



Toxicological profile for Caramel and or caramel color

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

Not applicable.

1.2. Synonyms

Caramel; caramel color; c.i. natural brown 10; caramel flavor; caramel liquid; caramel (color); natural brown 10; burnt sugar; (FDA 2022); FEMA No. 2235

Caramel Colour I (synonyms: plain caramel, caustic caramel, and spirit caramel);

Caramel Colour II (synonyms: caustic sulfite process caramel);

Caramel Colour III (synonyms: ammonia caramel, ammonia process caramel, closed-pan ammonia process caramel, open-pan ammonia process caramel, bakers' caramel, confectioners' caramel, and beer caramel);

Caramel Colour IV (synonyms: ammonia sulfite process caramel, sulfite ammonia caramel, sulfite ammonia process caramel, acid-proof caramel, beverage caramel, and soft-drink caramel);

WHO Food Additives Series 20

1.3. Molecular formula

No data available to us at this time.

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

8028-89-5

1.7. Properties

1.7.1. Melting point

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

1.7.2. Boiling point

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

1.7.3. Solubility

Miscible with water (IUCLID, 2000).

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

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

1.7.6. Flammability limits (vol/vol%)

Non-flammable (IUCRID, 2000).

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

Very good stability in most products (HSDB, 2002).

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

Cosmetics	Yes (Cosmetics Bench Ref. 1996)	Food	Yes, 0.05 - 543.1 ppm (Burdock GA, 2010)
Environment	No evidence (HSDB 2002; Merck 1996)	Pharmaceuticals	No evidence (Martindale 1993; HSDB 2002; Merck 1996)

EFSA (2011) reports the following:

The mean dietary exposure of European children including UK pre-school children ranged from:

- 76.9 to 427.2 mg/kg bw/day for Class I Plain Caramel
- 8.7 to 34.6 mg/kg bw/day for Class II Caustic Sulphite Caramel
- 21.7 to 302.4 mg/kg bw/day for Class III Ammonia Caramel
- 23.2 to 506.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel
- At the 95th or 97.5th percentile, estimates ranged from:
- 179.6 to 882.2 mg/kg bw/day for Class I Plain Caramel

- 18.5 to 117.3 mg/kg bw/day for Class II Caustic Sulphite Caramel
- 107.9 to 757.3 mg/kg bw/day for Class III Ammonia Caramel
- 129.7 to 1480.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel

“The anticipated dietary exposure reported for the UK adult population gives a mean of 136.6 mg/kg bw/day and a 97.5th percentile of 429.3 mg/kg bw/day for Class I Plain Caramel; a mean of 21.7 mg/kg bw/day and a 97.5th percentile of 109.5 mg/kg bw/day for Class II Caustic Sulphite Caramel; a mean of 60.8 mg/kg bw/day and a 97.5th percentile of 295.0 mg/kg bw/day for Class III Ammonia Caramel; and a mean of 89.4 mg/kg bw/day and 97.5th percentile of 368.9 mg/kg bw/day for Class IV Sulphite Ammonia Caramel” (EFSA, 2011).

“The dietary exposure to caramel (E150) food colours can be more accurately assessed using data on use levels obtained from the food industry. The National Institute for Public Health and the Environment (RIVM) drew this conclusion based on a study in which the total dietary exposure to E150a, E150b, E150c and E150d was estimated by applying use levels provided by the food industry. Previously, exposure to E150 was estimated using maximum use levels linked to aggregated food categories (e.g. all beers, including blond beer, were assumed to contain the maximum reported use level of dark beer), which results in overestimates of exposure. Collection of use levels of foods present on the Dutch market allows refined linkage of these concentrations to foods on the food group level (e.g. use levels of kidney bean soup were linked to the consumption of ‘legume soup’ instead of to all foods belonging to the food category ‘soups and broths’) or even on food level (e.g. use levels of ‘speculaas’ were linked to the consumption of this food instead of all foods belonging to the food category ‘fine bakery wares’). When refining the exposure assessment in this way using food consumption data from the Dutch National Food Consumption Survey (DNFCS) 2007-2010, a lower and more realistic exposure estimate for E150 was obtained for the adult population aged 17-30 years compared with a recent EFSA exposure assessment. For children aged 2-6 years (DNFCS-young children), the exposure estimate was comparable to that obtained by EFSA.” As taken from Sprong C et al. 2014. Available at <http://www.rivm.nl/bibliotheek/rapporten/050015001.pdf>.

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

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	1.02	2.48	Instant coffee, tea	0.05	0.19
Baked goods	2.34	4.30	Meat products	2.11	2.40
Breakfast cereals	1.20	1.20	Nonalcoholic beverages	1.53	1.67
Condiments, relishes	10.15	10.15	Nut products	0.05	0.08
Confection, frosting	2.30	3.21	Other grains	0.59	0.69
Fats, oils	0.67	0.67	Poultry	1.23	2.37
Fish	0.43	0.43	Processed vegetables	4.11	4.11
Frozen dairy	0.78	1.31	Reconstituted vegetables	20.86	47.77
Fruit juice	0.59	0.59	Snack foods	0.55	2.54

Gelatins, puddings	0.99	1.25	Soft candy	2.40	2.77
Gravies	539.30	543.10	Soups	0.55	1.61
Hard candy	0.07	0.39	Sweet sauce	2.53	2.67

Estimated intake from flavouring use: 0.132.6271 mg/kg bw/day.

The FEMA PADI (possible average daily intake) of caramel is 5.837 mg.

As taken from Burdock, 2010.

Used as a colourant and fragrance agent in cosmetics in the EU.

As taken from Cosing, undated.

“Caramel has been used as a synthetic colorant replacer in the baking and beverage industries.”
As taken from Sengar G & Sharma HK. 2014. J. Food. Sci. Technol. 51(9), 1686-1696. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25190825>

Caramel (CAS RN 8028-89-5) is listed as an ingredient in commercial / institutional, inside the home, personal care and pet care products by the CPID.

Typical uses of the four caramel colors include desserts, distilled spirits, spice blends (I), ice creams, brandies, liqueurs, vermouths (II), beer, gravies, baked goods (III), pet foods, soups, and soft drinks (IV).

Caramel color is one of the most commonly used food colors and produces pale yellow to dark brown colors in products intended for drug, cosmetic, and food uses. Furthermore, caramel color is used directly in practically every category of food product, including baked products, cola beverages, root beers, wines, gravies, jams, prepared meats, frozen desserts, and confections. Its ubiquitous presence in foods is matched perhaps only by salt or sugar. Highest average maximum use levels of caramel color are reported in gravies (5.4%) and reconstituted vegetables (4.8%).

As taken from Khan IA and Abourashed EA, 2010

Caramel color is listed as a fragrance ingredient by IFRA.

“4-Methylimidazole (4-MEI) is formed in caramel colours produced using ammonium compounds (Class III and Class IV caramel colours). 4-MEI can also form in food through Maillard reactions between reducing sugars and amino acids during cooking, roasting or dry-heating. The USFDA has analysed over 700 food and beverage samples collected from 2013 to 2015 for the presence of 4-MEI. These samples include foods containing added caramel colour and foods that are not labelled as containing added caramel colour, but which may contain 4-MEI resulting from thermal treatment. The 4-MEI levels in all food samples were quantified using LC-MS/MS. These data were used to develop a comprehensive dietary exposure assessment for 4-MEI for the U.S. population aged 2 years or more and several sub-populations, using two non-consecutive days of food consumption data from the combined 2009-2012 National Health and Nutrition Examination Survey and 10-14-day food consumption survey data for 2009-2012 from the NPD Group, Inc. National Eating Trends-Nutrient Intake Database. Dietary exposure estimates were prepared for each category of foods labelled as containing added caramel colour and of foods not labelled as containing added caramel colour, but which may contain 4-MEI from thermal treatment. Exposure to 4-MEI from consumption of foods containing added caramel colour was higher than that from foods that contain 4-MEI from thermal treatment for all population groups. Cola-type carbonated beverages were the highest contributors for most populations from foods containing added caramel colour. Coffee was the highest contributor for most populations from foods in which 4-MEI could be formed from thermal treatment. An overall combined exposure to 4-MEI was also estimated that included all foods identified as containing added caramel colour and foods in which 4-MEI could be formed by thermal treatment.” As taken from Folmer DE et al. 2018. Food Addit. Contam. Part A Chem. Anal.

Control Expo. Risk Assess. 35(10), 1890-1910. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/30130454>

“The aim of the study was to assess the dietary intake of caramel colours and their by-products 4-methylimidazole (4-MEI) and 2-acetyl-4-tetrahydroxybutylimidazole (THI) for the Chinese population. Based on the typical and maximum reported use levels of caramel colours in 15 food categories, the dietary intakes of combined and single-class caramel colours of Classes I, III and IV were estimated with the food consumption data from the China National Nutrient and Health Survey. Using the mean values of 4-MEI and THI contents in Class III and Class IV Caramel colour samples, the exposures to 4-MEI and THI from dietary caramel colours were derived. The results showed that the combined and individual average dietary caramel colour intakes for the Chinese population of different age groups were estimated to be 232-60.3 mg kg⁻¹ bw day⁻¹ for combined caramels, 5.9-29.2 mg kg⁻¹ bw day⁻¹ for Class I, 7.7-29.6 mg kg⁻¹ bw day⁻¹ for Class III, 21.2-54.3 mg kg⁻¹ bw day⁻¹ for Class IV, which were far below the group acceptable daily intake (ADI) and respective ADIs. The combined intake of 4-MEI from Class III and IV caramel colours was estimated to be 3.8-5.2 µg kg⁻¹ bw day⁻¹ on average, and 12.9-27.1 µg kg⁻¹ bw day⁻¹ at 95th-97.5th percentile for the general population. The anticipated exposure to THI from Class III caramel colours was estimated to be 0.1-0.3 µg kg⁻¹ bw day⁻¹ on average and 0.5-1.7 µg kg⁻¹ bw day⁻¹ at 95th-97.5th percentile for the general population. The dietary caramel colours intakes and the exposures to 4-MEI and THI from dietary caramel colour for the Chinese population were considered to be of low health concern based on the present toxicological data. Soy sauce, vinegar and compound seasonings were found to be the main contributors to the dietary intake of caramel colours.” As taken from Liang J et al. 2019. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 36(7), 1009-1019. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31100047/>

In non-medicinal and general natural health products, caramel (CAS RN 8028-89-5) is used as a colour additive and caramel flavour (no CAS RN listed) is used as a flavour enhancer for oral use (Health Canada, 2022).

2.2. Combustion products

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

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
furan	trace	trace	1368266	1.02
acetone	9570553	3.17	6617272	4.95
unknown	2528004	0.84	2036277	1.52
2-methylfuran	7743785	2.56	3532469	2.64
diacetyl + methyl ethyl ketone	6223514	2.06	3253586	2.43
2,5-dimethylfuran	9479801	3.14	3482210	2.61
acetic acid	46737248	15.48	24837151	18.58
acetol	28546377	9.45	10593694	7.93

2-cyclopenten-1-one	4335587	1.44	2053105	1.54
furfural	6216391	2.06	2626361	1.97
furfuryl alcohol	5881145	1.95	3692803	2.02
2-acetylfuran	4586177	1.52	2269566	1.70
5-methylfurfural	8533043	2.83	40624272	3.04
gamma-butyrolactone	3357887	1.11	trace	trace
unknown	8013726	2.65	2571879	1.92
unknown mixture	9098849	3.01	4284199	3.21
1,4:3,6-dianhydro-.alpha.-D-glucopyranose	5319496	1.76	2903107	2.17
5-hydroxymethylfurfural	31693394	10.49	15873792	11.88
unknown	trace	trace	2156238	1.61
levoglucosan	45528092	15.08	16614984	12.43
1,6-anhydro-beta-D-glucofuranose	33976466	11.25	14590447	10.92
Total ion chromatogram	302013031	100	133648869	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. J. Anal. Appl. Pyrolysis 74, 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (nüg)
Caramel 8028-89-5	500	Acetic acid 42.7 Theobromine 25.6 Long chain hydrocarbons 12.2 Methylfurfural 6.6 Acetylfuran 3.2 Furfural 2.5	110 64 31 17 8 6

2.3. Ingredient(s) from which it originates

No evidence of its presence in tobacco naturally (Stedman 1968; Lloyd et al. 1976).

