safety concern
2-Isopropylphenol	697	88-69-7 	No Europe: 16 USA: 0.3	NR	NR	See note 1	
4-(1,1-Dimethylethyl)-phenol (*p-tert*-butylphenol)	733	98-54-4 	No Europe: 0.01 USA: 0.01	NR	NR	See note 1	
Phenyl acetate	734	122-79-2 	No Europe: 0.01 USA: 0.01	NR	NR	See note 2	
o-Tolyl acetate	698	533-18-6 	No Europe: 0.1 USA: 40	NR	NR	See note 2	
p-Tolyl acetate	699	140-39-6 	No Europe: ND USA: 70	NR	NR	See note 2	
o-Tolyl isobutyrate	700	36438-54-7 	No Europe: 0.03 USA: 0.1	NR	NR	See note 2	

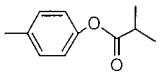
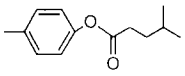
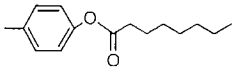
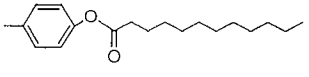
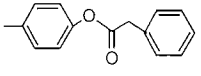
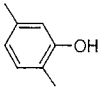
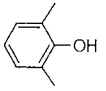
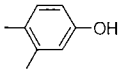
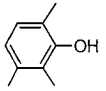
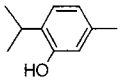
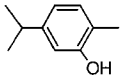
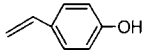
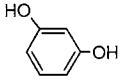
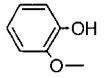
p-Tolyl isobutyrate	701	103-93-5 	No Europe: 0.04 USA: 0.01	NR	NR	See note 2	No safety concern
p-Tolyl 3-methyl-butyrate (*p*-tolyl isovalerate)	702	55066-56-3 	No Europe: 0.4 USA: 0.1	NR	NR	See note 2	
p-Tolyl octanoate	703	59558-23-5 	No Europe: 0.03 USA: 1	NR	NR	See note 2	
p-Tolyl laurate	704	10024-57-4 	No Europe: ND USA: 0.3	NR	NR	See note 2	
p-Tolyl phenyl-acetate	705	101-94-0 	No Europe: 0.7 USA: 0.1	NR	NR	See note 2	
2,5-Xylenol	706	95-87-4 	No Europe: 1 USA: 0.03	NR	NR	See note 1	
2,6-Xylenol	707	576-26-1 	No Europe: 2 USA: 1	NR	NR	See note 1	
3,4-Xylenol	708	95-65-8 	No Europe: 7 USA: 1	NR	NR	See note 1	

Table 3 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
2,3,6-Trimethylphenol	737	2416-94-6 	No Europe: 0.3 USA: 0.3	NR	NR	See note 1	No safety concern
Thymol (5-methyl-2-(1-methylethyl)phenol)	709	89-83-8 	No Europe: 59 USA: 160	NR	NR	See note 1	
Carvacrol (2-methyl-5-(1-methylethyl)-phenol)	710	499-75-2 	No Europe: 16 USA: 0.3	NR	NR	See note 1	
p-Vinylphenol (4-ethenylphenol)	711	2628-17-3 	No Europe: 0.1 USA: 6	NR	NR	See note 1	
Resorcinol	712	108-46-3 	No Europe: 1 USA: 0.3	NR	NR	See note 1	
Guaiacol (*o*-methoxyphenol)	713	90-05-1 	No Europe: 51 USA: 16	NR	NR	See note 1	

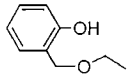
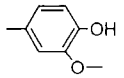
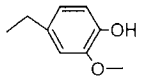
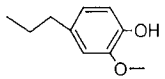
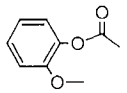
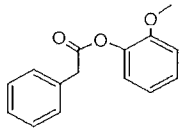
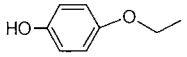
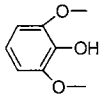
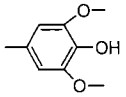
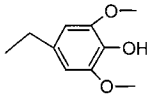
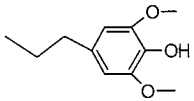
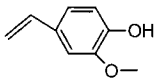
o-(Ethoxymethyl)-phenol	714	20920-83-6 	No Europe: 2 USA: 0.01	NR	NR	See note 1	No safety concern
2-Methoxy-4-methylphenol (2-methoxy-*p*-cresol)	715	93-51-6 	No Europe: 37 USA: 3	NR	NR	See note 1	
4-Ethylguaiacol	716	2785-89-9 	No Europe: 8 USA: 0.4	NR	NR	See note 1	
2-Methoxy-4-propylphenol	717	2785-87-7 	No Europe: 210 USA: 0.1	NR	NR	See note 1	
Guaiacyl acetate (2-methoxyphenyl acetate)	718	613-70-7 	No Europe: 0.01 USA: 0.1	NR	NR	See note 2	
Guaiacyl phenylacetate (2-methoxyphenyl phenylacetate)	719	4112-89-4 	No Europe: 0.4 USA: 2	NR	NR	See note 2	
Hydroquinone monoethyl ether (*p*-ethoxyphenol)	720	622-62-8 	No Europe: ND USA: 0.4	NR	NR	See note 1	

Table 3 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
2,6-Dimethoxyphenol	721	91-10-1 	No Europe: 6 USA: 12	NR	NR	See note 1	No safety concern
4-Methyl-2,6-dimethoxyphenol (2,6-dimethoxy-*p*-cresol)	722	6638-05-7 	No Europe: ND USA: 0.04	NR	NR	See note 1	
4-Ethyl-2,6-dimethoxyphenol	723	14059-92-8 	No Europe: ND USA: 1	NR	NR	See note 1	
4-Propyl-2,6-dimethoxyphenol	724	6766-82-1 	No Europe: ND USA: 0.1	NR	NR	See note 1	
2-Methoxy-4-vinylphenol	725	7786-61-0 	No Europe: 3 USA: 1	NR	NR	See note 1	

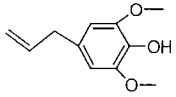
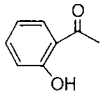
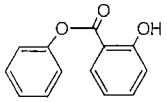
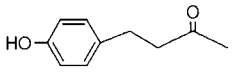
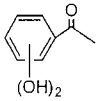
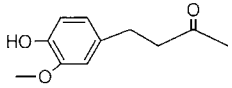
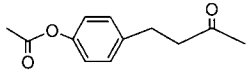
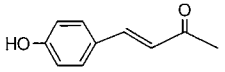
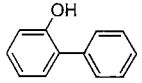
4-Allyl-2,6-dimethoxyphenol	726	6627-88-9 	No Europe: 0.01 USA: 6	NR	NR	See note 1	No safety concern
2-Hydroxyacetophenone (2′-hydroxyaceto-phenone)	727	118-93-4 	No Europe: 0.1 USA: 0.01	NR	NR	See note 1	
Phenyl salicylate (phenyl 2-hydroxy-benzoate)	736	118-55-8 	No Europe: 9 USA: 8	NR	NR	See note 1	
4-(*p*-Hydroxyphenyl)-2-butanone (4-(4-hydroxyphenyl)-butan-2-one)	728	5471-51-2 	Yes Europe: 2800 USA: 3800	No	Yes[a]	See note 1	
Dihydroxy-acetophenone (dihydroxy-1-phenylethanone)	729	28631-86-9 	No Europe: 0.01 USA: 0.1	NR	NR	See note 1	
Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone)	730	122-48-5 	No Europe: 40 USA: 83	NR	NR	See note 1	

Table 3 (*continued*)

Flavouring agent[b]	No.	CAS no. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
4-(*p*-Acetoxyphenyl)-2-butanone (4-(*p*-hydroxyphenyl)-2-butanone acetate)	731	3572-06-3 	No Europe: ND USA: 0.1	NR	NR	See note 1	No safety concern
Vanillylidene acetone (methyl 3-methoxy-4-hydroxystyryl ketone)	732	1080-12-2 	No Europe: ND USA: 0.1	NR	NR	See note 1	
Structural class III							
2-Phenylphenol[e] (biphenyl-2-ol)	735	90-43-7 	No Europe: 0.01 USA: 0.01	NR	NR	See note 1	No safety concern

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

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

[b] The names of the flavouring agents are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where flavouring agents were evaluated under their trivial name, the systematic name is given in parentheses.

[c] The thresholds for human intake for structural classes I and III are 1800 μg per day and 90 μg per day, respectively. All intake values are expressed in μg per day.

[d] The NOEL of 280 mg/kg of body weight per day in a 13-week study in rats is >1000 times the estimated intake of 4-(*p*-hydroxyphenyl)-2-butanone when used as a flavouring agent.

[e] An ADI of 0–0.4 mg/kg of body weight was established for this substance at the 1999 Joint FAO/WHO Meeting on Pesticide Residues (*6*).

Notes to Table 3

1. Detoxification of phenol primarily involves conjugation of the hydroxyl group with sulfate and glucuronic acid.
2. Phenyl esters undergo rapid hydrolysis, followed by conjugation with sulfate and glucuronic acid.

aryl-substituted phenols and their corresponding esters (Nos 691–711, 733, 735 and 737); alkoxy phenols and their corresponding esters (Nos 713–726); and phenol derivatives with alkyl side-chains containing a ketone function (Nos 727–732).

2-Phenylphenol (No. 735) was evaluated by the Committee at its eighth meeting, when an ADI of 0–0.2 mg/kg of body weight was established (Annex 1, reference *8*). The 1999 Joint FAO/WHO Meeting on Pesticide Residues evaluated 2-phenylphenol and established an ADI of 0–0.4 mg/kg of body weight (*6*).

Thirty-two of the 48 flavouring agents in this group are natural components of foods. They have been detected in berries, coffee and meat.

4.3.1 *Estimated daily per capita intake*

The total annual volume of production of the 48 flavouring agents considered here is approximately 25 tonnes in Europe and 32 tonnes in the USA. About 78% of the total annual volume in Europe and 90% of that in the USA is accounted for by 4-(*p*-hydroxyphenyl)-2-butanone ("raspberry ketone"; No. 728). The estimated daily per capita intake of this flavouring agent is 2.8 mg in Europe and 3.8 mg in the USA.

Other flavouring agents for which the estimated daily per capita intakes are in the range 37–300 µg include *o*-cresol (No. 691) (290 µg in Europe), *o*- and *p*-tolyl acetate (Nos 698 and 699) (40 µg and 70 µg, respectively, in the USA), 2-methoxy-4-methylphenol (No. 715) (37 µg in Europe), thymol (No. 709) (59 µg in Europe and 160 µg in the USA), guaiacol (No. 713) (51 µg in Europe), 2-methoxy-4-propylphenol (No. 717) (210 µg in Europe) and zingerone (No. 730) (40 µg in Europe and 83 µg in the USA). The estimated daily per capita intakes of all other flavouring agents in the group are in the range 0.01–16 µg. Annual production volumes were not reported in Europe for seven flavouring agents used in the USA.

4.3.2 *Absorption, metabolism and elimination*

Phenol (No. 690) and its derivatives are rapidly absorbed from the gastrointestinal tract and share common pathways of metabolism. Phenol (No. 690), phenyl acetate (No. 734), phenyl salicylate (No. 736), resorcinol (No. 712), and alkyl-, alkenyl- and aryl-substituted phenols and their corresponding esters (Nos 691–711, 733, 735 and 737) are conjugated with sulfate and glucuronic acid after hydrolysis of the esters and excreted primarily in the urine. Other

metabolic pathways, observed mainly at high doses, include hydroxylation of the phenol ring and oxidation of side-chains. Phenols containing alkoxy groups (Nos 713–717 and 720–726) and those that contain a ketone function on an alkyl side-chain (Nos 727–732) are also metabolized mainly by conjugation with sulfate and glucuronic acid.

Alternative metabolic pathways include dealkylation of alkoxyphenols, reduction of ketones on alkyl side-chains, oxidation of side-chains and ring hydroxylation. At very high doses (>500 mg/kg of body weight), small amounts of *p*-cresol (No. 693), *p*-ethylphenol (No. 694), 2-methoxy-4-methylphenol (No. 715), 2-methoxy-4-propylphenol (No. 717), 2-methoxy-4-vinylphenol (No. 725) and 4-allyl-2,6-dimethoxyphenol (No. 726) are oxidized to reactive quinone methide intermediates. However, given the presence of a detoxification pathway (glutathione conjugation) for such quinone methides, the toxicity of the intermediates potentially formed after high doses of these derivatives would not raise concern under the conditions of their use as flavouring agents.

4.3.3 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

Step 1

In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the above-mentioned substances, the Committee assigned 47 of the 48 flavouring agents with low toxic potential to structural class I. The remaining flavouring agent, 2-phenylphenol (No. 735), was assigned to structural class III.

Step 2

At current levels of intake, the flavouring agents can be predicted to be metabolized to innocuous products, and the pathways involved would not be expected to be saturated. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Step A3

The estimated daily per capita intakes of 46 of the 47 flavouring agents in structural class I and of the single agent in structural class III are below the thresholds of concern for these classes (1800 µg and 90 µg, respectively). The Committee concluded that these substances would not be expected to be of safety concern when used at their currently estimated levels of intake.

The estimated daily per capita intake of 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in Europe and the USA is above the threshold of

concern for structural class I (1800μg). Accordingly, the evaluation of this substance proceeded to step A4.

Step A4

4-(*p*-Hydroxyphenyl)-2-butanone (No. 728) does not occur endogenously in humans. The evaluation of this substance therefore proceeded to step A5.

Step A5

The NOEL of 280mg/kg of body weight per day for 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in a 13-week study in rats provides a margin of safety of >1000 in relation to the estimated intake in Europe (46μg/kg of body weight per day) and in the USA (63μg/kg of body weight per day).

Table 3 summarizes the evaluation of phenol and 47 phenol derivatives used as flavouring agents.

4.3.4 *Consideration of combined intakes*

In the unlikely event that all foods containing *p*-cresol (No. 693) and all six esters of *p*-cresol (Nos 699, 701–705) in structural class I were consumed concurrently on a daily basis, the combined intake of *p*-cresol equivalents (see Table 3) would not exceed the threshold for human intake for this class (1800μg per day). In the unlikely event that all foods containing all 47 substances in structural class I were consumed daily, the estimated combined intake would exceed the threshold for human intake for this class, but would not saturate the available high-capacity conjugation pathways involved in the metabolism of these substances. Moreover, approximately 78% of the annual volume consumed in Europe and approximately 90% of that consumed in the USA are accounted for by 4-(*p*-hydroxyphenyl)-2-butanone (No. 728), for which a NOEL providing an adequate margin of safety was available.

4.3.5 *Conclusions*

The Committee concluded that the safety of phenol and the 47 derivatives of phenol in this group would not raise concern at the currently estimated levels of intake. In using the Procedure, the Committee noted that all of the available data on the toxicity of phenol and its derivatives were consistent with the results of the safety evaluation.

The Committee took note of the ADI of 0–0.4mg/kg of body weight for 2-phenylphenol (No. 735) established by the 1999 Joint FAO/WHO Meeting on Pesticide Residues (*6*).

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

4.4 Pulegone and related flavouring agents

The Committee evaluated a group of flavouring agents that included pulegone (No. 753), isopulegone (No. 754), isopulegol (No. 755), isopulegyl acetate (No. 756), an unsaturated analogue of pulegone, *p*-menth-1,4(8)-dien-3-one (No. 757), and a principal metabolite of pulegone, menthofuran (No. 758) (Table 4) using the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). With the exception of the metabolite, menthofuran (No. 758), all of these substances contain a 3-menthyl (2-isopropyl-5-methyl-3-cyclohexyl) carbon skeleton. Isopulegone (No. 754), isopulegol (No. 755) and isopulegyl acetate (No. 756) contain an isopropenyl side-chain, while pulegone (No. 753) and *p*-menth-1,4(8)-dien-3-one (No. 757) contain an isopropylidene side-chain. None of these flavouring agents has been evaluated previously by the Committee.