Made by heating sugar or glucose, adding small amounts of alkali, alkaline carbonate or a trace of mineral acid during the heating.

Does not occur in nature (Burdock GA, 2010).

“Caramel coloring is a non-sweet and dark brown form of caramel that is commercially produced by the controlled action of chemicals and heat to sugar”.

“Caramel (CAS RN 8028-89-5) is divided into: Class I (prepared by heating carbohydrates with or without acids or alkalis, but no ammonium or sulfite compounds are used), Class II (sulfites involved in preparation, but not ammonium compounds), Class III (ammonium compounds involved

in preparation, but not sulfite compounds), and Class IV (ammonium and sulfite compounds involved in preparation) (Food Chemicals Codex).”

As taken from Health Canada, 2022.

Caramel Colour I (synonyms: plain caramel, caustic caramel, and spirit caramel); this class is prepared by the controlled heat treatment of carbohydrates with alkali or acid.

Caramel Colour II (synonyms: caustic sulfite process caramel); this class is prepared by the controlled heat treatment of carbohydrates with sulfite-containing compounds.

Caramel Colour III (synonyms: ammonia caramel, ammonia process caramel, closed-pan ammonia process caramel, open-pan ammonia process caramel, bakers' caramel, confectioners' caramel, and beer caramel); this class is prepared by the controlled heat treatment of carbohydrates with ammonium compounds.

Caramel Colour IV (synonyms: ammonia sulfite process caramel, sulfite ammonia caramel, sulfite ammonia process caramel, acid-proof caramel, beverage caramel, and soft-drink caramel); this class is prepared by the controlled heat treatment of carbohydrates with ammonium-containing and sulfite-containing compounds.

WHO Food Additives Series 20

3. Status in legislation and other official guidance

JECFA has allocated the following ADIs: caramel class I: Not limited (JECFA 1987) caramel class II: 0-160 mg/kg bw/day (JECFA 2001) caramels class III and IV: 0-200 mg/kg bw/day (or 0-150 mg/kg bw/day on a solids basis; specifications apply) (JECFA 1987) An EFSA Panel has concluded that, given the toxicological similarity of all four classes of caramel colours and the consistency in the toxicological database, the caramel colours can be considered as a single group in terms of assessing their safety, and that a group ADI can be derived. However, because of concerns about the immunotoxicity of 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI), present in Class III Ammonia Caramel, the Panel decided to define an individual ADI for this caramel within the group ADI, based on the currently available database. It therefore set a group ADI of 300 mg/kg bw/day for Caramel colours classes I-IV and, within this, a specific ADI of 100 mg/kg bw/day for Ammonia Caramel class II (EFSA, 2011).

“The Panel noted that anticipated combined dietary exposures of both adults and children to all caramel colours exceed the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile, while the ADI is also exceeded by the combined mean intake for children. In the case of children, this exceedance applies to the upper end of the exposure range only” (EFSA, 2011).

FDA Requirements:

FDA NUMBER 121.101, LIMITATIONS: GRAS, MISCELLANEOUS &/OR GENERAL-PURPOSE FOOD ADDITIVE, FDA NUMBER 8.303, LIMITATIONS: FOOD COLOR. PRODUCT SPECIFICATIONS APPLY. [Furia, T.E. (ed.). CRC Handbook of Food Additives. 2nd ed. Cleveland: The Chemical Rubber Co., 1972., p. 815]

As taken from HSDB, 2002

Caramel is included on the FDA's inventory of Substances Added to Food (formerly EAFUS) as a color or coloring adjunct, flavoring agent or adjuvant, nutritive sweetener and solvent or vehicle and is included under 21 CFR sections 182.1235 (multiple purpose GRAS food substances); 73.1085 (color additives exempt from certification: drugs); 73.2085 (color additives exempt from certification: cosmetics); 73.85 (color additives exempt from certification: caramel)) (FDA, 2022a, 2023a).

ADI “0-200 mg/kg b.w. (0-150 mg/kg b.w. on a solids basis; for caramel color III).”

ADI "0-200 mg/kg b.w. (0-150 mg/kg b.w. on a solids basis; for caramel color IV)." As taken from WHO Food Additives Series 20.

There is a REACH dossier on caramel (color) (CAS RN 8028-89-5) (ECHA).

Caramel (color) (CAS RN 8028-89-5) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Caramel color is listed as a fragrance ingredient by IFRA.

C.I. Natural brown 10 (CAS RN 8028-89-5) is included on the US EPA's list of Safer Chemical Ingredients (US EPA, 2023a).

Caramel is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products.

Caramel color (CAS RN 8028-89-5) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and 2020 CDR TSCA Inv.

US EPA Substance Registry Services (SRS)

Plain caramel (E150a), caramels (E150a-d), caustic sulphite caramel (E150b), ammonia caramel (E150c), sulphite ammonia caramel (E150d) and plain caramel, caustic sulphite caramel and sulphite ammonia caramel (E150a, b, d) are authorised for use as food additives in the EU (European Commission).

Caramel color has been given GRAS (generally recognised as safe) status as a food flavour by FEMA (FEMA no. 2235) (Hall and Oser, 1965).

Caramel, colour (CAS RN 8028-89-5) "poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework" and has been identified as "low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach" (AICIS, 2017).

INCI Name	CAMEL	
Description	Caramel is the concentrated solution obtained from heating sucrose or glucose solutions.	
CAS #	8028-89-5	
EC #	232-435-9	
Cosmetics Regulation provisions	IV/146	
Functions	COLORANT FRAGRANCE	
SCCS opinions		
Identified INGREDIENTS or substances e.g.		
Substance	Caramel	
CAS #		
EC #	232-435-9	
Colour index Number / Name of Common Ingredients Glossary	CAMEL	

INN/ISO/AN	
Regulation	(EC) 2009/1223
Regulated By	88/667/EEC
Other Directives/Regulations	
Annex/Ref #	IV/146
Colour	Brown
Product Type, body parts	
Maximum concentration in ready for use preparation	
Other	Purity criteria as set out in Commission Directive 95/45/EC (E150a-d)
Wording of conditions of use and warnings	
SCCS opinions	
Chemical/IUPAC Name	Caramel
Identified INGREDIENTS or substances e.g.	CARAMEL
Note	
Current Version	v.1

As taken from Cosing, undated.

Caramel (CAS RN 8028-89-5) is included on the FDA's list of inactive ingredients for approved drug products. It is permitted for use in various products at the following maximum potencies per unit dose and maximum daily exposure: (FDA, 2023b).

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
CARAMEL	ORAL	CAPSULE	8028895	T9D99G2B1R	NA	
CARAMEL	ORAL	GRANULE, FOR SUSPENSION	8028895	T9D99G2B1R	2.5 mg/5ml	
CARAMEL	ORAL	LIQUID	8028895	T9D99G2B1R	5.3 mg	
CARAMEL	ORAL	SOLUTION	8028895	T9D99G2B1R		11 mg

CARAMEL	ORAL	SUSPENSION	8028895	T9D99G2B1R	111.12 mg/5ml	
CARAMEL	ORAL	SYRUP	8028895	T9D99G2B1R	207 mg/5ml	
CARAMEL	RECTAL	SOLUTION	8028895	T9D99G2B1R	NA	
CARAMEL	TOPICAL	CREAM	8028895	T9D99G2B1R	0.26 %w/w	
CARAMEL	TOPICAL	LOTION	8028895	T9D99G2B1R	NA	

According to Health Canada's Natural Health Products Ingredients database, caramel (CAS RN 8028-89-5) is "permitted for internal and external use". "For Caramel prepared with ammonium compounds (Class III and Class IV Caramel) the upper limit for Acceptable Daily Intake (ADI) is 200 mg/kg bw. For Class II, the upper limit for ADI is 160 mg/kg bw".

As taken from Health Canada, 2022.

Caramel, color (CAS RN 8028-89-5) is listed on Australian Inventory of Industrial Chemicals (AICIS, formerly NICNAS). As taken from AICIS, undated.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

EFSA (2011) noted that data on the toxicokinetics of the caramel colours are very limited, but indicate little uptake of the high molecular weight fraction of the colours from the gastrointestinal tract, with the bulk of the material being excreted in the faeces.

"In groups of 2-4 rats, the absorption of the colour-giving components of caramel was determined by faecal extraction. Recoveries varied widely for the 10 or 20% caramel solutions examined despite pre-treatment for 100 days before testing. About one-third of the colour-giving components appeared to be absorbed, but no conclusions could be drawn regarding the absorption of colourless components (Haldi & Wynn, 1951)."

"After administration of large doses of the coloured components of caramel colour IV, only a small fraction was absorbed, distributed in lymphoreticular tissue, and eventually excreted in the urine; retention by the mesenteric lymph nodes appeared to account for the pigmentation observed in this

"Pigmentation of tissues of the lymphoreticular system, particularly of the mesenteric lymph nodes, has been a frequent observation in rats fed high levels of caramel colour. A study was undertaken to determine the distribution of the colour component of caramel colour in rats and to determine whether accumulation of caramel colour was the cause of pigmentation in the mesenteric lymph nodes."

"Groups of 4 rats were killed at intervals of 4, 8, 12, 24, and 96 hours after receiving the single oral dose of 14C-labelled material, and radioactivity was measured in the following tissues/organs; blood, brain, heart, lungs, liver, kidneys, spleen, thymus, mesenteric and cervical lymph nodes, gastrointestinal tract (contents and tissues), and carcass. Most of the radioactivity was located in

the gastrointestinal tract, and only low levels of radioactivity were found in blood and tissues. The specific activity in the thymus, mesenteric lymph nodes, spleen, kidneys, and liver exceeded that in blood, but with the exception of the mesenteric lymph nodes, the radioactivity was cleared rapidly over the 96-hour study period. With the exception of the gastrointestinal tract, the highest tissue levels attained, in the liver and kidneys, never exceeded 0.02% and 0.01% of the administered dose.”

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

“Caramel Colour IV prepared from [U-14C]glucose was ultrafiltered in order to isolate the high molecular weight colour fraction (HMCF). The colour fraction that was non-permeable to a 10,000-Da porosity membrane, contained 84% of the colour, 22% of the solids and 24% of the radioactivity of the [14C]Caramel Colour IV. The absorption, distribution and excretion of [14C]HMCF were evaluated in male rats after administration of single or multiple oral doses of the material at a dosage level of 2.5 g/kg body weight. Rats on the multiple oral dosage regimen were given unlabelled HMCF in their drinking water for 13 days before the administration of a bolus dose of [14C]HMCF on day 14. On both dosage regimens, the predominant route of excretion was by way of the faeces. Less than 3% of the administered radioactivity was excreted in the urine and only a negligible amount was found in the expired air. More than 99% of the administered radioactivity was excreted within 96 hr.” As taken from Selim S et al. 1992. Food Chem Toxicol. May; 30(5), 445-51. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=1644386&query_hl=35&itool=pubmed_docsum

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

“The CD50 and the LD50 of 4-MEI (the most toxic component in caramel color III) by i.p. injection in 1-day-old chicks were 174±10 mg/kg b.w. and 210±15 mg/kg b.w., respectively. Orally, the CD50 was 580±30 mg/kg b.w. and the LD50 was 599±50 mg/kg b.w. Doses of 100 mg/kg b.w. i.p. caused tremors, peeping, and spreading of the wings. Doses over 150 mg/kg b.w. i.p. caused opisthotonus, prostration with clonic leg movements, and terminal tonic extensor seizure (Nishie et al., 1970).”

Species	Route	Dose data LD ₅₀ (mg/kg bw)
Rat	Oral	> 2.3 mL approx. or eq. 1,900
Rat	Oral	> 25 mL approx or eq. 17,500
Rat	Oral	> 30 mL approx or eq. 20,400

Mice

Male albino mice (20-25 g) were used to determine the median convulsive dose (CD50) and the median lethal dose (LD50) of a few imidazoles. The results are given in the following table:

Convulsant and lethal effects of imidazoles:

CD50±SE

LD50±SE

	in mg/kg		in mg/kg b.w.	
	b.w i.p	oral	b.w i.p	oral
4-methylimidazole	155 ± 5	360 ± 18	165 ± 3	370 ± 15
1-methylimidazole	380 ± 8.2	1400 ± 79	380 ± 8.2	1400 ± 79
2-methylimidazole	500 ± 12	1300 ± 70	480 ± 18	1400 ± 114
imidazole	560 ± 34	1800 ± 45	610 ± 7.4	1880 ± 45

All the imidazoles tested produced varying degrees of tremor, running, restlessness, diarrhoea, Straub tail, opisthotonus and tonic extensor seizure that ended in death (Nishie *et al.*, 1970).

As taken from WHO Food Additives Series 20.

5.2. Repeated dose toxicity

EFSA (2011) reported the following:

“The available short-term studies on Class I Plain Caramel, Class II Caustic Sulphite Caramel, Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, employing generally high dose levels in drinking water, show some dose-related effects, including reduced body weight gain associated with reduced food and fluid consumption, pigmentation of mesenteric lymph nodes, enlargement of the caecum, reduced urinary output associated with increases in specific gravity of the urine, diarrhoea and increases in caecal and kidney weights, unaccompanied by any histopathological change....The Panel concluded that the effects on body-weight were in part due to the reduced water intake caused by poor palatability of the drinking water rather than toxic effects of the caramel colours per se, and that the other effects seen were secondary both to the reduced fluid intake and the intake of large quantities of osmotically-active caramel material. The Panel considered that these effects are not of toxicological significance in establishing the safety of the caramel colours.”

“Caramel colour I was administered to groups of 20 weanling female Wistar rats at dietary levels of 0, 15, or 30% for 8 weeks followed by a 4-week recovery period. Diarrhoea was observed in the treated animals and food efficiency was decreased, but the growth rate was normal. Haematological indices, in particular leucocyte counts, were normal throughout the study. The relative caecal weights were increased after eight weeks, but returned to normal by the end of the 4-week recovery period. Discolouration of the mesenteric lymph nodes was observed in animals of both treatment groups after eight weeks, but the discolouration diminished during the recovery period. No other gross or microscopic pathological changes were reported (Sinkeldam & van der Heyden, 1976).”

“Five groups of 20 male and 20 female weanling Fischer F-344 rats were given caramel colour II in drinking water at dose levels of 0, 4, 8, 12, or 16 g/kg b.w./day for 90 days. Body weights and food and water intakes were recorded weekly. Haematological examinations, blood biochemical studies, and urinalysis were performed during the seventh week and at termination on all animals. Animals were fasted overnight prior to bleeding from the orbital sinus under anaesthesia; the animals were also fasted during the 24-hour urine collection period. At necropsy, organs were weighed and all animals were examined macroscopically. Histopathological examinations were conducted on all animals in the control and high-dose groups and on animals from the low- and mid-dose groups that died or were sacrificed in extremis[...] Occasional statistically-significant changes were seen in some haematological and clinical chemical parameters, but the mean values were within the normal range for Fischer 344 rats and were not considered to be of toxicological significance.

There was a dose-related reduction in urinary volume and pH, and an increase in urine specific gravity. At necropsy, treatment-related increases were observed in kidney weights and in full and empty caecum weights, but no significant histopathological changes were observed in these or other tissues. Dose-related staining of the gastrointestinal tract and mesenteric lymph nodes was noted, and deposits of yellow pigment were seen histopathologically in the caecal submucosa and mesenteric lymph nodes of the top-dose groups (the only treated animals examined comprehensively). Reactive hyperplasia was not associated with the pigment deposition. The investigators concluded that the reduced body weights, food and fluid intakes, and increased kidney weights were due to water imbalance reflecting poor palatability of the drinking solution rather than toxic effects of caramel colour II per se (MacKenzie, 1985). The above data are insufficient to evaluate caramel colour II for an ADI, but the substance has a low sub-chronic toxicity and no frankly pathological effects were seen in the 90-day study."

"No treatment-related effects were detected during the observation of animals for 14 days after administration of single doses of 12 different caramels manufactured with ammonia or sulphate ammonia catalysts (Foote et al., 1958; Chacharonis, 1960; 1963). Single doses of caramel colour III of up to 10 g/kg b.w. in mice and 15 g/kg b.w. in rabbits did not cause convulsions or others signs of distress (Sharratt, 1971)."

"In a 4- to 6-week study, albino Swiss mice (10 males and 10 females/group) were fed caramel colour III containing 830 ppm 4-methylimidazole at concentrations of 0, 1, 2, 4, 8, or 16% in the diet. No influence of caramel colour III on appearance, behaviour, or food intake was observed. Growth was decreased, especially in the third and fourth weeks, in the groups fed 16% caramel colour III. The faeces of the animals fed the higher dose levels were soft, tarry in appearance, poorly-formed, and sticky or pasty in consistency. In the males fed 16% caramel colour III, an increase of neutrophilic leucocytes and a decrease in lymphocytes were observed. The mean relative weights of the caeca (full and empty) were increased at the 4, 8, and 16% dietary levels. No other remarkable findings were observed on gross examination; histopathological examinations were not conducted. No information is available on the THI content of the sample of caramel colour III used in this study, nor on the dietary levels of pyridoxine (Procter, 1976)."

"Four groups of 10 male and 10 female rats received either 0 or 10% of 2 different samples of caramel colour III in their diet for 90 days. Weight gains showed slight reductions compared with controls, but food consumption was normal in all groups. No abnormalities were noted regarding haematology, urinalysis, gross pathology, or histopathology. No information is available on the THI content of the samples of caramel colour III used in this study (Charcharonis, 1963)."

"Four groups of rats received 0, 4, 8, or 16% caramel colour III in their diet for 3 months. No convulsions or other behavioural abnormality or signs of neurological damage were seen. No macroscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971)."

"Six groups of 15 rats (CFE strain) of each sex were fed diets containing 4, 8, or 16% caramel colour III produced by either an "open" or a "closed" pan process for 13 weeks; a group of 25 rats of each sex served as controls. Body-weight gain was decreased at all dietary levels of both caramel colours. Haemoglobin concentrations were reduced at the highest dietary levels at week 6, while at the lower levels this effect was less clear. After 13 weeks, the males at all dose levels had significantly decreased haemoglobin concentrations, but this effect occurred in females only at the 8 and 16% levels. In some groups there was a less consistent decrease in the total number of red blood cells at 6 and 13 weeks. The total number of leucocytes was significantly decreased and a lymphocytopenia was present at all dose levels at 6 and 10 weeks; at 13 weeks these changes were observed only in males. At necropsy, decreased weights of the thymus and spleen were observed at the 8 and 16% dose levels. The caecal weights were increased at the 8 and 16% dose levels compared with controls. Increased relative liver and kidney weights suggested an effect on

these organs. Changes in the weights of other organs were considered to be related to the differences of body weight between the groups. The volumes of urine excreted during a 6-hour period without water or in the 2-hour period after a water load were lower than the control values. The latter differences were accompanied by higher values for the specific gravity of the urine. The histopathological study did not reveal treatment-related changes. No details of the THI content of the caramel colours used in this study were available (Gaunt et al., 1975)."

"Seven groups of 20 female Wistar rats were fed diets containing 0, 15, or 30% of 3 types of caramel colour, one of which was a sample of caramel colour III, for 8 weeks followed by a 4-week recovery period. The diets containing caramel colour III caused dose-related decreases in body weights and food efficiency, and also caused diarrhoea at the 30% dietary level. Leucocyte counts at 4 weeks were significantly increased at the highest dose level whereas at 8, 10, and 12 weeks no differences were observed between test groups and controls. The relative weights of the caeca of animals receiving caramel colour III, both filled and empty, were increased at 4 and 8 weeks but reverted to normal after the 4-week recovery period. Gross examination at autopsy after 8 weeks revealed a slight, dose-related brown discolouration of the mesenteric lymph nodes in a few animals of each test group. After recovery periods of 2 or 4 weeks the discolouration was less intensive, but still visible. Microscopically, the lymph nodes of the test rats showed accumulation of pigment-laden macrophages, which was not noticeably diminished after withdrawal of the caramel for 2 or 4 weeks. No details of the THI content of the caramel colour III used in this study were available (Sinkeldam & van der Heyden, 1976a)."

"In a 10-week feeding study, groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing caramel colour III at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 15.0%. In the animals receiving caramel colour III at levels of 5% or higher, the faeces became soft within two weeks and the water content of the faeces was higher than the faeces of controls. Body-weight gains were generally reduced in animals fed caramel colour III, particularly during the last 2-4 weeks of the study. In rats fed caramel colour III, there were no changes in haemoglobin levels or erythrocyte counts; a significant reduction in lymphocyte counts and a coincident increase in the number of segmented neutrophils was observed at all dose levels. No macroscopic evidence of abnormal pigmentation of the mesenteric lymph nodes was found. An increase in empty caecal weight was consistently evident in animals of both sexes given caramel colour III at the 5, 10, and 15% levels. Histopathological examination did not reveal changes in the structure of the ileal or caecal mucosa nor in the reticuloendothelial components of the central or peripheral systems. No abnormal pigmentation of the lymph nodes was found. No details of the THI content of the caramel colour used in this study were available (Procter et al., 1976)."

"Groups of 10 male and 10 female Wistar rats were fed diets containing caramel colour III at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 15.0% for 10 weeks. Caramel colour III caused loose stools, particularly at the 5% dietary level and body weights were slightly decreased in both sexes at this level. Leucocyte (lymphocyte) counts were decreased in males and in females fed 15% caramel colour III; at lower dose levels this effect occurred only in females. The relative weight of the caecum (both full and empty) was increased by feeding caramel colour III. Minimal amounts of pigment were observed in mesenteric lymph nodes of several rats fed 1.25% and higher levels of caramel colour III. From this experiment it appeared that caramel colour III was more active than two other types of caramel tested concurrently in regard to growth depression, enlargement of the caecum, and decrease in leucocyte counts. No details of the THI content were available (Sinkeldam & van der Heyden, 1976b)."

"In a 10-week study, weanling Wistar rats were fed caramel colour III in the diet at levels of 0, 0.5, 1.0, 2.0, 4.0, or 16%. There were 15 males and 15 females in each group except for the control group, which had 60 animals of each sex, and the highest dose group, which had 10 animals of each sex. Food intake and growth rates were recorded, and haematological examinations and

histopathological studies were carried out. In particular, the lymph nodes, thymus, spleen, and caecum were examined for distribution of pigment. Another 2 groups of 10 rats were given basal diet or diet containing 16% caramel colour III for 10 weeks followed by a 28-day period during which the basal diet was fed (recovery experiment). Five rats of each sex were killed after 7 days and the remainder after 28 days of the recovery period. Caramel colour III depressed body-weight gain at dietary levels greater than 1%. Total leucocyte counts were decreased in males in the groups receiving 2, 4, and 16% caramel colour III and in females receiving 4 and 16% caramel colour III. However, the lymphocyte/neutrophil ratio was significantly decreased in both sexes at all dose levels. Relative liver weights were increased at dietary levels of 2% and higher levels of caramel colour III, and reduced spleen weights were observed at the highest (16%) dose level. Increased relative kidney weights were observed in both sexes at 2% and higher dietary levels of caramel colour III. Increased caecal weights were observed at the 16% dietary level; microscopically, pigment was observed in the mesenteric lymph nodes of male and female rats at this dose level. During the recovery phase, white cell counts, cell ratios, and total numbers of lymphocytes rapidly returned to normal; recovery was complete in both sexes by 7 days. The caecal weights had returned to normal by 7 days and the relative liver and spleen weights returned to normal during the recovery phase, while kidney weights partially recovered. No details of the THI content of the caramel nor of the pyridoxine content of diet were available (BIBRA, 1977)."