Of the six flavouring agents reviewed, only isopulegyl acetate (No. 756) has not been found to occur naturally in food. All the other agents occur naturally in several plant and fruit juices and in oils such as peppermint and pennyroyal oil. Isopulegol (No. 755) has been found in citrus peel oils, cognac, rum and lemon balm. Isopulegone (No. 754) has a minty, herbaceous aroma and has been detected in ginger and buchu oil. *p*-Menth-1,4(8)-dien-3-one (No. 757) has been detected in orange and grapefruit juices.

4.4.1 *Estimated daily per capita intake*

The total annual volume of production of the six flavouring agents considered here is 250kg in Europe and 180kg in the USA. The flavouring agents produced in the highest volumes are menthofuran (No. 758) (170kg in Europe and 95kg in the USA) and isopulegol (No. 755) (50kg in Europe and 45kg in the USA). These two flavouring agents account for >90% of the total annual volume of production of this group of substances in Europe and the USA. On the basis of the reported total annual volume of production, the total estimated daily per capita intake of menthofuran from use of this substance as a flavouring agent is approximately 13μg in Europe and 25μg in the USA. Similarly, the total estimated daily per capita intake of isopulegol from use of this substance as a flavouring agent is 6μg in Europe and 7μg in the USA. The total estimated daily per capita intakes of the other substances in this group are 2μg or less in both Europe and the USA.

4.4.2 *Absorption, metabolism and elimination*

Isopulegone (No. 754) is predicted to be rapidly absorbed and metabolized in vivo (mainly by reduction) to yield isopulegol (No. 755) and undergoes reversible isomerization to pulegone (No. 753). Isopulegol, which may also be formed by hydrolysis of acetate isopulegyl (No. 756), is predicted to be conjugated with glucuronic acid and excreted in the urine. Pulegone (No. 753) and *p*-menth-1,4(8)-dien-3-one (No. 757) are either reduced to the corresponding alcohols and excreted or undergo allylic oxidation to yield the corresponding 9-hydroxy derivatives. In the case of pulegone (No. 753), the 9-hydroxy derivative cyclizes to yield menthofuran (No. 758) as the principal metabolite.

The metabolic pathway involving conversion of pulegone (No. 753) to menthofuran (No. 758) is considered to be a significant source of toxic products. Menthofuran is a proximate hepatotoxic agent that is transformed via an epoxide intermediate to the toxic agent, 8-pulegone aldehyde. This γ-ketoenal has been shown to bind covalently to mouse, rat and human liver microsomes, and this binding parallels the hepatotoxicity of menthofuran in these species. *p*-Menth-1,4(8)-dien-3-one (No. 757) is presumed to participate in the same pathway, since its effects are similar to those of pulegone, but there was no direct evidence of the mechanism of toxicity of this compound. Other major routes of metabolism can be considered to be detoxification pathways.

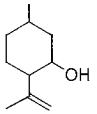
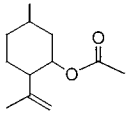
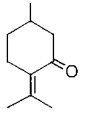
At low levels of intake, pulegone (No. 753), menthofuran (No. 758) and their metabolites, menthofuran epoxide and the γ-ketoenal, are conjugated with glutathione and glucuronic acid. Metabolism of these substances may lead to formation of a reactive metabolite, glutathione depletion and, eventually, hepatotoxicity at intakes of 100 mg/kg of body weight or more. Because these compounds may undergo metabolic bioactivation, the evaluation of their safety was based on a comparison with available data on toxicity, although the estimated daily per capita intakes would not be sufficient to result in appreciable depletion of hepatic glutathione.

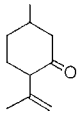
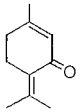
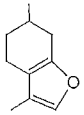
4.4.3 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

Step 1

In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the above-mentioned substances, the Committee assigned isopulegol (No. 755) and isopulegyl acetate

Table 4
Summary of the results of safety evaluations of pulegone and five related flavouring agents[a]

Flavouring agent[b]	No.	CAS no. and structure	*Step B3*[c] Does intake exceed the threshold for human intake?	*Step B4* Adequate NOEL for substance or related substance?	Conclusion based on current intake
Structural class I					
Isopulegol (*p*-menth-8-en-3-ol)	755	89-79-2 	No Europe: 6 USA: 7	Yes The NOEL of 0.44 mg/kg of body weight per day for related substance No. 753 is >1000 times the estimated intake of isopulegol when used as a flavouring agent	No safety concern
Isopulegyl acetate	756	57576-09-7 	No Europe: 0.4 USA: 1	Yes The NOEL of 0.44 mg/kg of body weight per day for related substance No. 753 is >10000 times the estimated intake of isopulegyl acetate when used as a flavouring agent	
Structural class II					
Pulegone (*p*-menth-4(8)-en-3-one)	753	89-82-7 	No Europe: 2 USA: 2	Yes The NOEL of 0.44 mg/kg of body weight per day in a 90-day study in rats is >10000 times the estimated intake of pulegone when used as a flavouring agent	No safety concern

Isopulegone (*trans-p*-menth-8-en-3-one)	754	29606-79-9 	No Europe: 1 USA: 0.01	Yes The NOEL of 0.44 mg/kg of body weight per day for related substance No. 753 is >10000 times the estimated intake of isopulegone when used as a flavouring agent	No safety concern
p-Menth-1,4(8)-dien-3-one (3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one)	757	491-09-8 	No Europe: 2 USA: 0.01	Yes The NOEL of 0.44 mg/kg of body weight per day for related substance No. 753 is >10000 times the estimated intake of *p*-menth-1,4(8)-dien-3-one when used as a flavouring agent	
Menthofuran (4,5,6,7-tetrahydro-3,6-dimethylbenzofuran)	758	494-90-6 	No Europe: 13 USA: 25	Yes The NOEL of 0.44 mg/kg of body weight per day for related substance No. 753 is >1000 times the estimated intake of menthofuran when used as a flavouring agent	

CAS: Chemical Abstracts Service.

[a] *Step 2*: None of the substances in this group is expected to be metabolized to innocuous products.

[b] The names of the flavouring agents are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where flavouring agents were evaluated under their trivial name, the systematic name is given in parentheses.

[c] The thresholds for human intake for structural classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

(No. 756) to structural class I. The remaining substances, which included three monocycloalkenones (Nos 753, 754 and 757) and a heterocyclic compound that is a common component of food (No. 758), were assigned to structural class II.

Step 2

At current levels of intake, the six flavouring agents in this group would not be expected to saturate the available metabolic pathways, but they are not completely metabolized to innocuous products. The evaluation of these substances therefore proceeded via the right-hand side of the decision-tree.

Step B3

The estimated daily per capita intakes of both substances in structural class I and all four substances in structural class II are below the thresholds for human intake for these classes (1800 μg and 540 μg, respectively). Accordingly, the evaluation of these substances proceeded to step B4.

Step B4

The lack of toxicity of pulegone (No. 753) at low levels of intake was demonstrated in a 90-day study in rats fed peppermint oil that contained 1.1% pulegone. The NOEL of 0.44 mg/kg of body weight per day for pulegone derived from this study is >10 000 times the estimated intake of 0.033 μg/kg of body weight from use of pulegone as a flavouring agent. Since pulegone is metabolized to menthofuran (No. 758) and *p*-menth-1,4(8)-dien-3-one (No. 757), data on pulegone can be used to evaluate the safety of these flavouring agents, although menthofuran was about three times more hepatotoxic than pulegone after single doses. Isopulegone (No. 754) was less hepatotoxic than pulegone after single doses. The NOEL of 0.44 mg/kg of body weight per day for pulegone in the 90-day study in rats is >1000 times the estimated intake of 0.4 μg/kg of body weight per day from use of menthofuran as a flavouring agent. Isopulegone (No. 754), isopulegol (No. 755) and isopulegyl acetate (No. 756) are expected to be partly metabolized to menthofuran. Even if these compounds are assumed to be metabolized to menthofuran to the same extent as pulegone, however, the NOEL for pulegone is >10 000 times the estimated intake from use of isopulegone and isopulegyl acetate and is >1000 times the estimated intake from use of isopulegol as a flavouring agent.

Table 4 summarizes the evaluation of pulegone and five related substances.

4.4.4 *Consideration of combined intakes*

In the unlikely event that all foods containing isopulegol (No. 755) and isopulegyl acetate (No. 756) were consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for class I (1800 μg per day). In the unlikely event that all foods containing isopulegone (No. 754), pulegone (No. 753), *p*-menth-1,4(8)-dien-3-one (No. 757) and menthofuran (No. 758) were consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for class II (540 μg per day). Furthermore, there is an adequate safety margin between the estimated combined intake of all six substances (approximately 40 μg/person per day) and the NOEL for pulegone.

4.4.5 *Conclusions*

The Committee concluded that the substances in this group would not be of safety concern at the current estimated levels of intake. In using the Procedure, the Committee noted that all of the available data on toxicity, including the results of short-term toxicity studies and genotoxicity studies on pulegone (No. 753) and related compounds, were consistent with the results of the safety evaluation.

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

5. Contaminants

5.1 Cadmium

Cadmium was evaluated by the Committee at its sixteenth, thirty-third and forty-first meetings (Annex 1, references *30*, *83* and *107*). At its sixteenth meeting, the Committee allocated a provisional tolerable weekly intake (PTWI) of 400–500 μg of cadmium per person. At its thirty-third meeting, the Committee retained this PTWI but expressed it in terms of intake per kg of body weight (7 μg/kg of body weight). In 1992, the International Programme on Chemical Safety (IPCS) produced a monograph on cadmium, which provided a detailed review of the available information on the health effects of cadmium and a description of the models on which the PTWI was based (*7*). At its forty-first meeting, the Committee maintained the PTWI of 7 μg/kg of body weight, pending future research.

In acknowledging the need for research in areas recommended in the monograph, the Committee at its forty-first meeting highlighted the following topics:

- Further studies on the dose–response relationship between the daily or accumulative intake of cadmium and renal dysfunction (β_2-microglobulinuria) in the general population.
- Re-examination of the existing epidemiological information correlating cadmium intake and β_2-microglobulinuria among inhabitants of a cadmium-polluted region.
- Examination of data on cadmium intake and its health effects among the general population in various countries, including data on cadmium concentrations in foods.
- Evaluation of the critical concentration of cadmium in the renal cortex in two groups exposed to high and low concentrations of cadmium.
- Studies on the chemical identity and bioavailability of cadmium compounds in food.
- Re-examination of mathematical models for estimating the biological half-life of cadmium.
- Studies on the involvement of renal glomeruli in chronic cadmium intoxication.

At that meeting, the Committee also reaffirmed that "there is only a relatively small safety margin between exposure in the normal diet and exposure that produces deleterious effects".

At its present meeting, the Committee based its evaluation on the IPCS monograph (*7*) and on updated information on the intake, bioavailability and health effects of cadmium.

5.1.1 *Bioavailability*

The bioavailability of cadmium can be affected markedly by nutritional factors. Low iron status, as determined from serum ferritin levels, which is prevalent among women, increases the uptake of cadmium from the gastrointestinal tract. Furthermore, the bioavailability of cadmium from some grains or seeds and foods in which cadmium is bound to phytates, metallothionein and other proteins may be reduced. The Committee examined the information on bioavailability that had become available since its forty-first meeting and concluded that it did not significantly differ from that considered previously. While information suggesting altered bioavailability due to dietary and nutritional factors exists, the bulk of the evidence indicates that the overall point estimate of 5% for bioavailability that was used in previous models of the relationship between cadmium intake and critical effects is appropriate. For specific populations, such as people with iron deficiency, the bioavailability of cadmium may range from 5% to 10%. The Committee also considered that studies in which experimental animals were given parenteral

injections of cadmium are not appropriate for determining the bioavailability of ingested cadmium.

5.1.2 *Health effects*

The presence of cadmium in food can result in long-term intake of low concentrations. Cadmium has an extremely long biological half-life in mammals (estimated to be at least 17 years in humans) and has a strong affinity for the liver and kidney. The toxic effects of this metal on the kidneys (e.g. tubule dysfunction) are the most sensitive for evaluation of its health effects. While cadmium can affect organs other than the kidneys, it generally does so at doses higher than those associated with renal effects. Acute effects can occur after ingestion of very high concentrations, and these may be fatal, owing to widespread systemic distribution. Such outcomes do not arise from typical dietary concentrations.

Non-renal effects

Neurodevelopmental and neurobehavioural effects have been demonstrated in experimental animals given repeated doses of cadmium by gavage. However, studies in which cadmium was administered by injection showed that it cannot easily enter the brain, its entry being blocked by the blood–brain barrier. Cadmium can replace zinc in a number of metallo-enzymes, proteins and ion channels, generally increases the brain concentrations of noradrenaline and dopamine, and impairs enzymes required for the production of neurotransmitters. The peripheral nervous system may also be susceptible to chronic exposure to cadmium, but the investigations of these effects are limited. Studies of occupationally exposed adults have shown increased prevalences of peripheral neuropathies and neurobehavioural deficits in specific domains (e.g. attention, psychomotor speed, memory). No population-based studies of neurotoxicity after environmental exposure of adults or children to cadmium were available in which validated biomarkers of exposure were used.

Experimental studies have shown that cadmium can induce metallothionein synthesis in the placenta and that cadmium is retained in the placenta at low concentrations. Large doses of cadmium compounds administered parenterally to several rodent species at a late stage of gestation induced severe placental damage and fetal deaths, whereas similar doses given parenterally in the early stages of gestation had teratogenic effects. Teratogenic effects have not been reported after oral intake of cadmium by animals or humans.

Cadmium is carcinogenic in experimental animals when given by injection or inhalation, and occupational exposure of humans by

inhalation has been shown to result in pulmonary cancer. There was no evidence that cadmium is carcinogenic to humans exposed by the oral route.

Large, population-based studies have provided little evidence that changes in blood pressure or in the prevalence of ischaemic heart disease are related to blood or urinary cadmium concentrations.

Excretion of cadmium in the urine is weakly but significantly associated with elevated urinary calcium concentrations and increased serum alkaline phosphatase activity. Studies of occupationally exposed persons suggest that high urinary and blood cadmium concentrations are associated with low bone mineral density. In environmentally exposed postmenopausal women, higher urinary cadmium excretion was associated with hypercalciuria, osteoporosis and reduced bone density, and an increased risk of fracture. The relationship between the effect of cadmium on calcium metabolism and osteoporosis should be investigated further in so far as the effects on bone might be a more sensitive indicator of the toxicity of cadmium than the renal effects.

Renal effects

The kidney is the critical target organ in mammals, including humans, exposed for long periods to small amounts of cadmium. Cadmium produces renal tubule dysfunction characterized by hypercalciuria and increased excretion of several proteins of low relative molecular mass. In particular, β_2-microglobulin has served as a biomarker of toxicity and may complement urinary cadmium as a biomarker of exposure.

The renal tubule dysfunction seen in non-human mammalian species exposed to low dietary concentrations of cadmium is analogous to that produced in humans; in animals, this may progress to interstitial nephropathy and glomerulopathy with longer exposure. The critical renal concentration of cadmium that is associated with cadmium-induced nephropathy in animals is 50–200 μg/g of renal cortex, which is consistent with the results of studies in humans. Recent studies in various species indicate that when the concentration of cadmium in the renal cortex exceeds 250 μg/g, continued exposure results in further increases in the concentration in the liver but not in the renal cortex. It has been suggested that this effect reflects increased loss of cadmium in the urine due to tubule dysfunction. Experimental studies have shown that impaired glomerular filtration (i.e. increased serum creatinine and blood urea nitrogen concentrations) is a less sensitive indicator of cadmium-induced nephropathy than are indicators of tubule dysfunction or injury.

Many reports from Japan and a large population-based study of environmental exposure to cadmium in Belgium confirm that the major risk factors for cadmium-induced renal effects in non-occupationally exposed humans include increasing age, high alcohol consumption, cigarette smoking and residence in a cadmium-contaminated region. In these studies, renal effects were investigated by using urinary biomarkers of renal dysfunction. Several markers of proximal tubule function, including *N*-acetyl-β-glucosaminidase activity and the concentrations of retinol-binding protein, β_2-microglobulin, amino acids and calcium, are related to the urinary cadmium concentrations of environmentally exposed individuals. No threshold was found for the relationship between *N*-acetyl-β-glucosaminidase activity and urinary cadmium concentration. The prevalence of abnormal values for these markers was 10% when urinary cadmium concentrations exceeded 2–4 μg/24 h or the estimated concentration in the renal cortex was >50 μg/g. Estimates of the relationship between urinary cadmium concentration and abnormal values for these markers were considerably lower in studies of environmental exposure than in studies of occupational exposure; however, past exposure would be underestimated in areas where cadmium concentrations in the environment had been reduced prior to the time the study was conducted. Certain individuals, such as patients with diabetes or pre-existing renal disorders, appear to be at increased risk for cadmium-related renal dysfunction.

Follow-up studies of workers with cadmium-related renal dysfunction suggest that many of the changes are irreversible, with continued declines in glomerular function for decades after cessation of heavy exposure. The low-relative-molecular-mass proteinuria associated with long-term exposure to cadmium is assumed to be irreversible. This assumption is based on the observation in studies of occupational and environmental exposure that for people who excrete >1000 μg of β_2-microglobulin in the urine per 24 h, renal tubule function does not improve or worsens within 5 years of a reduction in cadmium exposure. The prognosis appears to be more favourable for individuals with lower body burdens. In the study in Belgium, some of the subtle effects on renal tubule function seen at the time the participants entered the study were no longer apparent at follow-up or, at least, were not associated with a decline in glomerular function in the interim.

A comprehensive meta-analysis of the relevant epidemiological studies and a risk assessment suggested that the risk for renal dysfunction and progression to clinical disease could be lowered if exposure to cadmium were reduced such that the concentrations of

cadmium in the kidney and urine were maintained below 50 μg/g of renal cortex and 2.5 μg/g of creatinine, respectively.

5.1.3 *Dietary intake*

The diet is the major route of human exposure to cadmium. Contamination of foods with cadmium results from its presence in soil and water. Estimation of the intake of cadmium, like that of most contaminants, is complicated by the skewed distributions of residues, since cadmium does not reach foods through controlled or predictable agricultural or manufacturing processes. In addition, crops differ widely with respect to their absorption of cadmium from soil, depending on the type and salinity of the soil and the bioavailability of cadmium (see section 5.1.1). The cadmium concentrations in food samples vary widely, but the highest mean concentrations are found in molluscs, kidney, liver, cereals, cocoa and leafy vegetables. Estimates of mean cadmium intake from national food surveys and total diet studies generally range from 0.1 to 0.5 μg/kg of body weight per day. The estimates derived from the WHO GEMS/Food regional diets, based on food balance sheets, ranged from 0.35 to 0.63 μg/kg of body weight per day.

5.1.4 *Estimates of the relationship between dietary intake and renal tubule dysfunction*

Analysis of new data from population-based studies indicates that the early renal effects of cadmium are prevalent at lower intakes than those indicated by the model used by the Committee to confirm the PTWI at its forty-first meeting. That model was based on the assumption that about 10% of a population with a concentration of cadmium in the renal cortex of about 200 μg/g would experience renal tubule dysfunction.

In the recent meta-analysis of data from several studies of workers and general populations exposed to cadmium, the prevalence of cadmium-induced tubule proteinuria that would be expected to occur in individuals with specific levels of urinary cadmium was estimated. This analysis suggests that the risk of tubule dysfunction begins to increase when the urinary excretion of cadmium exceeds 2.5 μg/g of creatinine. The Committee considered this value to represent no excess prevalence of renal tubule dysfunction.

The Committee used data in the literature to investigate the empirical relationship between the concentration of cadmium in the diet and urinary concentrations. This relationship is a function of increasing cadmium concentration in the renal cortex and increasing urinary excretion with age. Furthermore, diets are made up of a mixture of

foods with different cadmium contents, and its bioavailability from some of the foods that contain high concentrations, such as shellfish and some grains, is low. The estimates were derived from data from Japan, Sweden and the USA. The mean dietary intake of cadmium by female non-smokers in many areas of Japan was 26 μg/day (range, 19–51 μg/day), and the mean urinary excretion of cadmium was 4.4 μg/g of creatinine (range, 3.6–7.0 μg/g of creatinine). These data indicate a ratio of dietary cadmium to urinary cadmium excretion of 6 (range, 3–14). The data from Sweden indicate that female non-smokers have a urinary cadmium excretion of 0.15 μg/g of creatinine and a median dietary intake of 10 μg/day (range, 5.7–26 μg/day). The estimated ratio of dietary cadmium to urinary cadmium excretion ranged from 40 to 170. The mean dietary intake of cadmium in the Total Diet Study in the USA was 5.5 μg/day, and the mean value for urinary excretion of cadmium obtained independently in the National Health and Nutrition Examination Survey in the USA was 0.5 μg/g of creatinine, resulting in a ratio of dietary cadmium to urinary cadmium excretion of 11.

The relationship between urinary cadmium excretion and dietary intake of cadmium can be predicted from a theoretical model. Possible dietary intakes can be predicted from the amount of cadmium excreted in the urine if it is assumed that there are no significant changes in the dietary intake of cadmium over time. Once a set of assumptions has been chosen, a table can be constructed, relating dietary intake to urinary cadmium excretion (see Table 5). The three sets of assumptions described in Table 5 are based on data on the toxicokinetics of cadmium. This table can be used to predict a range of dietary intakes for different urinary cadmium concentrations, which depend on the assumed values for bioavailability and for the percentage of absorbed cadmium that is excreted in urine. Table 5 can also be used to predict the prevalence rates of renal tubule dysfunction associated with different dietary intakes. For population groups in which it is reasonable to assume that 10% of dietary cadmium is bioavailable and that 100% of the absorbed cadmium is excreted in urine, the model predicts that dietary intakes of cadmium of more than 0.5 μg/kg of body weight per day would result in an increased prevalence of renal tubule dysfunction.

5.1.5 *Conclusion*

The estimates of excess prevalence shown in Table 5 were derived from studies of large, heterogeneous populations. As the confidence intervals for the point estimates are unknown and may be wide, the estimates may represent overestimates of the risks associated with

Table 5
Predicted intake of cadmium from the diet and excess prevalence of renal tubule dysfunction, based on three different sets of assumptions[a]

Assumptions	Urinary excretion of cadmium (µg/g of creatinine)[b]	Predicted intake of cadmium[c]		Predicted excess prevalence of renal tubule dysfunction (%)
		µg/day[d]	µg/kg of body weight per day[d,e]	
Bioavailability of cadmium in the diet = 10%; excretion of absorbed cadmium in urine = 100%[f]	2.5	30	0.5	0
	4.2	50	0.8	4
	8.2	100	1.7	20
Bioavailability of cadmium in the diet = 10%; excretion of absorbed cadmium in urine = 50%[g]	2.5	60	1.0	0
	4.2	100	1.7	4
	8.2	200	3.3	20
Bioavailability of cadmium in the diet = 5%; excretion of absorbed cadmium in urine = 50%[h]	2.5	120	2.0	0
	4.2	200	3.3	4
	8.2	400	6.7	20

[a] In each scenario, it is assumed that there are no significant changes in cadmium dietary intake over time and that 1.2 g of creatinine are excreted per day.
[b] Values derived primarily from studies of occupational exposure to cadmium.
[c] Predicted dietary intake = $\frac{\text{Urinary excretion of cadmium (µg of cadmium/g of creatinine)} \times 1.2}{\text{Fraction bioavailable} \times \text{Absorbed fraction excreted in urine}}$
[d] Cadmium intake corresponding to the excretion in urine in each scenario.
[e] The body weight is assumed to be 60 kg. The PTWI corresponds to a daily intake of 1 µg/kg of body weight.
[f] Ratio of dietary intake to urinary excretion = 12.
[g] Ratio of dietary intake to urinary excretion = 24.
[h] Ratio of dietary intake to urinary excretion = 48.

dietary intake of cadmium, especially at lower levels. The Committee concluded that the incidences of renal tubule dysfunction in populations with various dietary intakes of cadmium can serve as a reasonable basis for risk assessment if the assumptions made when applying the predictive model are scientifically based and clearly described.

Even though new information indicates that a proportion of the general population may be at increased risk of cadmium-induced tubule dysfunction when exposed at the current PTWI of 7 µg/kg of body weight, the Committee maintained this value because the risk estimates that can be made at present are imprecise. The range of predicted dietary intakes that may be associated with an excess prevalence of renal tubule dysfunction (see Table 5) can be used to

indicate the risk at various levels of intake for potentially sensitive groups within a population.

The Committee recommended that seven areas be investigated in order to increase confidence in the estimates of predicted excess prevalence of renal tubule dysfunction:

1. The toxicokinetics of cadmium should be investigated in controlled experimental studies in humans of the relationship between dietary intake and urinary excretion of cadmium in the general population and in groups at high risk, such as people with iron deficiency, renal disease or diabetes mellitus.
2. Dietary surveys should be conducted in which individual records of the food consumption of specific population subgroups are kept.
3. Studies should be conducted on the bioavailability of cadmium from specific foods and on the factors that affect its bioavailability, such as age, health status and dietary nutrients.
4. The relationship between biomarkers of renal tubule dysfunction and biomarkers of cadmium exposure should be elucidated.
5. The relationship between renal tubule dysfunction (as determined by specific biomarkers), clinical disease and mortality should be studied.
6. The influence of cadmium on calcium metabolism and osteoporosis should be examined.
7. Studies should be conducted to determine the effect of exposure to cadmium (integrated over a lifetime) on the subsequent development of osteoporosis.

5.2 Tin

Tin was previously evaluated by the Committee at its fourteenth, fifteenth, twenty-second, twenty-sixth and thirty-third meetings (Annex 1, references *22*, *26*, *47*, *59* and *83*). At its thirty-third meeting, the Committee converted the previously established provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg of body weight to a PTWI of 14 mg/kg of body weight. At its present meeting, the Committee retained the PTWI at its current value.

At its Thirty-first Session, the Codex Committee on Food Additives and Contaminants requested the Expert Committee to review information on the toxicity of tin in order to establish an acute reference dose (*4*). At its present meeting, the Expert Committee considered studies of the acute toxic effects seen after consumption of foodstuffs containing high concentrations of inorganic compounds of tin. It did not consider studies of organic tin compounds, since it had concluded at its twenty-second meeting (Annex 1, reference *47*) that

these compounds, which differ considerably with respect to toxicity, should be considered individually.

The major dietary source of tin is the tinplate of unlacquered or partially lacquered cans used for the preservation of foods. The migration of tin from tinplate into foods is greater:

— for highly acidic foods such as pineapples and tomatoes;
— with increased time and temperature of food storage;
— for foods, such as fruit juice, stored in opened cans.

The tin content of canned foods is variable, and some foods may have concentrations high enough to cause an acute toxic reaction. The mean dietary intakes of tin by individuals reported from seven countries ranged from <1 mg/day to about 14 mg/day and were considerably lower than the PTWI established previously by the Committee. Population groups with higher intakes of canned foods may have higher intakes of tin.

Inorganic tin may be present as Sn (II) or Sn (IV); it may occur in cationic form (stannous and stannic compounds) or as inorganic anions (stannites or stannates). Studies in rats provided evidence that the chemical form of inorganic tin is important in determining its toxicity. Inorganic tin compounds generally have little systemic toxicity in animals because of limited absorption from the gastrointestinal tract, low accumulation in tissues and rapid excretion, primarily in the faeces. Insoluble tin compounds, such as stannous sulfide, had minimal toxic effects in rats when administered for 28 days in the diet at concentrations similar to those at which soluble tin salts are clearly toxic. In short-term studies in rats in which several tin salts were used, histological examination revealed changes to the gastrointestinal tract, kidneys, liver and adrenal cortex. Alterations in haematological parameters indicative of anaemia have also been recorded. The acute toxicity of tin results from irritation of the mucosa of the gastrointestinal tract. Vomiting and diarrhoea were reported in cats given soluble salts of tin, but there was no clear dose–response relationship, and the vehicle in which the tin was administered may have affected its toxicity.

Episodes of human poisoning resulting from consumption of tin-contaminated foods and drinks have resulted in abdominal distension and pain, vomiting, diarrhoea and headache. These symptoms commonly appear within 0.5–3 h, and recovery occurs within 48 h. The doses of tin ingested in such episodes of poisoning were not estimated. In one study with five volunteers, all experienced symptoms when they ingested juice containing tin at a concentration of 1400 mg/kg (corresponding to a dose of 4.4–6.7 mg/kg of body weight).

Administration of the same dose 1 month later to these individuals resulted in symptoms in only one person.

The Committee concluded that insufficient data were available to establish an acute reference dose for tin. It noted that the gastric irritation that may occur after ingestion of a foodstuff containing tin may depend on the concentration and chemical form of the tin. It reiterated its opinion, expressed at its thirty-third meeting (Annex 1, reference *83*), that the limited human data available indicate that concentrations of 150mg/kg in canned beverages and 250mg/kg in other canned foods may produce acute manifestations of gastric irritation in certain individuals. In addition, the Committee reiterated its advice, given at its twenty-sixth and thirty-third meetings (Annex 1, references *59* and *83*), that consumers should not store food in open tin-coated cans. It welcomed the information that estimates of the intake of tin by the populations of several countries do not exceed the PTWI of 14mg/kg of body weight.

6. Intake assessments of specific food additives

6.1 Calcium from calcium salts of food additives

The Committee was asked by the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*4*) to estimate the contribution to the diet of calcium from food additives in relation to that from other sources (e.g. naturally occurring calcium, food fortificants, discretionary use of supplements, and pharmaceutical (antacid) preparations). No data on this source of calcium intake were available to the Committee for consideration at its present meeting.

The Committee concluded that it was not feasible to estimate the contribution of calcium salts of food additives to total calcium intake. Although these salts are widely used in the food supply, intake from this source alone could not be estimated because the actual food categories and levels of use are generally unknown. As an alternative, total calcium intake from food was evaluated, as reported in national surveys of the total diet and of nutrition, assuming that the calcium content of foods analysed in total diet surveys and reported in national tables of food composition includes calcium derived from food additives. This assumption has the limitation that the increased availability of calcium-fortified foods may not be reflected in national tables of food composition.

The best data for estimating the contribution of calcium salts of food additives to total calcium intake were considered to be those collected in surveys of the total diet, in which the actual content of the nutrient

of interest is measured at the time of the survey. However, no data from surveys conducted since 1994 were found. In addition, nutrients are rarely considered in such surveys.

Bearing in mind the limitations of the methods used to estimate calcium intake, the Committee noted that the total calcium intake from food of consumers with intakes at the 95th percentile reported from various sources did not exceed 2.7 g/person per day for any age group. The Committee also noted that although the available data on the adverse effects of calcium relate to intake from nutrient supplements and antacids and not from foods, many surveys cover only calcium intake from the diet. The potential contribution of calcium from food additives to total calcium intake will remain difficult to assess until data on food consumption are linked in national surveys to data on the intake of calcium from other sources at an individual level.

In order to determine the contribution of calcium salts of food additives to the total intake of calcium, the Committee would require data on the levels of use and the food groups in which those food additives are permitted.

7. Revision of certain specifications

7.1 Food additives

A total of 45 food additives (other than flavouring agents) were examined for specifications only at the present meeting (Annex 2). In general, all of the specifications were revised in line with the Committee's current policy on metal contaminants (see section 2.3.2).

7.1.1 *Food additives for which previous specifications were designated as "tentative"*

At its fifty-third meeting (Annex 1, reference *143*), the Committee noted that many of the specifications for food additives published in the *Compendium of food additive specifications* and its addenda (Annex 1, references *96*, *103*, *109*, *118*, *124*, *133*, *139* and *145*) were designated as "tentative", indicating that some data were missing or incomplete at the time the specifications were prepared.