"Test groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing 10 or 15% caramel colour III for 4 weeks; a control group of 20 rats of each sex received basal diet. During the course of the study, the rats fed diets containing caramel colour III had soft dark-coloured faeces, particularly at the highest dietary level. There were no consistent differences in body-weight gain or food consumption and no mortality occurred. Haematological studies were conducted prior to feeding caramel colour III and after 2 and 4 weeks. In males, total white cell and lymphocyte counts were unaffected by treatment at either sampling interval or dose level. In females, total white cell and lymphocyte numbers were significantly depressed after 4 weeks but not at 2 weeks at both dose levels. However, significant differences in differential white cell counts were observed in both sexes. In males, the differential lymphocytes (%) were significantly depressed at both dose levels and treatment intervals, and there was a concomitant increase in segmented neutrophils. In females, the differential counts of lymphocytes and segmented neutrophils were similarly affected after 4 weeks. At necropsy, increased caecal weights were observed in both sexes and a statistically significant increase in relative weights of the thymus in male rats fed both doses of caramel colour III was noted. Histopathological studies were not conducted. Details of the THI content of the caramel sample used were not available (Procter, 1977)."

"In a 13-week toxicity study, caramel colour III was administered in drinking water at concentrations of 0, 4, 6, 8, or 10% to groups of 10 male and 10 female weanling Wistar rats. The caramel colour III sample used was analysed and found to contain 78 mg/kg THI on an 'as is' basis, 105 mg/kg THI on a solids basis. The diet fed to the rats contained approximately 13 mg/kg pyridoxine. The general condition and behaviour of the animals was checked at frequent intervals, individual body weights were measured weekly, food consumption was measured (on a cage basis) over weekly periods during the whole experimental period, and fluid consumption was measured daily (on a cage basis). There was a dose-related decrease in fluid consumption, decreased urinary output of more concentrated urine, decreased food consumption, and decreased body-weight gain in both sexes. These changes were related to the palatability of the drinking fluid. No outstanding differences were observed in red blood cell analyses between test groups and controls. Total lymphocyte counts were relatively low in all test groups of both sexes. The differences from control values were statistically significant in all male treatment groups after 29/30 days, in the female 8% group after 29/30 days, and in the male 4 and 8% groups after 57/58 days. However, these differences did not show a clear dose-dependent relationship and at 13 weeks there were no significant differences among any of the treatment groups compared to controls. At necropsy, the relative weights of the kidneys and caecum were increased in several groups receiving caramel

colour III. The increase in kidney weight was dose-dependent, but no treatment-related histopathological changes were seen in any of the organs examined and the enlargement of the kidneys was attributed to the decreased fluid consumption by the treated rats. No effects on the relative weights of spleen or thymus were observed and these tissues were histologically normal (Sinkeldam et al., 1980b)."

"A 13-week toxicity study was conducted with caramel colour III given in the drinking water at concentrations of 0, 5, 10, 15, or 20% to groups of 10 male and 10 female weanling Wistar rats. The caramel colour III sample used in this study contained 0-3 mg/kg THI on an 'as is' basis. The diet fed to the rats contained 13.5 mg/kg pyridoxine. The protocol used was similar to that described in the previous study except that blood samples were collected at days 30/31, 57/58, and 80/81, and urinalysis was performed at day 85. Although the relative weights of the caecum, liver, brain, kidneys, and testes were increased in several test groups, there were no pathological changes in any of these organs. Treatment-related microscopic changes consisting of increased numbers of macrophages containing a yellow-brown PAS-positive pigment were found in the mesenteric lymph nodes. These were the only treatment-related changes noted (Sinkeldam et al., 1980a)."

"Groups of 10 male and 10 female weanling F344 rats were given caramel colour III in the drinking water at concentrations of 0, 0.5, 1.0, 2.0, 4.0, or 8.0% for 4 weeks. The sample of caramel colour III used was found to contain 70 mg/kg THI on an 'as is' basis and the NIH 07 Open Formula Mouse and Rat diet used in this study contained 17 mg/kg pyridoxine. No differences in body-weight gain were noted for any of the test groups, although food consumption of the males was significantly lower than controls throughout the study. Transient decreases in lymphocyte counts were noted among the treatment groups at the midpoint of the study, but no significant differences between control and treated groups were noted at 1 month. Other haematological and clinical chemistry parameters were normal for all groups. At necropsy no differences in organ weights were noted between treated and control groups and there were no gross or microscopic pathological changes related to treatment (Heidt and Rao, 1981)."

"Groups of 10 male and 10 female F344 rats were given caramel colour III in the drinking water at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 20.0% for 13 weeks in order to select doses for long-term carcinogenicity/chronic toxicity studies. Rats were given 20 ml/day of these solutions and basal diet (CRF-1, Charles River Japan, Inc.) was available ad libitum. The caramel colour III used contained 78 mg/kg THI on an 'as is' basis and the diet contained 11-12 mg/kg pyridoxine. During the experimental period, all animals were observed daily and clinical signs were recorded. Body weights were measured every other week and haematological examinations were performed every 4 weeks in the control and 20% groups. At the end of the study, all survivors were sacrificed for gross and microscopic examination. Weight gains were less in all experimental groups than in the control group from the first experimental week, and in week 13 the weight gains of the 1.25, 2.5, 5.0, 10.0, and 20.0% groups were 89, 94, 84, 76, and 76%, respectively, of that of controls for males and 96, 98, 80, 84, and 92%, respectively, for females. Except in the male 2.5% group and the female 1.25, 2.5, and 20.0% groups, the differences in weight gain from the controls were significant. No haematological changes were observed either during or at the end of the experimental period. At necropsy, no pronounced macroscopic changes were observed in any animals, although a few rats in the experimental groups were very emaciated. No histological changes related to caramel colour III administration were found in any experimental groups. From these results, the authors concluded that 1 and 4% caramel colour III in drinking water were the appropriate dose levels for a carcinogenicity study (Maekawa et al., 1983)."

"In a 90-day toxicity study, 2 samples of caramel colour III were used, one containing approximately 15 mg/kg THI on a solids basis (batch A) and the other containing 295 mg/kg THI on a solids basis (batch B). Groups of 20 male and 20 female weanling F344 rats were given caramel colour III in

drinking water at dose levels of 0, 10, 15, or 20 g/kg b.w. of batch A and 20 g/kg b.w. of batch B. The NIH 07 Open Formula Mouse and Rat diet used in this study contained more than 10 mg/kg pyridoxine. During the study, body weights and food intake were recorded weekly. Fluid consumption was recorded 3 times per week and concentrations of the test material were adjusted on the basis of body weight and fluid intake to give the required dose. Haematological analyses were performed at 2 and 6 weeks and at termination, and clinical chemistry analyses were carried out at 6 weeks and termination. At necropsy, all animals were examined macroscopically and selected organ weights were determined. Histopathological examinations were conducted on the 20 g/kg test groups and on the control animals. The only treatment-related microscopic changes noted were minimal to moderate accumulation of pigment in the tissues of the intestinal tract and mesenteric lymph nodes without pathologic alteration. Although statistically-significant changes were identified in some clinical and anatomical parameters, the authors did not consider them to be toxicologically important. On this basis, the no-adverse-effect level for ammonia caramel III was considered to be 20 g/kg b.w. (MacKenzie, 1985)."

"A paired-feeding study was conducted to determine whether poor palatability is the mechanism underlying the decreased body-weight gains frequently noted in toxicity studies of caramel colour III. A group of 10 male Wistar rats were fed 12% caramel colour III in the drinking water, and similar groups were permitted a limited intake of food or water equivalent to that consumed by the caramel colour III group. Body-weight gain and food and fluid intake were decreased in the group fed caramel colour III. A similar decrease in body-weight gain was noted in the groups of rats restricted to the equivalent intake of either food or water. The author concluded that the growth depression observed in rats when caramel colour III is fed in the drinking water is the result of decreased fluid and food intake. Poor palatability of drinking fluid containing caramel colour III was the probable cause of these changes (Sinkeldam, 1979)."

"In a number of animal studies with caramel colour III, a decrease in the total number of leucocytes associated with a decrease in the number of lymphocytes was noted. A pilot study in humans was carried out in which 1.5 g of caramel colour III (prepared by a closed-pan process) was ingested daily by 9 volunteers for 21 days. Total circulating leucocytes, lymphocytes, and erythrocytes, together with haemoglobin concentrations, were measured prior to and during the treatment. No changes were found that could be attributed to treatment with caramel colour III. In this experiment 6 of the subjects showed no differences from normal in stool frequency or condition. Three volunteers occasionally had soft stools; no control groups were used (BIBRA, 1976)."

"Groups of 10 B6C3F1 mice of each sex were given concentrations of 0, 10, 15, 20, or 30% caramel colour IV in drinking water for 4 weeks. The intake of caramel colour IV expressed in g/kg b.w./day was more than twice the percentage concentration in the drinking water. At 28 days the body weights of male animals at the 30%-dose level were significantly decreased compared to the controls, and transient depressions of body weights were noted at the highest-dose level in females. No significant depressions in body-weight gain were noted at the lower-dose levels in either male or female mice. Fluid consumption was depressed throughout the study among all treatment groups. However, these changes were not consistent with time or dosage. There were no statistically significant differences in food consumption between treated animals and controls. At necropsy, the only treatment-related effect reported was enlargement of the caecum (Tierney, 1979)."

"Groups of 5 male and 5 female rats were given 1 ml/kg b.w. of concentrated caramel colour for 21 days. Some diarrhoea was induced in all animals, but no other abnormalities were noted. Gross pathology and histopathology revealed no significant changes due to administration of the test compound (Foote et al., 1958)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

“Four groups of 10 male and 10 female Sprague-Dawley rats received 0, 0.1, 1.0 or 10% caramel colour IV in their diet for 12 weeks. No adverse effects were noted on growth, food consumption, urinalysis, haematology, gross pathology, or histopathology that were related to administration of the caramel colour (Prier, 1960).”

“Groups of 10 male and 10 female rats received 0, 5, or 10 g/kg caramel colour IV in their diet for 3 months. Weight gain was normal in all groups. Food consumption, haematology, and urinalysis were comparable in all groups. Gross pathology and histopathology showed no test-related adverse findings (Chacharonis, 1960).”

“Four groups of 10 male and 10 female Sprague-Dawley rats received 0, 5, 10, or 20% caramel colour IV (low colour intensity) or caramel colour IV (high colour intensity) in their diet for 90 days. In addition, a paired-feeding study involving 5 male rats in 2 groups was run for 23 days (one sample was at the 20% level), and there were no differences in the rate of growth. The only effects attributable to treatment were a mild depression in growth of male rats at the 10 and 20% levels due to unpalatability of the test diet. No other adverse findings were noted in growth, behaviour, mortality, haematology, urinalysis, gross pathology, organ weights, or histopathology (Kay and Calandra, 1962a; 1962b).”

“Four groups of rats received 0, 4, 8, or 16% caramel colour IV in their diet for 3 months. No convulsions or other behavioural abnormalities or signs of neurological damage were seen. No macroscopic or microscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971).”

“Five groups of 30 male and 30 female weanling F344 rats were given caramel colour IV in drinking water at concentrations which provided intakes of 0, 15, 20, 25, or 30 g/kg b.w./day for 13 weeks. After 43 days, 10 animals of each sex from each group were randomly selected for collection of data on urinalysis, haematology, and clinical chemistry, followed by sacrifice and necropsy; similar examinations were performed on survivors at termination. At interim and terminal sacrifice, a detailed necropsy was performed on all animals and a complete histopathological examination was performed on controls and animals in the top-dose group. Caramel colour IV at the dose levels tested did not significantly affect haematological values at interim or terminal examinations. All treatment groups of both sexes had significantly-reduced blood urea nitrogen and alkaline phosphatase levels at both 45 and 90 days. Total serum protein values of both sexes in the treatment groups were lower than controls at 90, but not at 45, days. These effects may be due to reduced food intake and growth retardation. Treated rats had reduced urine volume and increased urine specific gravity, protein, ketones, and acidity, which were associated with decreased water consumption. Increased kidney weights were observed at necropsy. Treatment-related decreases in thymus and spleen weights and increased caecal size, with staining of the mucosa of the caecum and colon, were noted. Accumulation of yellowish-tan pigment occurred in macrophages of the mesenteric lymph nodes. No treatment-related histopathological changes were observed in any organs at the highest-dose level (Heidt & Rao, 1980).”

“Four groups of 3 male and 3 female adult beagle dogs received 0, 6, 12.5, or 25% caramel colour IV in their diet 5 days per week for 90 days. No significant adverse effects on growth, behaviour, food consumption, mortality, liver function, kidney function, haematology, urinalysis, gross pathology, or histopathology were noted (Kay and Calandra, 1962c).”

“Tolerance studies of caramel colour IV (low colour intensity) and caramel colour IV (high colour intensity) were conducted in human volunteers. The subjects, 10 men and 10 women, ingested caramel colour once daily in simulated soft drinks over 3 test periods of 21 days each separated by

7-day rest intervals. The test doses were 6 g/day during the first test period, 12 g/day during the second period, and 18 g/day during the third test period. Haematological, clinical chemical, and routine urinary parameters were studied at the beginning of each ingestion period, after 10 days of ingestion, and at the end of each ingestion period. Most individual values for haemoglobin, haematocrit, RBC, corrected sedimentation rate, WBC, and differentials (neutrophils, basophils, eosinophils, monocytes, and lymphocytes) were found to be within normal limits. There were a few instances of values outside the normal range, indicating mild neutropenia and mild lymphocytosis, but these were not consistent and were unrelated to the ingestion of caramel colour IV. On the other hand, caramel ingestion was associated with an increased frequency of bowel movements and softening or increased liquidity of faeces (Marier et al., 1977a; 1977b)."

Level causing no toxicological effect for caramel color IV:

Mouse: 10 g/kg bw / day in drinking water Rat: 10 g/kg bw / day in drinking water Man: No adverse effects other than laxation at levels up to 18 g/day

"Groups of 5 rats received either 10 or 20% caramel colour IV solution (equivalent to about 10 or 20 g/kg b.w./day) as their sole source of fluid for 127 days. Only dark faeces and very mild diarrhoea were noted. No adverse effects were noted regarding general health, body weight, food and fluid consumption, haematology, gross pathology, or histopathology (Haldi & Wynn, 1951)."

"Six groups of 5 male and 5 female weanling rats received 0 or 10% caramel colour IV solution as their sole fluid source for 100, 200, or 300 days. No adverse effects were noted regarding growth, food and fluid intake, haematology, gross pathology, or histopathology (Haldi & Wynn, 1951)."

"Groups of 16 male and 16 female rats received either 0 or 10% caramel colour IV solution for 100 days and groups of 5 rats received 20% caramel solution for 100 days. At the lower test level, there were no observable abnormalities as regards growth, food consumption, haematology, gross pathology, or histopathology. Only growth and haematology were examined at the higher test level, and the results for both parameters were found to be normal (Haldi & Wynn, 1951)."

"Three groups of 20 male and female rats received either 0 or 11-14 g/kg b.w. of caramel colour IV solutions for 100 days. Growth and food intake did not differ significantly between test and control animals. Gross pathology and histopathology showed no abnormal findings related to administration of the test compound (Haldi & Wynn, 1958)."

"Three groups of 20 female Wistar rats received stock diet to which 0, 10, or 20% caramel colour IV containing 202 ppm 4-methylimidazole was added. During the second week of the experiment these levels of caramel colour IV were increased to 15 and 25% and in week 7, the levels were increased to 25 and 30% caramel colour IV. The diets were administered for 16 weeks followed by a 4-week recovery period. Gross examination at autopsy after 16 weeks of feeding caramel color IV revealed a dose-related, brown-greenish discolouration of the mesenteric lymph nodes in all test animals of the highest-dose level. After the recovery period of 4 weeks the colour change was less, but still visible. Microscopically, the lymph nodes of the test rats showed pigment accumulation which was not noticeably diminished after withdrawal of the caramel for 4 weeks. These results failed to confirm the decreased leucocyte counts that were observed in females fed 5 to 15% caramel colour IV in a 1-year feeding study (Sinkeldam & van der Heyden, 1975)."

"Six samples of caramel colour were tested in a long-term toxicity study, 3 of which were caramel colour IV (low colour intensity) and 3 of which were caramel colour IV (high colour intensity). Each group consisted of 40 male or 40 female weanling Wistar rats, except the control group which had double this number of animals. Animals were selected from the first litter of parents fed diets containing the various caramel colours from weaning age (see reproduction studies, Til & Spanjers, 1973). The dose levels tested were 5, 10, or 15% caramel colour IV (low colour intensity) and 2, 4,

or 6% caramel colour IV (high colour intensity). Two additional samples of caramel colour IV (low colour intensity) were tested at 10% and 2 additional samples of caramel colour IV (high colour intensity) were tested at 4% in the stock diet. Observations were made of general appearance, behaviour, mortality, growth, food intake, haematological factors, and clinical chemistry of blood and urine. After about 14 months mortality attributed to intercurrent disease was observed in both control and treated groups. Approximately three-quarters of the animals died or were killed before the experiment was terminated at week 104. Organs of animals that died or were killed were weighed and extensive histopathological examinations were carried out. However, one-third of the animals could not be examined histopathologically due to autolysis. At week 104 organs were weighed and extensive histopathological examinations were carried out on all surviving rats of all groups. No clinical changes were observed in this study except for slightly decreased haemoglobin and haematocrit values at weeks 78 and 98 in males fed caramel colour IV (high colour intensity). Leucocyte counts were decreased in females fed 10 and 15% of one sample of caramel colour IV (low colour intensity) at weeks 13 and 52, but these changes were not consistent and decreases in lymphocyte counts were not reported. At autopsy, an increased incidence of greenish discoloured mesenteric lymph nodes was observed in most groups fed high levels of caramel colour. Microscopically, an increased pigment-phagocytosis in the mesenteric lymph nodes in all test groups (except the group fed 2% caramel colour IV (high colour intensity)) was observed. No evidence of any other adverse structural or cellular alteration was found. Gross and microscopic examination of the other organs did not reveal any pathological changes attributable to the ingestion of caramel colour IV. An increase in the incidence of neoplastic lesions in the different groups was not found (Sinkeldam et al., 1976)."

"The toxicity of caramel colour II was evaluated in a 13-wk study in Fischer-344 (F344) rats. The test material was mixed with demineralised water and the solutions were given to the animals ad lib in the drinking fluid. The concentrations of caramel colour in the drinking fluid were adjusted periodically to achieve the desired caramel colour intake/kg body weight/day. Groups of 20 rats/sex were given caramel colour II at levels of 0, 4, 8, 12 or 16 g/kg bw/day for at least 13 weeks. There were no deaths in any of the groups. All rats fed caramel colour had soft faeces. All treated groups also had lower fluid consumption that was attributed to poor palatability due to the high concentrations of caramel colour that were fed. A number of observed changes (reduced food consumption in all treatment groups except males given 4 g/kg bw; significantly lower body weights for males given 12 g/kg bw or more and for females given 8 g/kg bw or more; lower urine volume and higher specific gravity) were attributed to the reduced water intake and not considered to be toxicologically significant. There were no consistent treatment-related alterations in haematology or blood chemistry variables, and random changes noted were not associated with macroscopic or microscopic pathological alterations. There were no toxicologically important pathological findings. Based on this study, caramel colour II was not toxic in F344 rats treated for 13 weeks. The highest dose tested in this study (16 g/kg bw/day) was considered to be the no-observed-adverse-effect level (MacKenzie et al 1992b)."

"Short-term oral toxicity studies were conducted on low-2-acetyl-4(5)-tetrahydroxybutylimidazole (THI) and high-THI samples of caramel colour III (13 wk) and on a sample of THI (28 days). In both studies, the test materials were mixed with demineralised water and the solutions were given to the animals ad lib in the drinking fluid. In the 13-wk subchronic toxicity study of caramel colour III, groups of 20 rats/sex were given concentrations of caramel colour equivalent to intakes of 0, 10, 15 or 20 g low-THI caramel colour/kg body weight/day or 20 g/kg bw/day of a high-THI caramel colour. In the 4-week toxicity study with THI, groups of 20 rats/sex were given 0, 8 or 64 ppm THI (equivalent to approx. 0, 0.9 or 7.2 mg/kg bw/day) and 10 rats/sex were given 1, 2, 4, 16 or 32 ppm THI (equivalent to approx. 0.1, 0.2, 0.5, 1.9 or 3.7 mg/kg bw/day) for four weeks followed by a 2-wk recovery phase for 10 rats/sex in the 0, 8 and 64 ppm groups. Rats given caramel colour III had soft faeces; there were no other treatment-related clinical observations and no treatment-related deaths occurred. All treated groups given caramel colour III had lower food and fluid consumption

than controls. Males given 15 or 20 g low-THI caramel colour/kg bw or 20 g high-THI caramel colour/kg bw/day and females given 20 g/kg bw/day or either type had lower body weights than controls. In the 4-wk toxicity study with THI, there were no treatment related ante-mortem observations, and no effects on body weights or food consumption. Fluid consumption by males and females treated with 64 ppm THI was lower than that of controls. Haematological changes noted in both studies included lower total white blood cell and lymphocyte (absolute and percentage) counts, and higher absolute and percentage neutrophil counts; these changes returned to normal by study termination in the caramel colour study, and by the third day post-treatment in the THI study. There were no consistent treatment-related alterations in blood chemistry or urinalysis variables in either study, and any changes noted were not associated with macroscopic or microscopic pathological alterations. There were no toxicologically important pathological findings for either study. Based on these studies the no-observed-adverse-effect level (NOAEL) for low-THI caramel colour III was considered to be 20 g/kg bw day. The acceptable daily intake for this caramel colour established by JEFCA is 200 mg/kg body weight. The NOAEL for THI was found to be 0.38 mg/kg bw/day for male rats and 0.12 mg/kg bw/day for females (MacKenzie et al 1992c)."

"Groups of 15 rats of each sex were given diet containing 4, 8 or 16% caramel produced by either an "open" or a "closed" pan ammonia process. A group of 25 rats of each sex served as controls. There was a reduced rate of body weight gain at all dietary levels although in those rats given 4% caramel there was evidence that this could be attributed to dilution of the diet. In the case of the open pan material this also applied to the rats given 8% in the diet. There were reduced haemoglobin concentrations at the highest dietary level although similar findings at the lower levels were inconsistent and of doubtful significance. Lymphocytopenia was present at all dietary levels in association with lower weights of thymus and of spleen at the two higher doses. The caecum weights were increased compared with the controls but this was not thought to represent a toxic effect. Increased relative liver and kidney weights suggested an effect on these organs. Changes in the weights of other organs were considered to be related to the differences of body weight between the groups. A no-untoward-effect level could not be established for either of the caramels (Gaunt et al 1977a)."