As no responses to the call for data for several of these substances were received for consideration at the current meeting, their specifications were withdrawn. These substances are: acetone peroxides, aluminium sodium sulfate, ammonium persulfate, benzoin gum, calcium iodate, calcium peroxide, carbohydrase from *Aspergillus awamori*, var., carbohydrase from *Aspergillus oryzae*, var., chlorine dioxide, diethyl pyrocarbonate, isoamyl gallate, lipase from

Aspergillus oryzae, var., potassium persulfate and rennet from *Endothia parasitica.*

Information was received on aluminium potassium sulfate, aluminium sulfate (anhydrous), ammonium salts of phosphatidic acid, diatomaceous earth and rennet from *Mucor* species. The specifications of these substances were revised, and the "tentative" designations deleted. The title of the specifications for rennet from *Mucor* species was changed to "rennet from *Rhizomucor* species" to reflect the new classification of the source organisms.

7.1.2 *Food additives considered for revision of specifications*

The existing specifications for curcumin, microcrystalline wax, shellac (bleached) and sorbitan monolaurate were revised, with minor changes.

The existing "tentative" specifications for *d*-α-tocopherol (concentrate), diethyl ether, pentasodium triphosphate and sodium sulfate were revised, and the "tentative" qualification was deleted.

The Committee received a request from the Thirty-first Session of the Codex Committee on Food Additives and Contaminants (*4*) for an alternative method of assay for microcrystalline cellulose, but no supporting information was received. Although the Expert Committee was unable to make the requested change, it revised the existing specifications, to include other minor changes.

The Committee also received a request from the Thirty-first Session of the Codex Committee on Food Additives and Contaminants (*4*) to delete the following sentence from the definition in the specifications for talc: "Talc derived from deposits that are known to contain associated asbestos is not food grade". The Committee decided to retain the sentence, but made other minor changes.

At its seventeenth meeting, the Committee requested information on the assay and method of assay of guaiac resin and designated the specifications as "tentative" (Annex 1, reference *32*). At its present meeting, the Committee concluded that no specific assay value was appropriate for guaiac resin, as with other gums, because of its complexity. The specifications were revised without inclusion of an assay value or a method of assay, and the "tentative" designation was removed.

The existing "tentative" specifications for smoke flavourings were extensively revised to include a modified definition, and a lower limit and an updated analytical procedure for benzo[*a*]pyrene. The specification for residual diethyl ether was deleted, and that for heavy metals (as lead) was replaced by a specific limit for lead. The "tentative" designation was maintained, pending the receipt of

information on an alternative solvent to benzene for use in the analysis of the carbonyl content. Proposals should be supported by a comparative test of the analytical method with benzene and the proposed alternative solvent.

The existing "tentative" specifications for oxystearin were revised. The "tentative" qualification was maintained, with the stipulation that the specifications would be withdrawn if information on the levels of, and a suitable analytical method for, epoxides was not provided by 1 May 2001. The Committee noted that this substance is no longer in commercial use.

The existing "tentative" specifications for blackcurrant extract were revised. The "tentative" qualification was maintained, pending the receipt of information on a chromatographic identification test and on the adequacy of the sample size for the test for sulfur dioxide.

The existing "tentative" specifications for tagetes extract were revised. The "tentative" qualification was maintained, pending the receipt of information on the composition of the commercial products, a test for the identification of xanthophylls, and a method of assay.

The existing "tentative" specifications for Quillaia extract were extensively revised, and the "tentative" status deleted. The name was changed to "Quillaia extracts", in order to include unrefined and semi-refined extracts obtained by aqueous extraction of the milled inner bark of *Quillaja saponaria* Molina or of the wood, including stems and branches.

2-Nitropropane was previously considered by the Committee at its twenty-third, twenty-fifth, twenty-eighth and thirty-fifth meetings (Annex 1, references *50*, *56*, *66* and *88*). The temporary acceptance of 2-nitropropane for use as a fractionating solvent in the production of fats and oils was not extended at the thirty-fifth meeting because of toxicological concerns. In the absence of further information on the use of this substance at its present meeting, the Committee withdrew the specifications.

At its twenty-seventh meeting, the Committee recommended that use of 1,1,2-trichloroethylene as an extraction solvent be limited because of toxicological concerns, and requested information on the nature, level(s) and methods of analysis for the added stabilizers and breakdown products. As the requested information was not submitted for consideration at the present meeting, the Committee withdrew the specifications.

The existing "tentative" specifications for eight enzyme preparations were revised on the basis of Annex 1 (General specifications for

enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*).

The existing "tentative" specifications for three enzyme preparations (α-amylase, α-amylase and glucoamylase, and protease) from *Aspergillus oryzae*, var., were revised to include the requirement that the microbial strain used as the source organism must be non-toxicogenic and non-pathogenic. The Committee deleted the maximum limits and associated methods of analysis for the known mycotoxins α-cyclopiazonic acid, β-nitropropionic acid and kojic acid, and removed the "tentative" designation from the specifications.

The existing "tentative" specifications for β-glucanase from *Trichoderma harzianum*, cellulase from *Penicillium funiculosum*, hemicellulase from *Aspergillus niger*, var., and pectinase from *Aspergillus niger*, var., were revised and the "tentative" qualifications removed.

The existing "tentative" specifications for amyloglucosidase from *Aspergillus niger*, var., were revised. The "tentative" qualification was maintained, pending the receipt of information on the assay for amyloglucosidase in formulated products with glucose.

7.1.3 *Food additives that are also flavouring agents*

In reviewing the specifications for flavouring agents, the Committee noted that monographs existed for acetic acid (glacial), triethyl citrate, *o*-phenylphenol, formic acid and butan-1-ol. The specifications had been prepared several years previously, however, and might be in need of revision to include uses other than those reviewed at the present meeting. The Committee concluded that these specifications should be reviewed at its next meeting on food additives in order to consider more up-to-date information.

7.2 Flavouring agents

7.2.1 *Procedure for evaluating proposed specifications for flavouring agents*

At its fifty-third meeting, the Committee developed a set of criteria for determining whether specifications for the purity of flavouring agents should be designated as "tentative" or full specifications (Annex 1, reference *143*). It agreed that specifications submitted for consideration should be designated as "tentative" if information had not been provided on:

- chemical formula and relative molecular mass, identity test, and the minimum amount that can be determined (minimum assay value);
- the additional criteria related to purity, including boiling-point (for liquids), melting-point (for solids), refractive index (for liquids) and specific gravity (for liquids).

The Committee also agreed, however, that it would consider assigning full specifications when the absence of one or more of the additional criteria relating to purity could be justified.

7.2.2 *Specifications established up to and including the fifty-third meeting*

At its fifty-third meeting, the Committee used the system described above to review the specifications for flavouring agents developed at its forty-sixth, forty-ninth and fifty-first meetings (Annex 1, references *122*, *131* and *137*) and to assess the specifications submitted for consideration at the fifty-third meeting. It agreed that further data were required on 284 of the 636 flavouring agents that had been evaluated at the forty-sixth, forty-ninth, fifty-first and fifty-third meetings, and these agents were included in the call for data for the present meeting. In addition, data were requested on 59 flavouring agents for which the minimum assay values were less than 95%.

At its present meeting, the Committee noted that data were still needed on 57 flavouring agents for which the minimum assay values were less than 95%. It reaffirmed that these data were needed in order to establish full specifications and agreed that the 57 specifications for which data were still lacking should be reclassified as "tentative", pending receipt of the information.

Information provided in response to the call for data for the present meeting enabled the Committee to assign "full" specifications to 475 of the 636 flavouring agents that had been considered up to and including the fifty-third meeting. Thus, 161 flavouring agents still have "tentative" specifications.

At its fifty-third meeting, the Committee indicated that unless the relevant data were supplied on the specifications designated as "tentative", they would be withdrawn. As a good response had been received from the manufacturers, the Committee relaxed this requirement, although it stressed that the data missing from the 161 flavouring agents that still have "tentative" specifications must be provided in time for consideration at its fifty-seventh meeting, to be held in 2001.

7.2.3 *Proposed specifications considered for the first time at the present meeting*

Draft specifications for 125 flavouring agents were submitted for consideration by the Committee at its present meeting. The Committee noted that specifications for three of the substances — furfural,

Table 6
Summary of specifications for flavouring agents considered up to and including the present meeting

Meeting at which the specifications were considered	Total no.	No. classified as "full"	No. classified as "tentative"
Forty-sixth, forty-ninth, fifty-first and fifty-third[a]	636	475 (75%)	161 (25%)
Present	122	83 (68%)	39 (32%)
Total	758	558 (74%)	200 (26%)

[a] For details, see Annex 1, references *122, 131, 137* and *143.*

furfuryl alcohol and allyl cinnamate — had been considered at earlier meetings. The information provided on these three substances was used to revise the existing specifications, and this enabled the previous "tentative" designation of the specifications for allyl cinnamate and furfuryl alcohol to be deleted.

The Committee considered the draft specifications for the remaining 122 flavouring agents and classified 83 as "full" and 39 as "tentative". The Committee requested that the data necessary to enable the tentative specifications to be designated as full specifications be provided by 2001.

Table 6 summarizes the status of the specifications for all the flavouring agents considered at the forty-sixth, forty-ninth, fifty-first, fifty-third and present meetings (Annex 1, references *122*, *131*, *137* and *143*).

The Committee noted that the information provided about the nature of the flavouring agents often did not specify which isomers were present. Unless more specific information is provided on the isomers present in individual flavouring agents, the Committee will proceed on the assumption that all the possible isomers may be present.

7.2.4 *Comments made at the Thirty-second Session of the Codex Committee on Food Additives and Contaminants*

At its Thirty-second Session, the Codex Committee on Food Additives and Contaminants asked the Expert Committee to consider three questions relating to specifications for flavouring agents (*2*):

- whether information on identification tests should include a relevant spectrum of a reference compound;
- whether it was necessary to use several different spectroscopic methods of identification, as one is usually sufficient; and

- whether boiling-points should be given in specifications for information only and not as a requirement.

The Codex Committee on Food Additives and Contaminants also asked the Expert Committee to consider using a mathematical formula for converting values for specific gravity obtained at 20 °C to values corresponding to 25 °C.

In response, the Expert Committee agreed that identification tests must be accompanied by a relevant spectrum of a reference compound. It noted that the spectra that were now being provided were of much better quality than those available at earlier meetings but that it was still important that unsatisfactory spectra be updated and that those still missing be provided.

The Committee also agreed that it was unnecessary to require more than one spectroscopic method of identification for a flavouring agent. When more than one method is given, the sponsor will be asked to indicate which it considers to be the most appropriate.

The Committee considered that, in principle, boiling-points for liquids should be submitted for inclusion in specifications as an additional check on the nature and/or purity of the material. However, the Committee agreed that these boiling-points may not always be relevant, for example, for flavouring agents that are mixtures of different substances.

The Committee reaffirmed that the values for specific gravity included in future proposed specifications for flavouring agents should be given at 25 °C. It considered it unlikely that a single mathematical formula could be developed for converting values for specific gravity measured at 20 °C to equivalent values at 25 °C. In the absence of supporting information, it did not consider the issue further.

7.3 Limits for metals in food additives

7.3.1 *Emulsifiers*

At its fifty-third meeting, the Committee reaffirmed its policy of replacing the outdated limit test for heavy metals (as lead) with limits for the individual metals of concern in all existing specifications (Annex 1, reference *143*). On the basis of data received at the present meeting on the organic emulsifiers listed in Table 7, the Committee deleted the limits for arsenic and heavy metals (as lead) and replaced them with a limit for lead of 2 mg/kg, in accordance with the principles stated in Section C of FAO Food and Nutrition Paper No. 52, Add. 7 (Annex 1, reference *145*).

Table 7
Limits for arsenic and lead in 43 organic and inorganic phosphate emulsifiers

Emulsifier	INS No.	Limit for arsenic (mg/kg)	Limit for lead (mg/kg)
Acetic and fatty acid esters of glycerol	472a	—	2
Ammonium polyphosphate	452(v)	3	4
Ammonium salts of phosphatidic acid	442	—	2
Calcium polyphosphate	452(iv)	3	4
Calcium stearoyl-2-lactylate	482(i)	—	2
Cholic acid	1000	—	2
Citric and fatty acid esters of glycerol	472c	—	2
Desoxycholic acid	—	—	2
Diacetyltartaric and fatty acid esters of glycerol	472e	—	2
Dicalcium pyrophosphate (diphosphate)	450(vi)	3	4
Dioctyl sodium sulfosuccinate	480	—	2
Disodium pyrophosphate (diphosphate)	450(i)	3	4
Glycerol ester of wood rosin	445	—	2
Lactic and fatty acid esters of glycerol	472b	—	2
Lecithin, partially hydrolysed	322	—	2
Mono- and diglycerides	471	—	2
Polyglycerol esters of fatty acids	475	—	2
Polyglycerol esters of interesterified ricinoleic acid	476	—	2
Polyoxyethylene (20) sorbitan monolaurate	432	—	2
Polyoxyethylene (20) sorbitan monooleate	433	—	2
Polyoxyethylene (20) sorbitan monopalmitate	434	—	2
Polyoxyethylene (20) sorbitan monostearate	435	—	2
Polyoxyethylene (20) sorbitan tristearate	436	—	2
Polyoxyethylene (8) stearate	430	—	2
Polyoxyethylene (40) stearate	431	—	2
Propylene glycol esters of fatty acids	477	—	2
Salts of fatty acids	470	—	2
Sodium aluminium phosphate, basic	541(ii)	3	4
Sodium metaphosphate, insoluble	—	3	4
Sodium polyphosphates, glassy	452(i)	3	4
Sodium stearoyl-2-lactylate	481(i)	—	2
Sorbitan monooleate	494	—	2
Sorbitan monopalmitate	495	—	2
Sorbitan monostearate	491	—	2
Sorbitan tristearate	492	—	2
Stearyl citrate	484	—	2
Stearyl monoglyceridyl citrate	—	—	2
Succinylated monoglycerides	472g	—	2
Sucroglycerides	474	—	2

Table 7 (*continued*)

Emulsifier	INS No.	Limit for arsenic (mg/kg)	Limit for lead (mg/kg)
Tetrapotassium pyrophosphate	450(v)	3	4
Tetrasodium pyrophosphate	450(iii)	3	4
Thermally oxidized soya bean oil	—	—	2
Thermally oxidized soya bean oil interacted with mono- and diglycerides of fatty acids	479	—	2

INS: International Numbering System.

Information on limits for lead in inorganic phosphates used as emulsifiers was also received, but no supporting analytical data were provided. The data were sufficient to replace the previous limits for arsenic and lead by limits of 3 mg/kg and 4 mg/kg, respectively, and to delete the limits for heavy metals (as lead) for the phosphate emulsifiers listed in Table 7.

When the limit test for heavy metals (as lead) is replaced by limits for individual metals, the absence of a particular metal from specifications indicates that the Committee has concluded that the level of contamination is so low as to be of no toxicological concern. Comments on the proposed limits are invited.

7.3.2 *Food additives other than emulsifiers*

The Committee agreed on priorities for reviewing the limits for metals in specifications for groups of food additives other than emulsifiers (Table 8).

8. Future work

1. The Committee noted that Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) was originally drawn up in 1984. It also recalled that Annex 1 and Appendix B of that annex (General considerations and specifications for enzyme preparations from genetically modified microorganisms) were reviewed and revised at its fifty-first and fifty-third meetings and included with the specifications (Annex 1, references *139* and *145*). The Committee reiterated its view, expressed at its fifty-third meeting, that Annex 1 required updating in the light of technological developments and to ensure consistency and coherence with the appendices, including Appendix B.