"Groups of 48 rats of each sex were given diets containing 0 (control), 1, 3 or 6% caramel produced by a straight ammonia-catalysed "half open-half closed pan" process for two years. There was no indication of carcinogenic effect at any level of treatment. Findings related to treatment were a reduced white cell count and an associated lymphocytopenia and a dose-related reduction in absolute and relative spleen weights. There was also a dose-related reduction in body weights, although the significance of this effect is in doubt. In males, there was a reduction also in the cumulative food intake, but when food conversion ratios were calculated taking into account a dilution effect of the caramel, the values for treated animals were similar to those for controls. Changes in other organ weights were considered to be related to the difference in body weight between groups. A no-untoward-effect level could not be established for the caramel used in this particular study, but there was no evidence of a carcinogenic effect (Evans et al 1977)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

"Because Caramel Colour III is used as a food additive in many products and consumption of such products may result in a Caramel Colour III intake higher than the current Acceptable Daily Intake (ADI) of 200 mg/kg body weight/day. Effects in (marginally vitamin B6 deficient) humans cannot be excluded. Therefore, a double-blind intervention study with human volunteers was performed. In blood samples taken at several time points before, during and after a 7 day test period during which human volunteers, consumed either a Caramel Colour III containing dessert or a control dessert twice a day. Routine haematological evaluation and measurements of parameters of the vitamin B6 status were conducted. In addition, immunoglobulin levels were measured, lymphocyte subsets

were determined by flow cytometry and the proliferative response of isolated mononuclear blood cells were determined well understood upon mitogenic stimulation. The results demonstrated that 200 mg Caramel Colour III/kg body weight/day induced no effects on the measured parameters in human subjects considered potentially sensitive to Caramel Colour III-induced changes because of a biochemically marginally vitamin B6 deficiency.” As taken from Houben GF and Penninks AH. 1994. Toxicology. Aug 12; 91(3):289-302. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8079366&query_hl=30&itool=pubmed_docsum

Subchronic toxicity – RAT / 90 DAY

Title		US FDA CFSAN PAFA Study					
Data Source		US FDA CFSAN PAFA					
Data inventory		US FDA CFSAN PAFA					
Study quality		Study completeness(US FDA CFSAN PAFA) : C					
Reference							
Study call							
Study Comments							
Study Background		· Document Type (Doc.Num) : US FDA CFSAN PAFA Record (198) · Study Start/End Date : - · Study Report Date : · Study Duration : 90 day					
Type	Value	Owner	Critical Sites	Critical Effects	Details		
LEL	4000 mg/kg bw/day	Study					
Species	Strain	Sex	Route Administration	Test Duration	Dose Levels / Range	Comments	Details
RAT			Oral	90 day	4000.0 mg/kg bw/day		

US FDA CFSAN

POD Method	POD Value	POD Owner
NOAEL	Not Calculated	US FDA CFSAN

Lowest observed effect level

Owner	Type	Value	Sites	Effects
US FDA CFSAN	LOAEL	Not established	NO EFFECTS	NO EFFECTS

No-observed effect

US FDA CFSAN: NOAEL: 500.0 mg/kg bw/day

Critical study: Target Organ Toxicity > Chronic Toxicity (Rat, Oral exposure) for 730 day

As taken from COSMOS Database, available at <https://ng.cosmosdb.eu>

5.3. Reproduction toxicity

EFSA (2011) noted that no data were available on the reproductive and developmental toxicity of Class I Plain Caramel and Class II Caustic Sulphite Caramel. However, it had no concerns about their reproductive and/or developmental toxicity because of the data available on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel and the toxicological similarities between all four classes of caramel colours. It considered there to be no indications that Class III or IV caramels

induce reproductive and/or developmental toxicity in mice, rats, or rabbits following gavage administration at levels of up to 1600 mg/kg bw/day. In a reproductive toxicity study with Class IV caramel in rats, pups at the top dose (25% of the mother's diet, equivalent to around 28 g/kg bw/day) showed a higher incidence of alopecia compared with pups in the control group, and a generalized poor condition during the last 7 days of suckling. The number of implantation sites, litter size and number of live pups at days 0, 4, and 21 of lactation in the 20% dose group were significantly lower than control values, but the Panel noted that there was no dose-related trend, as no such effect was seen in the 25% dose group. The Panel considered the no-observed-adverse-effect level (NOAEL) in this study to be 25-30 g/kg bw/day in female rats (EFSA, 2011).

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour III was administered by gavage to groups of 22 or 23 pregnant CD1 mice at the above doses beginning on day 6 and continuing through day 15 of gestation. On day 17, all dams were subjected to Caesarian section under anaesthesia and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The urogenital tract of each dam was examined for anatomical abnormalities and all fetuses were examined grossly for external abnormalities. One-third of the fetuses from each litter were examined for visceral abnormalities using the Wilson technique, and the remaining two-thirds were examined for skeletal defects after clearing and staining with Alizarin Red. Caramel colour III had no clearly discernible effects on nidation nor on maternal or foetal survival. The number of abnormalities of either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974)."

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour III was administered by gavage to groups of 21-24 pregnant Wistar rats at the above doses beginning on day 6 and continuing daily through day 15 of gestation. On day 20, all dams were subjected to Caesarian section under anaesthesia and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of live pups were also recorded. All fetuses were examined grossly for external abnormalities. One-third of the fetuses from each litter were examined for visceral abnormalities using the Wilson technique, and the remaining two-thirds were examined for skeletal defects after clearing and staining with Alizarin Red. Caramel colour III had no clearly discernible effect on nidation nor on maternal or foetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974)."

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour III was administered by gavage to groups of 11 or 12 pregnant Dutch-belted female rabbits at the above doses beginning on day 6 and continuing daily through day 18 of pregnancy. On day 29, all does were subjected to Caesarian section under anaesthesia and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. The live fetuses from each litter were then placed in an incubator for 24 hours for evaluation of neonatal survival. Surviving pups were sacrificed and all pups examined for visceral abnormalities by dissection. All fetuses were then cleared and stained with Alizarin Red and examined for skeletal defects. Caramel colour III treatment had no effect on nidation nor on maternal or foetal survival. The number of abnormalities in either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974)."

"Fifteen male and female Wistar rats were given 0 or 10% caramel colour IV solution as their sole fluid source until day 100 and were then mated. Animals of the F1-generation (25 males and 25 females) were weaned and again given 0 or 10% caramel solution until day 100. There were no adverse effects with regard to the number of litters born and the number of pups/litter. No influence on haematology, growth, food consumption, gross pathology, or histopathology of the F1-generation at 100 days of age was observed (Haldi & Wynn, 1951)."

"Twelve groups of 10 male and 20 female weanling Wistar rats were allocated to the above dietary groups and, at week 12, the rats were mated in sub-groups of 5 males and 10 females. After a 3-week mating period, the females were caged individually. At weaning age of the F1 generation, 40 males and 40 females were selected from as many different litters as possible within each caramel-colour group and continued on the same diet as the parent generation. Ten males and 10 females of each group were sacrificed after one year (see Sinkeldam et al., 1975, under short-term toxicity studies); the remaining 30 males and 30 females of each group were fed the test diets for 2 years (see Sinkeldam et al., 1976, under long-term toxicity studies). After weaning the F1 litters, the dams were killed and the implantation sites were counted. No consistent, dose-related effects on growth of the F0 animals were noted. No adverse effects were seen on female fertility, litter size, average weight and growth of the pups, or number of implantation sites or sex ratio of the young. In one group (10% SS caramel colour IV + 600 ppm 4-methylimidazole) there was a slight increase in mortality at birth. No teratogenic effects were found (Til & Spanjers, 1973)."

"range-finding reproduction study was conducted on F344 rats. Five groups of 12 male and 12 female mature rats were given caramel colour IV at concentrations of 0, 10, 15, 20, or 25% in drinking water for 21 days prior to mating and throughout mating, gestation, and lactation. Animals of the F0 generation were killed when animals from the F1 generation were weaned. At weaning, 2 pups/sex/litter were randomly selected from litters that had a minimum of 2 males and 2 females at day 21 and treatment of these animals was continued for 13 weeks post-weaning. Haematology and clinical chemistry were performed on days 45/46 and at termination. Complete gross post-mortem examinations of both F0 and F1 animals were performed at sacrifice. Selected organs (caecum, spleen, thymus, liver, kidneys, heart, adrenals, and gonads from animals of the F0 generation were weighed at autopsy. Although the dose levels of caramel colour IV in this study were very high (ranging from 8-28 g/kg b.w.), no specific toxic effects were observed. All F0-generation animals survived the duration of the study, but 3 F1-generation animals were killed accidentally while sampling for clinical chemistry studies at day 45. In the 20- and 25%-dose groups of both generations there was a higher incidence of soft stools than in the controls, and all animals of the F0 generation showed slight to statistically significant, dose-related decreases in body weight. Dose-related depression of body-weight gain was also noted in animals of the F1 generation. Mating, pregnancy, and fertility rates were comparable for all groups, but the number of implantation sites and of pups alive at days 0, 4, and 21 of lactation in the 20%-dose groups was significantly lower than control values. Litter size was decreased at the 15-, 20- and 25%-dose levels. Pups in the 25%-dose group showed a markedly higher incidence of alopecia and arched spine than controls, and a generalized poor condition during the last 7 days of suckling. No significant haematological changes were observed at 45 or 90 days post-weaning, except that prothrombin time of the F1 females in the 15 and 25% groups were significantly greater than controls at day 45. Blood urea nitrogen values were lower than controls at 45 and 90 days but other clinical chemistry values were normal. At necropsy, dose-related increases in absolute and relative weights of the liver, kidneys, and caecum (full and empty) were observed from animals in the 15%- and higher-dose groups. The only gross treatment-related morphological changes reported were brown/black or green colouration of the contents and mucous membrane of the lower gut and mesenteric lymph nodes (Tierney, 1980)."

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour IV was administered by gavage to groups of 19-22 pregnant albino CD1 mice at the doses above, beginning on day 6 and continuing through day 15 of gestation. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. On day 17 all dams were subjected to Caesarean section under surgical anaesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external congenital abnormalities, one-third of the fetuses from each litter underwent visceral examination (Wilson technique), and the remaining two-thirds were cleared, stained with Alizarin Red, and examined for skeletal defects. The number of

abnormalities seen in either soft or skeletal tissues did not differ from the number occurring spontaneously in sham-treated controls (Morgareidge, 1974)."

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour IV was administered by gavage to groups of 21-24 pregnant Wistar rats at the dose levels above, beginning on day 6 and continuing daily through day 15 of gestation. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. On day 20 dams were subjected to Caesarean section under surgical anaesthesia and the number of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined for gross, visceral, and skeletal abnormalities as in the mouse experiment. No clearly discernible effects on nidation or on maternal or foetal survival were observed. The number of abnormalities seen in the test groups did not differ from the number occurring spontaneously in sham-treated controls (Morgareidge, 1974)."

"The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. Caramel colour IV was administered by gavage to groups of 11-12 pregnant Dutch-belted female rabbits at the doses above beginning on day 6 and continuing daily through day 18 of pregnancy. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. The does were subjected to Caesarian section under surgical anaesthesia on day 29 and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. Body weights of the live pups were also recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. The live fetuses from each litter were then placed in an incubator for 24 hours for evaluation of neonatal survival. Surviving pups were sacrificed and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared with potassium hydroxide, stained with Alizarin Red, and examined for skeletal defects. No clearly discernible effects on nidation or on maternal or foetal survival were observed. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in sham-treated controls (Morgareidge, 1974)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

Species	Test conditions	Effects	Reference
Rats, groups of 21-24 females	Caramel class III or IV were administered via stomach tube (0, 16, 74.3, 345 or 1600 mg/kg bw/day) on days 6-15 of gestation. On day 20 all dams were subject to caesarean section and the number of implantation and resorption sites recorded. Offspring were examined for gross external malformations, visceral and skeletal abnormalities. Maternal survival, foetal viability, and foetal body weight were also monitored.	None	Morgareidge, 1974
Rats, groups of 15/sex (F ₀), and groups of 25/sex (F ₁).	Animals (F ₀ generation) were given a 10% caramel class IV solution as their sole fluid source for 100 days and then mated. The offspring (F ₁ generation) after weaning also received the same treatment as their parents for 100 days. Numbers of litters and pups/litter, haematology, growth and food	None	Haldi & Wynn, 1951

	consumption were monitored. Gross and microscopic examination [unspecified tissues] of the offspring was carried out at 100 days.		
Rats, 12 groups of 10 male & 20 female (F ₀) and a total of 40/sex (F ₁).	Animals were fed six different samples of caramel class IV (single or double strength products), each containing between 200 and 850 ppm of 4-methylimidazole, in the diet at up to 15%, and mated at week 12. At weaning age, the F ₁ generation were continued on the same diet as their parents for up to two years. Effects on growth of the F ₀ & F ₁ generations, female fertility, litter size, body weight and sex ratio of pups, number of implantation sites and teratogenic effects were reported.	None	Til & Spanjers 1973
Rats, groups 12/sex (F ₀), and 2/sex (F ₁) per dose level	Caramel class IV was administered in the drinking water at concentrations of 0, 10, 15, 20 or 25% (8-28 g/kg bw/day) for 21 days prior to mating through to lactation. At weaning the F ₁ generation (2/sex/litter) continued to receive the same dose as their parents for 13 weeks. Monitored for mating, pregnancy and fertility rates, for body weight gain, implantation sites, pup survival, organ weights, overt condition and behaviour, clinical chemistry. Complete gross examination of both generations was undertaken.	No effects at up to 10%. At 15%, rats showed maternal toxicity (increased liver, kidney and caecum weights). At 20%, growth was affected in both generations and implantation site numbers and pup survival were reduced. At 25%, pups showed hair loss, arched spine and poor condition. The lower gut and lymph nodes were "discoloured"	Tierney, 1980
Rabbits, groups of 11-12 females	Caramel class III or IV was given via stomach tube at 0, 16, 74.3, 345 or 1600 mg/kg bw/day on days 6-18 of gestation. On day 29 all dams were subject to caesarean section and the number of corpora lutea, implantation and resorption sites were recorded. Offspring were examined for gross external malformations, visceral and skeletal abnormalities. Maternal survival, foetal viability, and foetal body weight was also monitored.	None	Morgareidge, 1974
Mice, groups of 19-23 females	Caramel class III or IV was given via stomach tube (0, 16, 74.3, 345 or 1600 mg/kg bw/day) on days 6-15 of gestation. On day 17 all dams were subject to caesarean section and the number of implantation and resorption sites recorded. Offspring were examined for gross external malformations, visceral and skeletal abnormalities. Maternal and fetal	None	Morgareidge, 1974

	survival, and fetal body weight were also noted.		