Table 8
Priorities for the review of specifications for metals in food additives other than emulsifiers

Group[a]	Category	No. of specifications
1	Flavour enhancers	19
	Sweeteners	14
	Thickeners	30
	Anti-caking agents	15
	Subtotal	78
2	Acidity regulators	52
	Antioxidants	35
	Subtotal	87
3	Preservatives	43
	Other additives	45
	Subtotal	88
4	Glazing agents	11
	Flour treatment agents	16
	Colours	54
	Subtotal	81
	Total	334

[a] The Group numbers refer to the order of priority for reviewing the specifications.

2. The Committee reiterated its recommendation, made at its forty-sixth meeting, concerning the need for periodic updating of the *Compendium of food additive specifications* (Annex 1, reference *96*) and the *Guide to specifications* (Annex 1, reference *100*) to take into account developments in analytical procedures and new methods for the production of food additives. The Committee recommended that such revision should be undertaken without delay.

9. Recommendation

In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role of the recommendations of the Committee in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.

Acknowledgement

The Committee thanked Mrs E. Heseltine, Communication in Science, Lajarthe, Saint-Léon-sur-Vézère, France, for her assistance in the preparation of the report.

References

1. *Joint FAO/WHO Conference on Food Additives.* Rome, Food and Agriculture Organization of the United Nations, 1956 (FAO Nutrition Meetings Report Series, No. 11); Geneva, World Health Organization, 1956 (WHO Technical Report Series, No. 107).
2. Codex Alimentarius Commission. *Report of the Thirty-second Session of the Codex Committee on Food Additives and Contaminants, Beijing, People's Republic of China, 20–24 March 2000.* Rome, Food and Agriculture Organization of the United Nations, 2000 (unpublished FAO document ALINORM 01/12; available from FAO or WHO).
3. Lucas CD et al., eds. *1995 Poundage and technical effects update summary.* Washington, DC, Flavor and Extract Manufacturers' Association of the United States, 1999.
4. Codex Alimentarius Commission. *Report of the Thirty-first Session of the Codex Committee on Food Additives and Contaminants, The Hague, The Netherlands, 22–26 March 1999.* Rome, Food and Agriculture Organization of the United Nations, 1999 (unpublished FAO document ALINORM 99/12A; available from FAO or WHO).
5. Food safety. In: *Fifty-third World Health Assembly, Geneva, 15–20 May 2000. Part 1. Resolutions and decisions, annex.* Geneva, World Health Organization, 2000 (unpublished document WHA53/2000/REC/1): 18–21.
6. *Pesticide residues in food — 1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Rome, Italy, 20–29 September 1999.* Rome, Food and Agriculture Organization of the United Nations, 1999 (FAO Plant Production and Protection Paper, No. 153).
7. *Cadmium.* Geneva, World Health Organization, 1992 (WHO Environmental Health Criteria, No. 134).

Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. I. *Antimicrobial preservatives and antioxidants*. Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. II. *Food colours*. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25 (out of print).
11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-*

treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C, 1967; WHO/Food Add/67.29 (out of print).
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967 (out of print).
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968 (out of print).
15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33 (out of print).
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31 (out of print).
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969 (out of print).
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Report Series, No. 45A, 1969; WHO/Food Add/69.34 (out of print).
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970 (out of print).
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36 (out of print).
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37 (out of print).
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971 (out of print).
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39 (out of print).
24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40 (out of print).

25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41 (out of print).
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972 (out of print).
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972 (out of print).
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum (out of print).
31. *Evaluation of mercury, lead, cadmium, and the food additives amaranth, diethylpyrocarbonate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974 (out of print).
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum (out of print).
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents, and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975 (out of print).

39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others.* FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978 (out of print).
48. *Summary of toxicological data of certain food additives and contaminants.* WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives.* FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.

59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda (out of print).
63. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications — General notices, general methods, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours.* FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives.* FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 20).
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 21).
75. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 37, 1986.
76. *Principles for the safety assessment of food additives and contaminants in food.* Geneva, World Health Organization, 1987 (WHO Environmental Health Criteria, No. 70) (out of print).[1]
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. *Toxicological evaluation of certain food additives.* Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 22).

[1] The full text is available electronically on the Internet at http://www.who.int/pcs.

79. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. *Toxicological evaluation of certain veterinary drug residues in food.* Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 23).
82. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41, 1988 (out of print).
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. *Toxicological evaluation of certain food additives and contaminants.* Cambridge, Cambridge University Press, 1989 (WHO Food Additives Series, No. 24).
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 25, 1990.
87. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 26, 1990.
90. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 49, 1990.
91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990.* Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1992.
98. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 29, 1992.
99. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/4, 1991.

100. *Guide to specifications — General notices, general analytical techniques, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.
102. *Toxicological evaluation of certain food additives and naturally occurring toxicants.* WHO Food Additives Series, No. 30, 1993.
103. *Compendium of food additive specifications, addendum 1.* FAO Food and Nutrition Paper, No. 52, Add. 1, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 32, 1993.
109. *Compendium of food additive specifications, addendum 2.* FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/7, 1995.
116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 35, 1996.
118. *Compendium of food additive specifications, addendum 3.* FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/8, 1996.

122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 37, 1996.
124. *Compendium of food additive specifications, addendum 4.* FAO Food and Nutrition Paper, No. 52, Add. 4, 1996 (out of print).
125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/10, 1998.
131. *Evaluation of certain food additives and contaminants* (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 40, 1998.
133. *Compendium of food additive specifications, addendum 5.* FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. *Evaluation of certain veterinary drug residues in food* (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999, and corrigendum.
135. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 41, 1998.
136. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/11, 1999.
137. *Evaluation of certain food additives* (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 42, 1999.
139. *Compendium of food additive specifications, addendum 6.* FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. *Evaluation of certain veterinary drug residues in food* (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 43, 2000.
142. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/12, 2000.
143. *Evaluation of certain food additives and contaminants* (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.

144. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 44, 2000.
145. *Compendium of food additives specifications, addendum 7*. FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. *Evaluation of certain veterinary drug residues in food* (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 45, 2000.
148. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/13, 2000.

Annex 2

Acceptable Daily Intakes, other toxicological information and information on specifications

Specific food additives

Substance	Specifications[a]	Acceptable Daily Intake (ADI) in mg/kg of body weight and other toxicological recommendations
Flavouring agents		
Furfural (No. 450)	R	0–0.5 (group ADI)[b]
Paprika oleoresin	S	Use of paprika oleoresin as a spice is acceptable[c]
Food colours		
Caramel colour II	R[d]	0–160
Cochineal extract	R	May be allergenic[e]
Carmines	R	May be allergenic[e]
Sweetening agents		
Aspartame–acesulfame salt	N	Aspartame and acesulfame moieties are covered by the ADIs established previously for aspartame (0–40) and acesulfame potassium (0–15)
D-Tagatose	N	No ADI allocated[f]
Miscellaneous substances		
Benzoyl peroxide	R	Additional uses could not be evaluated[g]
Nitrous oxide	R	Use as a packaging gas could not be evaluated[h]
Stearyl tartrate	R	Additional uses could not be evaluated[g]
Trehalose	N	ADI "not specified"[i]

[a] N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required.

[b] Group ADI for furfural, furfuryl alcohol, furfuryl acetate, furfuryl propionate, furfuryl pentanoate, furfuryl octanoate, furfuryl 3-methylbutanoate, methyl 2-furoate, propyl 2-furoate, amyl 2-furoate, hexyl 2-furoate and octyl 2-furoate.

[c] Paprika oleoresin was not evaluated at the present meeting. The Committee's recommendation was based on the report of its fourteenth meeting (WHO Technical Report Series, No. 462, 1971).

[d] Included in the existing specifications for caramel colours.

[e] The Committee concluded that cochineal extract, carmines, and, possibly, carminic acid in foods and beverages may initiate or provoke allergic reactions in some individuals.

[f] An ADI could not be allocated to D-tagatose because of concern about its potential to induce liver glycogen deposition and hypertrophy and to increase serum uric acid concentrations; see Annex 3.

[g] No conclusions could be drawn about the acceptability of the uses proposed in the draft General Standard for Food Additives of the Codex Committee on Food Additives and Contaminants because information on toxicity and intake was not available.

[h] No information on intake of nitrous oxide resulting from the proposed use was available.

[i] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary

intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in the individual evaluation, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

Flavouring agents

The substances listed here were evaluated by the Procedure for the Safety Evaluation of Flavouring Agents. For further details, see sections 2.2.1 and 4 of the main report.

Flavouring agent[a]	No.	Specifications[b]	Conclusion based on current intake
Cinnamyl alcohol and related flavouring agents			
Structural class I			
3-Phenyl-1-propanol (3-phenylpropanol)	636	N	
3-Phenylpropyl formate (benzenepropanol formate)	637	N	
3-Phenylpropyl acetate (benzenepropanol acetate)	638	N	
3-Phenylpropyl propionate (benzenepropanol propionate)	639	N	
3-Phenylpropyl isobutyrate	640	N	
3-Phenylpropyl isovalerate	641	N	
3-Phenylpropyl hexanoate	642	N, T	
Methyl 3-phenylpropionate	643	N	
Ethyl 3-phenylpropionate	644	N	
3-Phenylpropionaldehyde (benzenepropanal)	645	N, T	
3-Phenylpropionic acid (benzenepropionic acid)	646	N	No safety concern
Cinnamyl alcohol	647	N	
Cinnamyl formate	649	N	
Cinnamyl acetate	650	N	
Cinnamyl propionate	651	N	
Cinnamyl butyrate	652	N, T	
Cinnamyl isobutyrate	653	N	
Cinnamyl isovalerate	654	N	
Cinnamyl benzoate	760	N, T	
Cinnamyl phenylacetate	655	N	
Cinnamaldehyde (3-phenyl-2-propenal)	656	N, T	
Cinnamic acid (3-phenyl-2-propenoic acid)	657	N	
Methyl cinnamate	658	N	
Ethyl cinnamate	659	N	

Flavouring agent[a]	No.	Specifications[b]	Conclusion based on current intake
Propyl cinnamate	660	N, T	
Isopropyl cinnamate	661	N	
Allyl cinnamate (2-propenyl 3-phenyl-2-propenoate)	19	R	
Butyl cinnamate	663	N, T	
Isobutyl cinnamate	664	N	
Isoamyl cinnamate (isopentyl cinnamate)	665	N	
Heptyl cinnamate	666	N, T	
Cyclohexyl cinnamate	667	N	
Linalyl cinnamate	668	N, T	
Terpinyl cinnamate ((*Z*)-1-methyl-1-(4-methyl-3-cyclohexen-1-yl)ethyl cinnamate)	669	N, T	
Benzyl cinnamate	670	N	
Phenethyl cinnamate	671	N, T	
3-Phenylpropyl cinnamate	672	N, T	
Cinnamyl cinnamate	673	N, T	
5-Phenylpentanol (benzenepentan-1-ol)	675	N	
α-Amylcinnamyl formate (2-(phenylmethylene)heptyl formate)	676	N, T	No safety concern
α-Amylcinnamyl acetate (2-(phenylmethylene)heptyl acetate)	677	N, T	
α-Amylcinnamyl isovalerate (2-(phenylmethylene)heptyl isovalerate)	678	N, T	
3-Phenyl-4-pentenal (3-phenylpent-4-enal)	679	N	
3-(*p*-Isopropylphenyl)propionaldehyde (3-(*p*-cumenyl)propionaldehyde)	680	N	
α-Amylcinnamaldehyde dimethyl acetal ((2-(dimethoxymethyl)-1-heptenyl) benzene)	681	N, T	
p-Methylcinnamaldehyde (3-(4-methylphenyl)-2-propenal)	682	N	
α-Methylcinnamaldehyde	683	N	
p-Methoxycinnamaldehyde	687	N	
o-Methoxycinnamaldehyde	688	N	
p-Methoxy-α-methylcinnamaldehyde (4′-methoxy-2-methylcinnamaldehyde)	689	N	
Structural class II			
Cinnamaldehyde ethylene glycol acetal (2-styryl-1,3-dioxolane)	648	N, T	
α-Amylcinnamyl alcohol (2-pentyl-3-phenylprop-2-en-1-ol)	674	N	No safety concern
α-Butylcinnamaldehyde	684	N	
α-Amylcinnamaldehyde (α-pentyl cinnamaldehyde)	685	N	
α-Hexylcinnamaldehyde	686	N	

Flavouring agent[a]	No.	Specifications[b]	Conclusion based on current intake
Furfuryl alcohol and related flavouring agents[c]			
Structural class II			
Furfuryl alcohol[d] (2-hydroxymethylfuran)	451	R	No safety concern
Furfuryl acetate[d] (2-furanmethanol acetate)	739	N	
Furfuryl propionate[d] (2-furanmethanol propionate)	740	N, T	
Furfuryl pentanoate[d] (2-furanylmethyl pentanoate)	741	N, T	
Furfuryl 3-methylbutanoate[d] (2-furanylmethyl-3-methylbutanoate)	743	N, T	
Furfural[d] (2-furfuraldehyde)	450	R	
5-Methylfurfural (5-methyl-2-furfuraldehyde)	745	N	
Methyl 2-furoate[d]	746	N	
Propyl 2-furoate[d]	747	N	
Structural class III			
Furfuryl octanoate[d] (2-furanylmethyl octanoate)	742	N, T	No safety concern
Amyl 2-furoate[d] (pentyl 2-furoate)	748	N, T	
Hexyl 2-furoate[d]	749	N, T	
Octyl 2-furoate[d]	750	N, T	
2-Benzofurancarboxaldehyde	751	N	
2-Phenyl-3-carbethoxyfuran (ethyl 2-phenyl-3-furoate)	752	N, T	
Phenol and phenol derivatives			
Structural class I			
Phenol	690	N	No safety concern
o-Cresol	691	N	
m-Cresol	692	N	
p-Cresol	693	N	
p-Ethylphenol (4-ethylphenol)	694	N	
o-Propylphenol (2-propylphenol)	695	N	
p-Propylphenol (4-propylphenol)	696	N	
2-Isopropylphenol	697	N	
4-(1,1-Dimethylethyl)phenol (*p-tert*-butylphenol)	733	N	
Phenyl acetate	734	N	
o-Tolyl acetate	698	N, T	
p-Tolyl acetate	699	N	
o-Tolyl isobutyrate	700	N	
p-Tolyl isobutyrate	701	N	
p-Tolyl 3-methylbutyrate (*p*-tolyl isovalerate)	702	N	
p-Tolyl octanoate	703	N	

Flavouring agent[a]	No.	Specifications[b]	Conclusion based on current intake
p-Tolyl laurate	704	N, T	
p-Tolyl phenylacetate	705	N	
2,5-Xylenol	706	N	
2,6-Xylenol	707	N	
3,4-Xylenol	708	N	
2,3,6-Trimethylphenol	737	N, T	
Thymol (5-methyl-2-(1-methylethyl)-phenol)	709	N	
Carvacrol (2-methyl-5-(1-methylethyl)-phenol)	710	N	
p-Vinylphenol (4-ethenylphenol)	711	N, T	
Resorcinol	712	N	
Guaiacol (*o*-methoxyphenol)	713	N	
o-(Ethoxymethyl)phenol	714	N	
2-Methoxy-4-methylphenol (2-methoxy-*p*-cresol)	715	N	
4-Ethylguaiacol	716	N	
2-Methoxy-4-propylphenol	717	N	
Guaiacyl acetate (2-methoxyphenyl acetate)	718	N	
Guaiacyl phenylacetate (2-methoxyphenyl phenylacetate)	719	N, T	
Hydroquinone monoethyl ether (*p*-ethoxy-phenol)	720	N, T	No safety concern
2,6-Dimethoxyphenol	721	N	
4-Methyl-2,6-dimethoxyphenol (2,6-dimethoxy-*p*-cresol)	722	N	
4-Ethyl-2,6-dimethoxyphenol	723	N, T	
4-Propyl-2,6-dimethoxyphenol	724	N, T	
2-Methoxy-4-vinylphenol	725	N	
4-Allyl-2,6-dimethoxyphenol	726	N, T	
2-Hydroxyacetophenone (2′-hydroxy-acetophenone)	727	N	
Phenyl salicylate (phenyl 2-hydroxy-benzoate)	736	N	
4-(*p*-Hydroxyphenyl)-2-butanone (4-(4-hydroxyphenyl)butan-2-one)	728	N	
Dihydroxyacetophenone (dihydroxy-1-phenylethanone)	729	N, T	
Zingerone (4-(4-hydroxy-3-methoxy-phenyl)-2-butanone)	730	N	
4-(*p*-Acetoxyphenyl)-2-butanone (4-(*p*-hydroxyphenyl)-2-butanone acetate)	731	N	
Vanillylidene acetone (methyl 3-methoxy-4-hydroxystyryl ketone)	732	N, T	

Flavouring agent[a]	No.	Specifications[b]	Conclusion based on current intake
Structural class III			
2-Phenylphenol[e] (biphenyl-2-ol)	735	N, T	No safety concern
Pulegone and related flavouring agents			
Structural class I			
Isopulegol (*p*-menth-8-en-3-ol)	755	N	No safety concern
Isopulegyl acetate	756	N	
Structural class II			
Pulegone (*p*-menth-4(8)-en-3-one)	753	N	No safety concern
Isopulegone (*trans-p*-menth-8-en-3-one)	754	N	
p-Menth-1,4(8)-dien-3-one (3-methyl-6-(1-methylethylidene)cyclohex-2-en-1-one)	757	N	
Menthofuran (4,5,6,7-tetrahydro-3,6-dimethylbenzofuran)	758	N	

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

[b] N, new specifications prepared; R, existing specifications revised; T, the existing new or revised specifications are tentative and further information is required.