5.4. Mutagenicity

EFSA (2011) noted that “Caramel colours have been extensively tested for genotoxic potential in a variety of assays in vitro and in vivo. The results in in vitro systems were generally negative, with a few marginally positive findings, and no positive findings have been reported in in vivo assays. Overall the Panel concluded that there were no concerns regarding the genotoxic potential of caramel colours.”

“Two samples of caramel colour I with different colour intensities were subjected to the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538.

Caramel colour I was neither mutagenic nor cytotoxic, either with or without activation by rat liver S-9 fraction, at concentrations up to 20 µl per plate (Richold & Jones, 1980a,b).”

“Caramel colour II was tested for potential mutagenic activity based on induction of DNA repair (unscheduled DNA synthesis) in cultured human epithelial (HeLa 53) cells. Caramel colour II was incorporated in the culture medium at concentrations of 25-51,200 µg/ml and the test was performed on 2 occasions both in the presence and absence of rat liver S-9 mix. In both tests, in the absence of the S-9 mix a small but statistically significant increase in the number of silver grains over nuclei was observed at a concentration of 25,600 µg/ml; no significant increases were seen at higher concentrations in this test nor at any concentration in the repeat test (Allen & Proudlock, 1984).”

“Caramel colour II was not clastogenic to cultured Chinese hamster ovarian cells at concentrations of 500, 2500, or 5000 µg/ml either in the presence or absence of rat liver S-9 mix (Allen et al., 1984).”

“Caramel colour III (a blend of 3 commercial samples) was evaluated by the Ames *Salmonella*/microsome plate test and the *Saccharomyces*/microsome plate test. The *Salmonella* test organisms used were TA98, TA100, TA1535, TA1537, and TA1538; the *Saccharomyces* was strain D4, and the tests were conducted in the presence and absence of liver S-9 mix from Aroclor-induced rats. The dose range employed was 1-50 mg/plate for TA100 and 1-20 mg/plate for all other test strains. Caramel colour III was non-mutagenic to the test organisms under these conditions (Jagannath & Brusick, 1978a).”

“In a similar study using the same *Salmonella* tester strains, caramel colour III was non-mutagenic in a range of concentrations up to 20 µl/plate with or without metabolic activation (Richold & Jones, 1980).”

“Caramel colour III was non-mutagenic in the Ames test against *Salmonella* strains TA98 and KTA100 at concentrations of 0-1 mg/plate, with or without metabolic activation. Acidic and basic organic extracts of the caramel were also non-mutagenic (Ashoor & Monte, 1983).”

“Thirteen commercial caramel colours (not identified) were examined for mutagenicity in the Ames test, using *Salmonella* strains TA98 and TA100 with and without metabolic activation, and for DNA-damaging effects in *E. coli* (Wild/pol A-, Wild/rec A-); none of the samples tested was active under the test conditions (Kawana et al., 1980).”

"Five samples of commercial caramel colour (not identified) were tested for mutagenicity in the Ames test using Salmonella strains TA98 and TA100. All samples gave equivocal results with TA100 and 2 of the 5 samples were equivocal with TA98 (Kawachi et al., 1980)."

"Five samples of caramel colour were tested in the Ames Salmonella plate assay using strains TA98, TA100, and TA1537 without metabolic activation but with a 20-minute preincubation step. The same samples were used in chromosome aberration tests performed on a cultured Chinese hamster lung fibroblast cell line, both in the presence and absence of S-9 fraction. All samples of caramel colour were designated as positive both in the Ames assay and in the chromosome aberration test (aberrations in 20% of metaphase cells) (Ishidate & Yoshikawa, 1980)."

"The same series of caramel colour samples was tested by Jagannath and Brusick, and all were found to be non-mutagenic in the Ames test using Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, and Saccharomyces strain D4, in the presence or absence of S-9 mix. One of the samples in this study and in the Ishidate & Yoshikawa (1980) study was caramel colour III (Jagannath & Brusick, 1978b)."

"The mutagenicity of a series of samples taken at various stages in the manufacture of caramel colour III were assayed in the Ames test using Salmonella strains TA98, TA100, and TA1535, with and without metabolic activation. No mutagenicity was seen with any sample against all three tester strains in the presence of S-9 fraction but an increased number of revertants was found with strain TA100 in the absence of S-9 fraction. Mutagenic activity was associated with samples taken late in the process (Jensen et al., 1983)."

"A sample of caramel colour III was non-mutagenic in the Ames test against Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 both in the presence and absence of rat hepatic S-9 fraction at caramel colour concentrations up to 5 mg/plate (Richold et al., 1984)."

"The same sample as that used by Richold et al. (1984b) was tested for potential mutagenic activity based on induction of unscheduled DNA synthesis in cultured human epithelial (HeLa 53) cells both in the presence and the absence of rat liver S-9 fraction. The test was performed on two occasions at caramel colour III concentrations of 25-51,200 µg/ml in the culture medium. In both tests, caramel colour III caused a significant increase in the number of silver grains found over cell nuclei at concentrations of 6,400 and 12,800 µg/ml in the absence of S-9 mix; significant increases were not observed at higher or lower concentrations in the absence of S-9 mix nor at any of the concentrations tested in the presence of S-9 mix. The authors concluded that, although reproducible effects on unscheduled DNA synthesis were demonstrated in the test, this effect may be moderated by metabolism (Allen & Proudlock, 1984)."

"Caramel colour III was not clastogenic to cultured Chinese hamster ovarian cells at concentrations of 500, 2500, or 5000 µg/ml either in the presence or absence of rat liver S-9 mix (Allen et al., 1984)."

"The clastogenicity of caramel colour III was evaluated in an in vitro cytogenetic assay using cultured Chinese hamster ovarian cells. A significant increase in chromosome aberrations was observed at concentrations of 3 mg/ml or higher in the absence of metabolic activation; similar findings were reported for sodium ascorbate at 2 mg/ml (Galloway & Brusick, 1981a)."

"These tests were repeated in the presence of rat liver S-9 fraction, and neither caramel colour III at a concentration up to 5 mg/ml nor sodium ascorbate at a concentration of 5 mg/ml was active (Galloway & Brusick, 1981b)."

"In an in vivo mouse micronucleus test, caramel colour III was administered by gavage to 5 males and 5 females at doses of 0, 1.05, or 3.5 g/kg b.w. (2 doses 24 hours apart). Caramel colour III did

not increase the incidence of micronuclei in polychromatic erythrocytes obtained from marrow and was considered by the authors not to exhibit clastogenic activity under the conditions of the test (Cimino & Brusick, 1981)."

"Caramel colour IV was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. *Salmonella typhimurium* (strains TA1535, TA1537, and TA1538) and *Saccharomyces cerevisiae* were used. Caramel colour IV was not genetically active under the test conditions employed in this study (Brusick, 1974)."

"Caramel colour IV was evaluated for mutagenicity using the Ames *Salmonella*/microsome plate test and the *Saccharomyces*/microsome plate test. The samples tested were blends of 3 samples of caramel colour IV (low colour intensity) and 3 samples of caramel colour IV (high colour intensity). The *Salmonella* test organisms used were TA98, TA100, TA1535, TA1537, and TA1538. Tests were conducted directly or in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. Tests were conducted over a range of concentrations from 1-50 mg/plate. No signs of genetic activity were observed with any of the samples of caramel colour IV tested using either the *Salmonella* or *Saccharomyces* test organisms (Jagannath & Brusick, 1978a)."

"The clastogenicity of caramel colour IV (low colour intensity) was evaluated in an in vitro cytogenetic assay using cultured Chinese hamster ovary cells without metabolic activation. No increase in chromosome aberrations was observed at concentrations of test material between 5 µg/ml and 5 mg/ml. In the same test, sodium ascorbate produced a positive response at 2 mg/ml (Galloway & Brusick, 1981a)."

"The clastogenicity of caramel colour IV (high colour intensity) was evaluated in an in vitro cytogenetic assay using Chinese hamster ovary cells without metabolic activation. No increase in chromosome aberrations was observed at concentrations of test material between 5 µg/ml and 5 mg/ml. In the same test, sodium ascorbate produced a positive response at 2 mg/ml (Galloway & Brusick, 1981b)."

"Two samples of caramel colour IV of medium and high colour intensity were assayed for mutagenic activity in the Ames test using *Salmonella* strains TA98 and TA100 in the absence or presence of rat hepatic S-9 fraction. Neither sample showed mutagenic activity under the conditions of the test (Ashoor & Monte, 1983)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

Chinese hamster ovary cells were exposed for 3 hours to commercial caramel powder (a positively-charged material prepared by heating a sugar-ammonium solution), or to caramelised solutions of sucrose, fructose, glucose, mannose, maltose or arabinose (prepared by heating solutions at 180°C for 1 hour). The caramel and all the caramelised sugars induced a high frequency of chromosome breaks and exchanges, the potency of caramel itself (which produced 46% metaphase plates with chromosome aberrations) being exceeded only by caramelised fructose. Non-caramelised sugars did not increase the aberration frequency. The transition metals Cu²⁺ and Mn²⁺ had no effect on caramels' potency, but Fe²⁺ and Fe³⁺ had a strong inhibitory effect. Addition of an S-9 microsomal preparation from PCB-pretreated rats abolished the clastogenic activity of the caramel powder, and this property was unimpaired by removal of NADP or MgCl₂ from the S-9 mixture. However, heating for 20 min at 80°C did destroy its inhibitory actions. It was concluded that the effects of the S-9 preparation were not due to the mixed-function-oxidase system, but might result from a physical binding of caramel to S-9 components (Stich et al 1981).

A food grade caramel gave negative results in an Ames test with five *Salmonella* strains (Bonin & Baker 1980).

A very weak mutagenicity of caramel to *S. typhimurium* TA100 has been reported (Sugimura et al 1979).

There was no evidence of mutagenicity in an Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA*, when tested at up to 5 mg caramel/plate with and without metabolic activation (Harigaea et al. 2000).