[c] Specifications were prepared for furfuryl butyrate, but its safety was not assessed because the Committee had no information on its intake.

[d] A group ADI of 0–0.5 mg/kg of body weight was established by the Committee at its present meeting for furfural, furfuryl alcohol, furfuryl acetate, furfuryl propionate, furfuryl pentanoate, furfuryl octanoate, furfuryl 3-methylbutanoate, methyl 2-furoate, propyl 2-furoate, amyl 2-furoate, hexyl 2-furoate and octyl 2-furoate.

[e] An ADI of 0–0.4 mg/kg of body weight was established for this substance by the 1999 Joint FAO/WHO Meeting on Pesticide Residues (FAO Plant Production and Protection Paper, No. 153, 1999).

Contaminants

Cadmium

The provisional tolerable weekly intake (PTWI) of 7 μg/kg of body weight was maintained. Ranges of predicted dietary intakes that may be associated with an excess prevalence of renal tubule dysfunction were estimated, as summarized in the following table. These values provide an indication of the risk at various levels of intake for potentially sensitive groups within the population. See Annex 3 for recommended studies.

Predicted intake of cadmium from the diet and excess prevalence of renal tubule dysfunction, based on three different sets of assumptions[a]

Assumptions	Urinary excretion of cadmium (μg/g of creatinine)[b]	Predicted intake of cadmium[c]		Predicted excess prevalence of renal tubule dysfunction (%)
		μg/day[d]	μg/kg of body weight per day[d,e]	
Bioavailability of cadmium in the diet = 10%; excretion of absorbed cadmium in urine = 100%[f]	2.5	30	0.5	0
	4.2	50	0.8	4
	8.2	100	1.7	20
Bioavailability of cadmium in the diet = 10%; excretion of absorbed cadmium in urine = 50%[g]	2.5	60	1.0	0
	4.2	100	1.7	4
	8.2	200	3.3	20
Bioavailability of cadmium in the diet = 5%; excretion of absorbed cadmium in urine = 50%[h]	2.5	120	2.0	0
	4.2	200	3.3	4
	8.2	400	6.7	20

[a] In each scenario, it is assumed that there are no significant changes in cadmium dietary intake over time and that 1.2 g of creatinine are excreted per day.

[b] Values derived primarily from studies of occupational exposure to cadmium.

[c] Predicted dietary intake = $\frac{\text{Urinary excretion of cadmium (μg of cadmium/g of creatinine)} \times 1.2}{\text{Fraction bioavailable} \times \text{Absorbed fraction excreted in urine}}$

[d] Cadmium intake corresponding to the excretion in urine in each scenario.

[e] The body weight is assumed to be 60 kg. The PTWI corresponds to a daily intake of 1 μg/kg of body weight.

[f] Ratio of dietary intake to urinary excretion = 12.

[g] Ratio of dietary intake to urinary excretion = 24.

[h] Ratio of dietary intake to urinary excretion = 48.

Tin

The PTWI of 14 mg/kg of body weight was not reconsidered and was maintained. The Committee assessed the acute toxicity of tin, but the data were insufficient for establishing an acute reference dose. The Committee reiterated its opinion, expressed at its thirty-third meeting (WHO Technical Report Series, No. 776, 1989), that the limited human data available indicate that concentrations of 150 mg/kg in canned beverages and 250 mg/kg in other canned foods may produce acute manifestations of gastric irritation in certain individuals. In addition, the Committee reiterated its advice, given at its twenty-sixth and thirty-third meetings (WHO Technical Report Series, No. 683, 1982 and No. 776, 1989), that consumers should not store food in open tin-coated cans.

Intake assessments of specific food additives

Calcium from calcium salts of food additives

Data on the levels of use and the food groups in which calcium salts of food additives are permitted would be required in order to determine their contribution to the total intake of calcium.

Substances considered for specifications only

Substance	No.	Specifications[a]
Food additives for which previous specifications were designated as "tentative"		
Acetone peroxides[b]	—	W
Aluminium potassium sulfate	—	R
Aluminium sodium sulfate[b]	—	W
Aluminium sulfate (anhydrous)	—	R
Ammonium persulfate[b]	—	W
Ammonium salts of phosphatidic acid	—	R
Benzoin gum[b]	—	W
Calcium iodate[b]	—	W
Calcium peroxide[b]	—	W
Carbohydrase from *Aspergillus awamori*, var.[b]	—	W
Carbohydrase from *Aspergillus oryzae*, var.[b]	—	W
Chlorine dioxide[b]	—	W
Diatomaceous earth	—	R
Diethyl pyrocarbonate[b]	—	W
Isoamyl gallate[b]	—	W
Lipase from *Aspergillus oryzae*, var.[b]	—	W
Potassium persulfate[b]	—	W
Rennet from *Endothia parasitica*[b]	—	W
Rennet from *Rhizomucor* species (*Mucor* species)	—	R
Food additives considered for revision of specifications		
α-Amylase from *Aspergillus oryzae*, var.	—	R
α-Amylase and glucoamylase from *Aspergillus oryzae*, var.	—	R
Amyloglucosidase from *Aspergillus niger*, var.[c]	—	R, T
Blackcurrant extract[c]	—	R, T
Cellulase from *Penicillium funiculosum*	—	R
Curcumin	—	R
Diethyl ether	—	R
β-Glucanase from *Trichoderma harzianum*	—	R
Guaiac resin	—	R
Hemicellulase from *Aspergillus niger*, var.	—	R
Microcrystalline cellulose	—	R
Microcrystalline wax	—	R
2-Nitropropane[d]	—	W
Oxystearin[c]	—	R, T
Pectinase from *Aspergillus niger*, var.	—	R
Pentasodium triphosphate	—	R

Substance	No.	Specifications[a]
Protease from *Aspergillus oryzae*, var.	—	R
Quillaia extracts	—	R
Shellac, bleached	—	R
Smoke flavourings[c]	—	R, T
Sodium sulfate	—	R
Sorbitan monolaurate	—	R
Tagetes extract[c]	—	R, T
Talc	—	R
d-α-Tocopherol, concentrate	—	R
1,1,2-Trichloroethylene[e]	—	W
Flavouring agents[f]		
Allyl propionate (2-propenyl propanoate)	1	R
Allyl tiglate (2-propenyl *trans*-2-methyl 2-butenoate)	10	R, T
Allyl phenylacetate (2-propenyl phenylacetate)	17	R
Allyl anthranilate (2-propenyl 2-aminobenzoate)	20	R
Allyl 2-furoate (2-propenyl furan-2-carboxylate)	21	R
Ethyl undecanoate	36	R
Ethyl hexadecanoate	39	R
Ethyl octadecanoate	40	R
Ethanol	41	R
Isoamyl octanoate (3-methylbutyl octanoate)	47	R
Isoamyl nonanoate (3-methylbutyl nonanoate)	48	R
Isoamyl 2-methylbutyrate (3-methylbutyl 2-methylbutanoate)	51	R, T
Geranyl acetate (3,7-dimethyl-2,6-octadien-1-yl acetate)	58	R, T
Rhodinyl propionate (3,7-dimethyl-7-octen-1-yl propionate)	64	R, T
Citronellyl valerate (3,7-dimethyl-6-octen-1-yl pentanoate)	69	R
Geranyl hexanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl hexanoate)	70	R, T
Geranyl isobutyrate (3,7-dimethyl-2,6-octadienyl-2-methylpropanoate)	72	R, T
Rhodinyl isobutyrate (3,7-dimethyl-7-octen-1-yl-2-methylpropanoate)	74	R, T
Geranyl isovalerate (3,7-dimethyl-2,6-octadienyl-3-methylbutanoate)	75	R
Rhodinyl isovalerate (3,7-dimethyl-7-octen-1-yl-3-methylbutanoate)	77	R, T
Formic acid	79	R
Acetaldehyde	80	R
Acetic acid[g]	81	R
Propionaldehyde (propanal)	83	R
Propionic acid (propanoic acid)	84	R
Butyl alcohol (1-butanol)	85	R
Butyraldehyde (butanal)	86	R
Hexyl alcohol (1-hexanol)	91	R
Octanoic acid	99	R
Decanoic acid	105	R
Undecanoic acid	108	R
Lauric acid (dodecanoic acid)	111	R, T
Myristic acid (tetradecanoic acid)	113	R, T

Substance	No.	Specifications[a]
Palmitic acid (hexadecanoic acid)	115	R, T
Stearic acid (octadecanoic acid)	116	R, T
Heptyl formate	121	R
Lauryl acetate (dodecyl acetate)	133	R
2-Ethylbutyl acetate	140	R
cis-3- and *trans*-2-Hexenyl propionate (*cis*-3- and *trans*-2-hexenyl propanoate)	147	R
Heptyl butyrate (heptyl butanoate)	154	R
Octyl butyrate (octyl *n*-butyrate)	155	R
Decyl butyrate	156	R
cis-3-Hexenyl hexanoate (*cis*-3-hexen-1-ol hexanoate)	165	R
Isobutyl hexanoate (2-methylpropyl hexanoate)	166	R
Propyl heptanoate	168	R
Butyl heptanoate	169	R
Octyl heptanoate	171	R
Isobutyl heptanoate (2-methylpropyl heptanoate)	172	R, T
Heptyl octanoate	176	R
Octyl octanoate	177	R
Nonyl octanoate	178	R, T
Isoamyl laurate (3-methylbutyl dodecanoate)	182	R, T
Butyl stearate (butyl octadecanoate)	184	R, T
Dodecyl isobutyrate (dodecyl-2-methylpropanoate)	193	R
2-Methylbutyl 3-methylbutanoate	204	R
Ethyl 2-methylbutyrate (ethyl-2-methylbutanoate)	206	R
n-Butyl 2-methylbutyrate (butyl-2-methylbutanoate)	207	R
Hexyl 2-methylbutanoate	208	R
Octyl 2-methylbutyrate (octyl 2-methylbutanoate)	209	R
Isopropyl 2-methylbutyrate (1-methylethyl-2-methylbutanoate)	210	R
3-Hexenyl 2-methylbutanoate (3-hexenylethyl-2-methylbutanoate)	211	R
Methyl 2-methylpentanoate	213	R
Ethyl 2-methylpentanoate	214	R
Ethyl 3-methylpentanoate	215	R
Methyl 4-methylvalerate	216	R
Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid)	218	R
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	222	R
γ-Heptalactone (5-propyldihydro-2(3*H*)-furanone)	225	R
γ-Octalactone (5-butyldihydro-2(3*H*)-furanone)	226	R
4,4-Dibutyl-γ-butyrolactone (5,5-dibutyldihydro-2(3*H*)-furanone)	227	R
δ-Octalactone (6-propyltetrahydro-2-pyrone)	228	R
γ-Nonalactone (5-pentyldihydro-2(3*H*)-furanone)	229	R
δ-Decalactone (6-pentyltetrahydro-2-pyrone)	232	R
δ-Dodecalactone (6-heptyltetrahydro-2-pyrone)	236	R
ω-Pentadecalactone (oxacyclohexadecan-2-one)	239	R
ω-6-Hexadecenlactone (oxacycloheptadec-7-en-2-one)	240	S, T
ε-Decalactone (7-butyl-2-oxooxacycloheptane)	241	R
ε-Dodecalactone (7-hexyl-2-oxooxacycloheptane)	242	R
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (3-hydroxy-4,5-dimethyl-2(5)-furanone)	243	R
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	244	R, T

Substance	No.	Specifications[a]
5-Hydroxy-2,4-decadienoic acid δ-lactone (6-pentyl-2-pyrone)	245	R
5-Hydroxy-7-decenoic acid δ-lactone (6-pentyltetrahydro-2-pyrone)	247	R
5-Hydroxy-8-undecenoic acid δ-lactone (6-hexyltetrahydro-2-pyrone)	248	R
Isobutyl alcohol (2-methylpropanol)	251	R
Isobutyraldehyde (2-methylpropanal)	252	R
2-Ethylbutyraldehyde (2-ethylbutanal)	256	R
2-Methylpentanal	260	R, T
4-Methyloctanoic acid	271	R
2-Tridecanone (tridecan-2-one)	298	R
2-Pentadecanone (pentadecan-2-one)	299	R
3-Methyl-2-butanol (3-methylbutan-2-ol)	300	R
Isopropyl propionate	306	R, T
Isopropyl hexanoate	308	R, T
Isopropyl isovalerate (isopropyl 3-methylbutanoate)	310	R
Isopropyl myristate (isopropyl tetradecanoate)	311	R
cis-3-Hexenal ((*Z*)-hex-3-enal)	316	R
3-Hexenoic acid (hex-3-enoic acid)	317	R
cis-4-Hexenal ((*Z*)-hex-4-enal)	319	R
cis-4-Heptenal ((*Z*)-hept-4-en-1-al)	320	R
cis-6-Nonen-1-ol ((*Z*)-non-6-en-1-ol)	324	R
5- and 6-Decenoic acid (mixture)	327	R
9-Undecenal (undec-9-en-1-al)	329	R, T
Oleic acid ((*Z*)-octadeca-9-enoic acid)	333	R
Methyl 3-hexenoate (methyl hex-3-enoate)	334	R, T
Methyl *cis*-4-octenoate ((*Z*)-methyl oct-4-enoate)	337	R, T
Ethyl *cis*-4-octenoate ((*Z*)-ethyl oct-4-enoate)	338	R, T
Ethyl *cis*-4,7-octadienoate (ethyl (*Z*)-octa-4,7-dienoate)	339	R
Methyl 3-nonenoate (methyl non-3-enoate)	340	R
Ethyl *trans*-4-decenoate (ethyl (*E*)-4-decenoate)	341	R
Methyl 9-undecenoate (methyl undec-9-enoate)	342	R
Butyl 10-undecenoate (butyl undec-10-enoate)	344	R, T
2-Methyl-3-pentenoic acid (2-methyl pent-3-enoate)	347	R, T
Ethyl 2-methyl-3,4-pentadienoate (ethyl 2-methylpenta-3,4-dienoate)	353	R
Methyl 3,7-dimethyl-6-octenoate (methyl 3,7-dimethyloct-6-enoate)	354	R
Linalyl isovalerate (1,5-dimethyl-1-ethenylhex-4-enyl 3-methylbutyrate)	363	R
Linalyl octanoate (1,5-dimethyl-1-ethenylhex-4-enyl octanoate)	365	R
Terpinyl formate (*p*-menth-1-en-8-yl formate)	367	R, T
Terpinyl butyrate (*p*-menth-1-en-8-yl butyrate)	370	R, T
Terpinyl isobutyrate (*p*-menth-1-en-8-yl isobutyrate)	371	R
Terpinyl isovalerate (*p*-menth-1-en-8-yl isovalerate)	372	R, T
p-Menth-3-en-1-ol	373	R
p-Menthan-2-ol	376	R
Dihydrocarvone (*p*-menth-8-en-2-one)	377	R
l-Carvone (*p*-mentha-6,8-dien-2-one)	380.2	R
Carvyl propionate (1-*p*-mentha-6,8-dien-2-yl propionate)	383	R

Substance	No.	Specifications[a]
β-Ionol (4-(2,6,6-trimethyl-1-cyclohexenyl)-3-butene-2-ol)	392	R
Dihydro-β-ionone (4-(2,6,6-trimethyl-1-cyclohexenyl)-3-butan-2-one)	394	R
Methyl-α-ionone (5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-4-penten-3-one)	398	R
Methyl-δ-ionone (4-(2,6,6-trimethyl-3-cyclohexen-1-yl)-3-methyl-3-buten-2-one)	400	R
1,4-Dimethyl-4-acetyl-1-cyclohexene	402	R
2-Acetoxy-3-butanone (1-methyl-2-oxopropyl acetate)	406	R
Butan-3-one-2-yl butanoate (1-methyl-2-oxopropyl butyrate)	407	R
3-Hydroxy-2-pentanone (3-hydroxypentan-2-one)	409	R, T
4-Methyl-2,3-pentanedione (4-methylpentane-2,3-dione)	411	R
2,3-Hexanedione (hexane-2,3-dione)	412	R
5-Methyl-2,3-hexanedione (5-methylhexane-2,3-dione)	414	R
5-Hydroxy-4-octanone (5-hydroxyoctan-4-one)	416	R, T
2,3-Undecadione (undeca-2,3-dione)	417	R, T
Methylcyclopentenolone (3-methylcyclopentane-1,2-dione)	418	R
3,4-Dimethyl-1,2-cyclopentanedione (3,4-dimethylcyclopentane-1,2-dione)	420	R
3,5-Dimethyl-1,2-cyclopentanedione (3,5-dimethylcyclopentane-1,2-dione)	421	R
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (2-hydroxy-3,5,5-trimethylcyclohex-2-en-1-one)	426	R
l-Menthyl lactate	433	R
5-Hydroxy-2-dodecenoic acid δ-lactone (6-heptyl-2*H*-dihydro-2-pyrone)	438	R
4-Carvomenthenol (*p*-menth-1-en-4-ol)	439	R
4-Thujanol (2-methyl-5-(1-methylethyl)bicyclo[3.1.0]-hexan-2-ol)	441	R
dl-Menthone 1,2-glycerol ketal (1,4-dioxaspiro[4,5]decane-2-menthanol)	446	R
1,4-Dithiane	456	R
Allyl sulfide (diallyl sulfide)	458	R
4-(Methylthio)butanol (4-(methylthio)-1-butanol)	462	R
2-Methyl-4-propyl-1,3-oxathiane	464	R
3-(Methylthio)propionaldehyde	466	R
3-(Methylthio)butanal	467	R
Ethyl 2-(methylthio)acetate	475	R
3-(Methylthio)propyl acetate	478	R, T
3-(Methylthio)hexyl acetate	481	R
S-Methyl thioacetate (*S*-methyl acetothioate)	482	R
Methyl thiobutyrate (*S*-methyl butanethioate)	484	R
S-Methyl 2-methylbutanethioate	486	R
S-Methyl 3-methylbutanethioate	487	R
4-(Methylthio)-2-butanone	497	R
4,5-Dihydro-3(2*H*)-thiophenone	498	R
2-Methyltetrahydrothiophen-3-one (4,5-dihydro-2-methyl-3(2*H*)-thiophenone)	499	R
4-(Methylthio)-4-methyl-2-pentanone (4-methyl-4-(methylthio)-2-pentanone)	500	R

Substance	No.	Specifications[a]
Di(butan-3-one-1-yl) sulfide (di-(3-oxobutyl) sulfide)	502	R, T
o-(Methylthio)phenol (2-(methylthio)phenol)	503	R
Methylsulfinylmethane	507	R
Methyl mercaptan (methanethiol)	508	R
Propanethiol (1-propanethiol)	509	R
2-Propanethiol	510	S, T
2-Methyl-1-propanethiol	512	R
3-Methylbutanethiol (3-methyl-1-butanethiol)	513	R
2-Pentanethiol	514	R
Cyclopentanethiol	516	R
1-Hexanethiol	518	R
2-, 3- or 10-Mercaptopinane (mixture of 2,6,6-trimethylbicyclo-[3.1.1]heptane-(2-, 3- and 10-)thiols)	520	R
Allyl mercaptan (allylthiol)	521	R
1-*p*-Menthene-8-thiol	523	R
Thiogeraniol (3,7-dimethyl-2(*trans*),6-octadiene-1-thiol)	524	R
o-Toluenethiol (2-methylbenzenethiol)	528	R
2-Ethylthiophenol (2-ethylbenzenethiol)	529	R
bis(Methylthio)methane	533	R
1,2-Propanedithiol	536	R
3-Mercapto-3-methyl-1-butanol	544	R
2-Mercapto-3-butanol (3-mercapto-2-butanol)	546	R
Ethyl 2-mercaptopropionate	552	R
3-Mercapto-2-butanone	558	R
3-Mercapto-2-pentanone	560	R
p-Mentha-8-thiol-3-one (8-mercapto-3-*p*-menthanone)	561	R
Methyl propyl disulfide	565	R
Allyl disulfide (diallyl sulfide)	572	R
3,5-Dimethyl-1,2,4-trithiolane	573	R
3-Methyl-1,2,4-trithiane	574	R
Dicyclohexyl disulfide	575	R
Benzyl disulfide (dibenzyl disulfide)	579	R
Dimethyl trisulfide	582	R
Methyl propyl trisulfide	584	R
Dipropyl trisulfide	585	R
Diallyl trisulfide	587	R
Diallyl polysulfide (mixture of diallyl di-, tri-, tetra- and pentasulfides)	588	R
2-Oxobutyric acid	589	R
Citronelloxyacetaldehyde (6,10-dimethyl-3-oxa-9-undecenal)	592	R, T
Ethyl 3-hydroxybutyrate	594	R
Butyl acetoacetate (butyl 3-oxobutyrate)	596	R
Isobutyl acetoacetate (2-methylpropyl 3-oxobutyrate)	597	R
Isoamyl acetoacetate (3-methylbutyl 3-oxobutyrate)	598	R
Methyl 3-hydroxyhexanoate	600	R
3-(Hydroxymethyl)-2-heptanone	604	R, T
1,3-Nonanediol acetate (mixed esters) (mixture of 3-acetoxynonyl acetate, 3-hydroxynonyl acetate and 1-(2-hydroxyethyl)heptyl acetate)	605	R, T
Hydroxycitronellol (2,6-dimethyl-2,8-octanediol)	610	R

Substance	No.	Specifications[a]
Hydroxycitronellal diethyl acetal (8,8-diethoxy-2,6-dimethyl-2-octanol)	613	R
Fumaric acid (2(*trans*)-butenedioic acid)	618	R
l-Malic acid (2-hydroxybutanedioic acid)	619	R
Diethyl malate (diethyl 2-hydroxybutanedioate)	620	R
Triethyl citrate (triethyl 2-hydroxy-1,2,3-propanetricarboxylate)	629	R
3-Methyl-2-oxobutanoic acid, sodium salt (sodium 3-methyl-2-oxobutyrate)	631.1	R, T
Tributyl acetylcitrate (tributyl 2-acetoxy-1,2,3-propane-tricarboxylate)	630	R
3-Methyl-2-oxopentanoic acid, sodium salt (sodium 3-methyl-2-oxovalerate)	632.1	R, T
4-Methyl-2-oxopentanoic acid, sodium salt (sodium 4-methyl-2-oxovalerate)	633.1	R, T
Furfuryl butyrate (2-furanylmethyl butanoate)	759	N, T

[a] R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new or revised specifications are tentative and comments are invited; W, existing specifications withdrawn.

[b] Relevant information was not provided so the tentative specifications were withdrawn.

[c] See Annex 3.

[d] The temporary acceptance of 2-nitropropane for use as a fractionating solvent in the production of fats and oils was not extended by the Committee at its thirty-fifth meeting (WHO Technical Report Series, No. 789, 1990). In the absence of further information on use, the Committee withdrew the specifications.

[e] At its twenty-seventh meeting (WHO Technical Report Series, No. 696, 1983), the Committee recommended that use of 1,1,2-trichloroethylene as an extraction solvent be limited because of toxicological concerns and requested information on the nature, level(s) and methods of analysis for stabilizers and breakdown products. As the requested information was not submitted for consideration at the present meeting, the Committee withdrew the specifications.

[f] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 8, 2000). In cases where substances were considered under their trivial name, the systematic name is given in parentheses.

[g] The Committee concluded that the specifications of this substance should be reviewed at its next meeting on food additives in order to consider more up-to-date information.

Annex 3
Further information required or desired

Toxicological information

D-Tagatose

Before reviewing the compound again, the Committee would wish to evaluate the final reports of the two studies in Sprague-Dawley and Wistar rats that were available in draft form, which might help to resolve the relevance of the induction of liver glycogen deposition and hypertrophy. It also wished to evaluate data to clarify the extent, mechanism and toxicological consequences of the increased serum uric acid concentrations observed in humans exposed to D-tagatose.

Cadmium

The Committee recommended that seven areas be investigated in order to increase confidence in the estimates of predicted excess prevalence of renal tubule dysfunction:

1. The toxicokinetics of cadmium should be investigated in controlled experimental studies in humans of the relationship between dietary intake and urinary excretion of cadmium in the general population and in groups at high risk, such as people with iron deficiency, renal disease or diabetes mellitus.
2. Dietary surveys should be conducted in which individual records of the food consumption of specific population subgroups are kept.
3. Studies should be conducted on the bioavailability of cadmium from specific foods and on the factors that affect its bioavailability, such as age, health status and dietary nutrients.
4. The relationship between biomarkers of renal tubule dysfunction and biomarkers of cadmium exposure should be elucidated.
5. The relationship between renal tubule dysfunction (as determined by specific biomarkers), clinical disease and mortality should be studied.
6. The influence of cadmium on calcium metabolism and osteoporosis should be examined.
7. Studies should be conducted to determine the effect of exposure to cadmium (integrated over a lifetime) on the subsequent development of osteoporosis.

Information on specifications

Amyloglucosidase from Aspergillus oryzae, var.

Information is required on the assay for amyloglucosidase in formulated products with glucose. Comments on other aspects of the monograph are invited.

Blackcurrant extract

Information is required on a chromatographic identification test and on the adequacy of the sample size for the test for sulfur dioxide. Comments on other aspects of the monograph are invited.

Oxystearin

Information on the levels of, and a suitable analytical method for, epoxides is required for consideration by the Committee at its meeting in June 2001. Comments on other aspects of the monograph are invited. If no information is received by 1 May 2001, the monograph will be withdrawn.

Smoke flavourings

Information is required on an alternative solvent to benzene for use in the analysis of the carbonyl content (proposals should be supported by a comparative test of the analytical method with benzene and the proposed alternative solvent). Comments on other sections of the monograph are invited.

Tagetes extract

Information is required on the composition of the commercial products, a test for the identification of xanthophylls, and a method of assay. Comments on other sections of the monograph are invited.

World Health Organization Technical Report Series

Recent reports:

No.		Sw.fr.*
857	(1995) **Vector control for malaria and other mosquito-borne diseases** Report of a WHO Study Group (97 pages)	15.–
858	(1995) **WHO Expert Committee on Biological Standardization** Forty-fifth report (108 pages)	17.–
859	(1995) **Evaluation of certain food additives and contaminants** Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (63 pages)	11.–
860	(1996) **Nursing practice** Report of a WHO Expert Committee (37 pages)	12.–
861	(1996) **Integration of health care delivery** Report of WHO Study Group (74 pages)	14.–
862	(1996) **Hypertension control** Report of a WHO Expert Committee (89 pages)	16.–
863	(1996) **WHO Expert Committee on Specifications for Pharmaceutical Preparations** Thirty-fourth report (200 pages)	35.–
864	(1996) **Evaluation of certain veterinary drug residues in food** Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives (66 pages)	12.–
865	(1996) **Control of hereditary diseases** Report of a WHO Scientific Group (89 pages)	16.–
866	(1996) **Research on the menopause in the 1990s** Report of a WHO Scientific Group (114 pages)	20.–
867	(1997) **The use of essential drugs** Seventh report of the WHO Expert Committee (80 pages)	15.–
868	(1997) **Evaluation of certain food additives and contaminants** Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives (77 pages)	14.–
869	(1997) **Improving the performance of health centres in district health systems** Report of a WHO Study Group (70 pages)	14.–
870	(1997) **Promoting health through schools** Report of a WHO Expert Committee on Comprehensive School Health Education and Promotion (99 pages)	20.–
871	(1997) **Medical methods for termination of pregnancy** Report of a WHO Scientific Group (117 pages)	23.–
872	(1998) **WHO Expert Committee on Biological Standardization** Forty-sixth report (97 pages)	20.–
873	(1998) **WHO Expert Committee on Drug Dependence** Thirtieth report (56 pages)	14.–
874	(1998) **WHO Expert Committee on Leprosy** Seventh report (49 pages)	14.–
875	(1998) **Training in diagnostic ultrasound: essentials, principles and standards** Report of a WHO Study Group (52 pages)	14.–
876	(1998) **Evaluation of certain veterinary drug residues in food** Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives (91 pages)	19.–
877	(1998) **Cardiovascular disease and steroid hormone contraception** Report of a WHO Scientific Group (96 pages)	20.–

* Prices in developing countries are 70% of those listed here.

878 (1998) **WHO Expert Committee on Biological Standardization**
Forty-seventh report (107 pages) 20.–

879 (1998) **Evaluation of certain veterinary drug residues in food**
Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives (80 pages) 16.–

880 (1998) **Preparation and use of food-based dietary guidelines**
Report of a Joint FAO/WHO Consultation (114 pages) 23.–

881 (1998) **Control and surveillance of African trypanosomiasis**
Report of a WHO Expert Committee (119 pages) 23.–

882 (1998) **The use of essential drugs**
Eighth report of the WHO Expert Committee (83 pages) 19.–

883 (1999) **Food safety issues associated with products from aquaculture**
Report of a Joint FAO/NACA/WHO Study Group (62 pages) 14.–

884 (1999) **Evaluation of certain food additives and contaminants**
Report of a Joint FAO/WHO Expert Committee on Food Additives (104 pages) 20.–

885 (1999) **WHO Expert Committee on Specifications for Pharmaceutical Preparations**
Thirty-fifth report (162 pages) 35.–

886 (1999) **Programming for adolescent health and development**
Report of a WHO/UNFPA/UNICEF Study Group (266 pages) 56.–

887 (1999) **WHO Expert Committee on Drug Dependence**
Thirty-first report (28 pages) 14.–

888 (1999) **Evaluation of certain veterinary drug residues in food**
Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives (102 pages) 20.–

889 (1999) **WHO Expert Committee on Biological Standardization**
Forty-eighth report (117 pages) 23.–

890 (1999) **High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy**
Report of a Joint FAO/IAEA/WHO Study Group (203 pages) 42.–

891 (2000) **Evaluation of certain food additives**
Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives (176 pages) 35.–

892 (2000) **WHO Expert Committee on Malaria**
Twentieth report (76 pages) 14.–

893 (2000) **Evaluation of certain veterinary drug residues in food**
Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives (109 pages) 20.–

894 (2000) **Obesity: preventing and managing the global epidemic**
Report of a WHO Consultation (264 pages) 56.–

895 (2000) **The use of essential drugs**
Ninth report of the WHO Expert Committee on the Use of Essential Drugs (including the revised Model List of Essential Drugs) (65 pages) 14.–

896 (2000) **Evaluation of certain food additives and contaminants**
Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives (136 pages) 25.–

897 (2000) **WHO Expert Committee on Biological Standardization**
Forty-ninth report (112 pages) 20.–

898 (2000) **Home-based long-term care**
Report of a WHO Study Group (48 pages) 14.–

899 (2001) **Chemistry and specifications of pesticides**
Sixteenth report of the WHO Expert Committee on Vector Biology and Control (72 pages) 14.–

900 (2001) **Evaluation of certain veterinary drug residues in food**
Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (102 pages) 20.–

SAFETY DATA SHEET

500420 RASPBERRY KETONE (OXYPHENYLON)

1. IDENTIFICATION

Product Description: **RASPBERRY KETONE (OXYPHENYLON)**

CAS # 5471-51-2

FEMA Number 2588

Other means of identification

Vigon Item # 500420

Recommended use Concentrated aromatic and flavor ingredient which may be used in flavor and fragrance compounds according to legal and IFRA or FEMA GRAS/FDA guidelines.

Recommended restrictions For Manufacturing Use Only

Company

Vigon International, LLC
127 Airport Road
E. Stroudsburg, PA 18301
For information call: 570-476-6300
Web Site: www.vigon.com

24 Hour Emergency Response Information

INFOTRAC (ACCT# 78928);
1-800-535-5053 WITHIN THE U.S.A.
1-352-323-3500 OUTSIDE THE U.S.A.

Manufacturer/Importer/Supplier/Distributor information

Manufacturer

Company name	Vigon International, LLC	
Address	127 Airport Road E. Stroudsburg, PA 18301 United States	
Telephone	For information call:	570-476-6300
Website	www.vigon.com	
E-mail	regulatory@vigon.com	
Emergency phone number	INFOTRAC 1-800-535-5053 1-352-323-3500	(ACCT# 78928); WITHIN THE U.S.A. OUTSIDE THE U.S.A.

2. HAZARD(S) IDENTIFICATION

Physical hazards Not classified.

Health hazards Not classified.

Environmental hazards Not classified.

Label elements

Hazard symbol None.

Signal word None.

Hazard statement The substance does not meet the criteria for classification.

Precautionary statement

Prevention Observe good industrial hygiene practices.

Response Wash hands after handling.

Storage Store away from incompatible materials.

Disposal	Dispose of waste and residues in accordance with local authority requirements.
Hazard(s) not otherwise classified (HNOC)	WARNING! May form combustible dust concentrations in air. Avoid breathing dust.
Supplemental information	100% of the substance consists of component(s) of unknown acute dermal toxicity. 100% of the substance consists of component(s) of unknown acute inhalation toxicity. 100% of the substance consists of component(s) of unknown acute hazards to the aquatic environment. 100% of the substance consists of component(s) of unknown long-term hazards to the aquatic environment.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Substances

Chemical name	Common name and synonyms	CAS number	%
4-(4-HYDROXYPHENYL)-2-BUTANONE	4-(4-Hydroxyphenyl)butan-2-one 2-Butanone, 4-(4-hydroxyphenyl)- OXYPHENYLON	5471-51-2	100

4. FIRST-AID MEASURES

Inhalation	If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing. For breathing difficulties, oxygen may be necessary. Call a physician if symptoms develop or persist.
Skin contact	Take off immediately all contaminated clothing. Get medical attention if irritation develops and persists. Wash skin thoroughly with soap and water for several minutes.
Eye contact	Remove contact lenses, if present and easy to do. Get medical attention if irritation develops and persists. Promptly wash eyes with plenty of water while lifting the eye lids.
Ingestion	Call a physician or poison control center immediately. If swallowed, rinse mouth with water (only if the person is conscious). Do not induce vomiting. If vomiting occurs, the head should be kept low so that stomach vomit doesn't enter the lungs.
Most important symptoms/effects, acute and delayed	Dusts may irritate the respiratory tract, skin and eyes.
Indication of immediate medical attention and special treatment needed	Not available.
General information	Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves. Show this safety data sheet to the doctor in attendance.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray, fog, CO2, dry chemical, or alcohol resistant foam.
Unsuitable extinguishing media	Do not use a solid water stream as it may scatter and spread fire.
Specific hazards arising from the chemical	Explosion hazard: Avoid generating dust; fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard. Fire may produce irritating, corrosive and/or toxic gases.

Special protective equipment and precautions for firefighters	Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA. Structural firefighters protective clothing will only provide limited protection. Wear self-contained breathing apparatus with a full facepiece operated in the positive pressure demand mode when fighting fires.
Fire fighting equipment/instructions	In case of fire and/or explosion do not breathe fumes. Use standard firefighting procedures and consider the hazards of other involved materials. Move containers from fire area if you can do so without risk. Water runoff can cause environmental damage. Ventilate closed spaces before entering them. Keep run-off water out of sewers and water sources. Dike for water control.
Specific methods	Use water spray to cool unopened containers.
General fire hazards	Static charges generated by emptying package in or near flammable vapor may cause flash fire.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures	Keep unnecessary personnel away. Eliminate all sources of ignition. Avoid contact with skin or inhalation of spillage, dust or vapor. Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Ventilate closed spaces before entering them.
Methods and materials for containment and cleaning up	Eliminate all ignition sources (no smoking, flares, sparks or flames in immediate area). Sweep up and place in a clearly labeled container for chemical waste. Wash contaminated area with water. Use only non-sparking tools. Avoid the generation of dusts during clean-up. Collect and dispose of spillage as indicated in section 13 of the SDS. This material and its container must be disposed of as hazardous waste. Never return spills in original containers for re-use.
Environmental precautions	Prevent further leakage or spillage if safe to do so. Do not contaminate water. Avoid release to the environment. Retain and dispose of contaminated wash water. Contact local authorities in case of spillage to drain/aquatic environment.

7. HANDLING AND STORAGE

Precautions for safe handling	Take precautionary measures against static discharges when there is a risk of dust explosion. Minimize dust generation and accumulation. Routine housekeeping should be instituted to ensure that dusts do not accumulate on surfaces. Dry powders can build static electricity charges when subjected to the friction of transfer and mixing operations. Provide adequate precautions, such as electrical grounding and bonding, or inert atmospheres. Do not handle or store near an open flame, heat or other sources of ignition. Assume that this material is capable of producing a dust explosion if ignited as a dust cloud. Take precautionary measures against static discharges. Avoid breathing vapor. Avoid breathing dust. Avoid contact with eyes, skin, and clothing. Avoid prolonged exposure. Wash thoroughly after handling.
Conditions for safe storage, including any incompatibilities	Keep container closed. Handle containers with care. Open slowly in order to control possible pressure release. Store in a cool, well-ventilated area.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Occupational exposure limits	This substance has no PEL, TLV, or other recommended exposure limit.
Biological limit values	No biological exposure limits noted for the ingredient(s).

Appropriate engineering controls	Use explosion-proof ventilation equipment to stay below exposure limits. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen-deficient environment. Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment). Use only appropriately classified electrical equipment and powered industrial trucks.
Individual protection measures, such as personal protective equipment	
Eye/face protection	Wear safety glasses with side shields (or goggles).
Skin protection	
Hand protection	Chemical resistant gloves.
Other	Not available.
Respiratory protection	Dust mask.
Thermal hazards	Wear appropriate thermal protective clothing, when necessary.
General hygiene considerations	When using, do not eat, drink or smoke. Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	Refer to Spec Sheet
Physical state	Powder\Crystal.
Form	Powder. Crystalline powder.
Color	Refer to Spec Sheet
Odor	Characteristic.
Odor threshold	Not available.
pH	Not available.
Melting point/freezing point	181.4 °F (83 °C)
Initial boiling point and boiling range	413.6 °F (212 °C)
Flash point	212.0 °F (100.0 °C) Closed Cup
Evaporation rate	Not available.
Flammability (solid, gas)	Not available.
Upper/lower flammability or explosive limits	
Explosive limit - lower (%)	Not available.
Explosive limit - upper (%)	Not available.
Vapor pressure	0.003 mm Hg at 20°C
Vapor density	5.7
Relative density	Not available.
Solubility(ies)	
Solubility (water)	Insoluble

Partition coefficient (n-octanol/water)	Not available.
Auto-ignition temperature	Not available.
Decomposition temperature	Not available.
Viscosity	Not available.
Other information	
Density	1.17 g/cm3 at 20 °C
Explosive properties	Not explosive.
Flammability class	Combustible IIIB estimated
Molecular formula	C10H12O2
Molecular weight	164.2 g/mol 164.2 g/mol
Oxidizing properties	Not oxidizing.

10. STABILITY AND REACTIVITY

Reactivity	The product is stable and non-reactive under normal conditions of use, storage and transport.
Chemical stability	Material is stable under normal conditions.
Possibility of hazardous reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	Keep away from heat, sparks and open flame. Avoid temperatures exceeding the flash point. Contact with incompatible materials. Minimize dust generation and accumulation.
Incompatible materials	Strong oxidizing agents.
Hazardous decomposition products	No hazardous decomposition products if stored and handled as indicated.

11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Inhalation	Irritating to respiratory system.
Skin contact	Causes mild skin irritation.
Eye contact	Dust may irritate the eyes. Causes mild eye irritation.
Ingestion	Knowledge about health hazard is incomplete.
Symptoms related to the physical, chemical and toxicological characteristics	Dusts may irritate the respiratory tract, skin and eyes.

Information on toxicological effects

Acute toxicity	Not known.
Skin corrosion/irritation	Due to partial or complete lack of data the classification is not possible.
Serious eye damage/eye irritation	Due to partial or complete lack of data the classification is not possible.
Respiratory or skin sensitization	
Respiratory sensitization	Due to partial or complete lack of data the classification is not possible.

Skin sensitization	Due to partial or complete lack of data the classification is not possible.
Germ cell mutagenicity	Due to partial or complete lack of data the classification is not possible.
Carcinogenicity	Due to partial or complete lack of data the classification is not possible.

IARC Monographs. Overall Evaluation of Carcinogenicity

Not listed.

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1053)

Not listed.

US. National Toxicology Program (NTP) Report on Carcinogens

Not listed.

Reproductive toxicity	Due to partial or complete lack of data the classification is not possible.
Specific target organ toxicity - single exposure	Due to partial or complete lack of data the classification is not possible.
Specific target organ toxicity - repeated exposure	Due to partial or complete lack of data the classification is not possible.
Aspiration hazard	Due to partial or complete lack of data the classification is not possible.

12. ECOLOGICAL INFORMATION

Ecotoxicity	The product is not classified as environmentally hazardous. However, this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment.
Persistence and degradability	No data is available on the degradability of this substance.
Bioaccumulative potential	No data available.
Mobility in soil	No data available.
Other adverse effects	No other adverse environmental effects (e.g. ozone depletion, photochemical ozone creation potential, endocrine disruption, global warming potential) are expected from this component.

13. DISPOSAL CONSIDERATIONS

Disposal instructions	Do not discharge into drains, water courses or onto the ground. Do not allow this material to drain into sewers/water supplies. Do not contaminate ponds, waterways or ditches with chemical or used container. Dispose of contents/container in accordance with local/regional/national/international regulations.
Local disposal regulations	Dispose in accordance with all applicable regulations.
Hazardous waste code	Not established.
Waste from residues / unused products	Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner (see: Disposal instructions).
Contaminated packaging	Since emptied containers may retain product residue, follow label warnings even after container is emptied. Empty containers should be taken to an approved waste handling site for recycling or disposal.

14. TRANSPORT INFORMATION

ADN

Not regulated as dangerous goods.

ADR

Not regulated as dangerous goods.

RID

Not regulated as dangerous goods.

DOT

BULK

Not regulated as dangerous goods.

DOT

NON-BULK

Not regulated as dangerous goods.

IATA

Not regulated as dangerous goods.

IMDG

Not regulated as dangerous goods.

15. REGULATORY INFORMATION

US federal regulations This product is a "Hazardous Chemical" as defined by the OSHA Hazard Communication Standard, 29 CFR 1910.1200.

Toxic Substances Control Act (TSCA)

TSCA Section 12(b) Export Notification (40 CFR 707, Subpt. D)

Not regulated.

CERCLA Hazardous Substance List (40 CFR 302.4)

Not listed.

SARA 304 Emergency release notification

Not regulated.

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1053)

Not listed.

Superfund Amendments and Reauthorization Act of 1986 (SARA)

SARA 302 Extremely hazardous substance

Not listed.

SARA 311/312 Hazardous chemical Yes

Classified hazard categories Combustible dust

SARA 313 (TRI reporting)

Not regulated.

Other federal regulations

Clean Air Act (CAA) Section 112 Hazardous Air Pollutants (HAPs) List

Not regulated.

Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130)

Not regulated.

Safe Drinking Water Act (SDWA)	Not regulated.

16. OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION

Issue date	03-16-2013
Revision date	11-08-2021
Version #	07
Further information	Refer to NFPA 654, Standard for the Prevention of Fire and Dust Explosions from the Manufacturing, Processing, and Handling of Combustible Particulate Solids, for safe handling.
HMIS® ratings	Health: 1 Flammability: 1 Physical hazard: 0
List of abbreviations	ACGIH: American Conference of Governmental Industrial Hygienists. ADR: European Agreement concerning the International Carriage of Dangerous Goods by Road. AICIS: Australian Inventory of Industrial Chemicals. CAS: Chemical Abstract Service. IARC: International Agency for Research on Cancer. IATA: International Air Transport Association. IBC Code: International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk. IMDG: International Maritime Dangerous Goods. MARPOL: International Convention for the Prevention of Pollution from Ships. RID: Regulations concerning the International Carriage of Dangerous Goods by Rail. STEL: Short term exposure limit. TWA: Time Weighted Average.
Disclaimer	Vigon International, LLC cannot anticipate all conditions under which this information and its product, or the products of other manufacturers in combination with its product, may be used. It is the user's responsibility to ensure safe conditions for handling, storage and disposal of the product, and to assume liability for loss, injury, damage or expense due to improper use. The information in the sheet was written based on the best knowledge and experience currently available. The above information relates only to this product and not to its use in combination with any other material or any particular process and is designed only as guidance for the safe handling, use, processing, storage, transportation, and disposal and should not be considered as a guarantee or quality specification. This product has not been evaluated for safe use in e-cigarettes or any vaping application where the product(s) is/are intentionally vaporized and inhaled. Vigon International, Inc. has performed no testing on these products in e-cig/vaping applications. It is the sole responsibility of the individual(s) purchasing this product to assess its' safety in the final application. The above information relates only to this product and not to its use in combination with any other material or any particular process and is designed only as guidance for the safe handling, use, processing, storage, transportation, disposal, and should not be considered as a guarantee or quality specification. The above information is based on data provided by and collected from recognized sources such as distributors, manufacturers, and technical groups and is considered to be accurate to the best of Vigon's knowledge as of the date of this document. It is the responsibility of the user to review all safety information about this product and determine its safety and suitability in their own processes and operations. Appropriate warnings and safe handling procedures should be provided to all handlers and users, taking into account the intended use and the specific conditions and factors relating to such use in accordance with all applicable laws and regulations.

Revision information

Product and Company Identification: Alternate Trade Names
HAZARD(S) IDENTIFICATION: Prevention
HAZARD(S) IDENTIFICATION: Response
HAZARD(S) IDENTIFICATION: Storage
COMPOSITION/INFORMATION ON INGREDIENTS: Composition comments
EXPOSURE CONTROLS/PERSONAL PROTECTION: Appropriate engineering controls
EXPOSURE CONTROLS/PERSONAL PROTECTION: Other
Physical & Chemical Properties: Multiple Properties
Toxicological Information: Toxicological Data
TOXICOLOGICAL INFORMATION: Eye contact
OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION: Reference s OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION: List of abbreviations