In vivo					
Species	Test conditions	Endpoint	Result	Reference	
Mice, 15 of each sex	A class I caramel was given by gavage at 5 g/kg bw (a limit dose approach, as no toxicity was observed). Five per sex were killed at 24, 48 and 72 hours, and the bone marrow examined for micronuclei	Chromosome damage	-ve	Adams et al. 1992	
Mice, 5 of each sex per dose	A class III caramel was given at 1 or 3.5 g/kg bw/day for 2 days by gavage, mice were killed 6 hours after the second dose, and the bone marrow examined for micronuclei	Chromosome damage	-ve	Brusick et al. 1992; JECFA, 1987 (citing Cimino & Brusick, 1981)	
Rat	A paper summarising Japanese studies contains a table indicating that three unspecified caramels were tested for the ability to cause chromosome aberrations in the bone marrow. No further details, and it is possible that the table is in error, and caramels were not tested.	Chromosome damage	-ve	Kawachi et al. 1980	
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]					
In vitro					
Test system	Test conditions	Endpoint	Activation	Result	References
Chinese hamster ovary cells	Two caramels (a class II and a class III caramel) were tested at up to 5 mg/ml, and examined for chromosome aberrations	Chromosome damage	With and without S9	-ve	Allen et al. 1984 and 1992; JECFA, 1987
Chinese hamster ovary cells	Three caramels (a class III and two class IV caramels) were tested at up to 4 or 5 mg/ml, and examined for chromosome aberrations	Chromosome damage	With S9 Without	-ve -ve (IV) +ve (III)	Brusick et al. 1992; JECFA, 1987 (citing Galloway & Brusick, 1981)
Chinese hamster ovary cells	A class I caramel was tested at up to 10 mg/ml (which was non-toxic), cells examined for chromosome aberrations	Chromosome damage	With S9 Without	-ve +ve	Adams et al. 1992

Chinese hamster lung cells	5 unidentified caramels tested at undisclosed concentrations, cells examined for chromosome aberrations	Chromosome damage	With and without S9	+ve	JECFA, 1987 (citing Ishidate & Yoshikawa, 1980)
Mouse lymphoma cells	A class I caramel was tested at up to 9 mg/ml (cell survival was decreased at 6 mg/ml and above), and plates evaluated for mutant colonies	Somatic cell mutation	With and without S9	+ve	Adams et al. 1992
Human embryo fibroblast cells	5 caramels (unspecified) were tested for the ability to induce chromosome aberrations and sister chromatid exchange. No further details, results given in a table	Chromosome damage and chromosome effects	Without	-ve	Kawachi et al. 1980
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	15 caramels from caramel classes I, II, III and IV were tested, typically up to 20 mg/plate, some assays repeated using a pre-incubation step	Mutation	With and without S9	-ve	Allen et al. 1992
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538, and in Saccharomyces cerevisiae D4 yeast	9 caramels from caramel classes III and IV were tested, typically up to 50 mg/plate. Several colours were retested using a pre-incubation step	Mutation	With and without S9	-ve	Brusick et al. 1992
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	A class I caramel was tested at up to 10 mg/plate	Mutation	With and without S9	-ve	Adams et al. 1992
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Two class I caramels, a class II caramel and a class III caramel were tested at up to 20 mg/plate (non-toxic)	Mutation	With and without S9	-ve	JECFA, 1987 (citing Richold & Jones, 1980)
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	A class II and a class III caramel were tested at up to 5 mg/plate (non-toxic)	Mutation	With and without S9	-ve	JECFA, 1987 (citing Richold et al. 1984)

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	A class III caramel was tested at up to 20 mg/plate or (in TA100 only) 100 mg/plate. Three class IV caramels and a blend of three other class IV caramels were tested at up to 50 mg/plate	Mutation	With and without S9	-ve	JECFA, 1987 (citing Jagannath & Brusick, 1978)
Salmonella typhimurium strains TA98 and TA100	Ames tests on Caramel III at up to 1 mg/plate and on two class IV caramels, doses unspecified	Mutation	With and without S9	-ve (limited study, only 2 strains tested)	JECFA, 1987 (citing Ashoor & Monte, 1983)
Salmonella typhimurium strains TA98 and TA100	13 unidentified caramels, no further details given	Mutation	With and without S9	-ve (limited study, only 2 strains tested)	JECFA, 1987 (citing Kawana et al. 1980)
Salmonella typhimurium strains TA98 and TA100	5 unidentified caramels, no further details given. No detailed data, result given in a table.	Mutation	With and without S9	?	Kawachi et al. 1980
Salmonella typhimurium strains TA98, TA100 and TA1537	5 unidentified caramels, no further details given	Mutation	Without (but with 20 min pre-incubation)	+ve	JECFA, 1987 (citing Ishidate & Yoshikawa, 1980)
Retested in the above strains and TA1535, TA1538	Same 5 caramels (Note that publication date of "retest" precedes that of the first test – publication date may not reflect date of testing)	Mutation	With and without S9	-ve	JECFA, 1987 (citing Jagannath & Brusick, 1978)
Human epithelial HeLa (cancer) cells	A caramel class II and a class III caramel were tested up to 51.2 mg/ml, UDS assay	DNA repair (indicative of DNA damage)	With and without S9	+ve (weak)	JECFA, 1987 (citing Allen & Proudlock, 1984)
Bacillus subtilis	From a tabulated summary of Japanese studies. Rec assay measuring differential killing on 4 unspecified caramels. No further details.	DNA damage (indicative test)	With and without S9	-ve	Kawachi et al. 1980
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic					

activation]

Record for caramel (color) (CAS RN 8028-89-5):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
Mutation in microorganisms		Bacteria - Salmonella typhimurium	50 mg/plate	SKEZAP Shokuhin Eiseigaku Zasshi. Food Hygiene Journal. (Nippon Shokuhin Eisei Gakkai c/o Shokuhin Eisei Senta, 2-6-1 Jingumae, Shibuya-ku, Tokyo 150, Japan) V.1- 1960- Volume(issue)/page/year: 23,86,1982
Mutation in microorganisms		Bacteria - Salmonella typhimurium	50 mg/plate	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 22,623,1984
Cytogenetic analysis		Rodent - hamster Fibroblast	8 gm/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 22,623,1984
Cytogenetic analysis		Rodent - hamster Lung	6900 mg/L	GMCRDC Gann Monograph on Cancer Research. (Plenum Pub. Corp., 233 Spring St., New York, NY 10013) No. 11- 1971- Volume(issue)/page/year: 27,95,1981
Cytogenetic analysis		Rodent - hamster Ovary	10 gm/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 30,397,1992
Mutation in mammalian somatic cells		Rodent - hamster Ovary	6 gm/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 30,397,1992

As taken from RTECS, 2019a

Record for Caramel A ("Food additive obtained through sulfite ammonia process, glucose") (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
Mutation in microorganisms		Bacteria - Salmonella typhimurium	1 mg/plate	AMONDS Applied Methods in Oncology. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY

				10017) V.1- 1978- Volume(issue)/page/year: 3,253,1980
Cytogenetic analysis		Rodent - hamster Lung	100 gm/L	AMONDS Applied Methods in Oncology. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY 10017) V.1- 1978- Volume(issue)/page/year: 3,253,1980

As taken from RTECS, 2019b

Record for Caramel C ("Food additive obtained through ammonia process, glucose") (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
Mutation in microorganisms		Bacteria - Salmonella typhimurium	1 mg/plate	AMONDS Applied Methods in Oncology. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY 10017) V.1- 1978- Volume(issue)/page/year: 3,253,1980
Cytogenetic analysis		Rodent - hamster Lung	1 gm/L	AMONDS Applied Methods in Oncology. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY 10017) V.1- 1978- Volume(issue)/page/year: 3,253,1980

As taken from RTECS, 2019c

5.5. Cytotoxicity

"Caramel colour II at concentrations of 2.5-20 µl/plate was neither mutagenic nor cytotoxic in the Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, with or without metabolic activation by rat liver S-9 fraction (Richold & Jones, 1980)."

"In a similar test, caramel colour II was neither mutagenic nor cytotoxic at concentrations of 50-5000 µg/plate (Richold et al., 1984)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

"The aim of the work was early identification of preventable risk factors connected with the consumers usage of products of everyday use, such as cosmetics, toys and children products, and other materials intended for contact with human skin. The risk factor is represented by substances with irritation potential and subsequent possible sensitisation, resulting in negative impact on human physical and psychical health with social and societal consequences. The legislation for cosmetics, chemical substances and other products requires for hazard identification the application of alternative toxicological methods in vitro without the use of animals. For this reason we used a battery of alternative assays in vitro, based on cell cultures. Progressive methods of molecular biology, based on fluorimetry and fluorescence, were employed for identification of early morphological and functional changes on cellular level. Four colorants frequently used in cosmetics (P-WS Caramel, Chlorophyllin, Unicert Red K 7054-J and Unicert Red K 7008-J) were tested on cell line NIH3T3 (mouse fibroblast cell) and 3T3 Balb/c with/without UV irradiation (dose 5 J cm⁻²). Fluorescence methods for the study of cell damage using fluorescence probes offer results for

the evaluation of cytotoxicity and cell viability of adherent cells. We detected intracellular production of ROS investigated by molecular probe CM-H(2)DCFDA, which is primarily sensitive to the increased production of hydrogen peroxide or its downstream products. Toxic effects on the cellular level were identified by viability tests using Neutral Red uptake and MTT assay, where the live cells reduce yellow soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to insoluble formazan crystals. The reaction was investigated on mitochondrial membrane of living cells and the type of cell death was determined using Apoptosis detection kit. Cytotoxicity tests revealed health risks of using Chlorophyllin and Unicert Red K 7054-J" (Tomankova et al. 2011).

5.6. Carcinogenicity

EFSA (2011) reported the following:

"The findings in the long-term toxicity studies carried out with Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel were similar to, and did not reveal any pattern of toxicity not already seen in, the 90-day oral toxicity studies carried out with these caramel colours. No evidence of carcinogenicity was seen in 2-year studies in rats on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. In a parallel study in mice, there was similarly no evidence for a carcinogenic potential of Class IV Sulphite Ammonia Caramel. The Panel noted that no long-term toxicity or carcinogenicity data were available for Class I Plain Caramel and Class II Caustic Sulphite Caramel. The Panel considered however that given the fact that Class I Plain Caramel is likely to be produced in normal cooking processes, also considering the long-term toxicity and carcinogenicity data available on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, and the rather similar toxicological profile of all the caramel colours, there are no concerns regarding the long-term toxicity or carcinogenicity of Class I Plain Caramel or Class II Caustic Sulphite Caramel, nor regarding the carcinogenicity of Class III Ammonia Caramel or Class IV Sulphite Ammonia Caramel."

"Three groups of 50 male and 50 female B6C3F1 mice were given drinking water containing 0, 1.25, or 5% caramel colour III for 96 weeks followed by 8 weeks of drinking water without caramel colour; the animals were fed CFR diet ad libitum. The caramel colour III used in this study contained less than 25 mg/kg THI. The cumulative mortality of males given 5% caramel colour III was higher than that of controls from week 100 to the end of the experiment, but there were no clear pathological differences in any organs and no treatment-related abnormalities in urinalyses. The only statistically significant differences in haematological parameters between control and treated groups were elevations of the total leucocyte counts in males of both treatment groups, but the observed values were within the range encountered for the strain of B6C3F1 mice used in the study. No treatment-related gross pathology was noted during or at the end of the experiment. Malignant lymphoma/leukaemia, hepatocellular carcinoma, and sub-cutaneous fibrosarcoma and/or malignant fibrous histiocytoma were frequent, but no significant differences were found in their incidences between treated and control groups. Adenomas and adenocarcinomas of the lungs, hyperplastic nodules of the liver, and fibroma of the sub-cutis were frequent in males, but their incidences were similar in treated and untreated mice. The authors concluded that, under the conditions used, caramel colour III was not carcinogenic for B6C3F1 mice (Hagiwara et al., 1983)."

"Three groups of 50 male and 50 female F344 rats were given caramel colour III in drinking water at levels of 0, 1, or 4% for 104 weeks followed by drinking water without caramel for 9 weeks. The animals were fed ad libitum basal diet that contained 11-12 mg/kg pyridoxine, and the caramel colour III used contained less than 25 mg/kg THI. During the experimental period, all animals were observed daily, and clinical signs and mortality were recorded. Body weights were recorded weekly during the first 13 weeks of the study and then every 4 weeks. Moribund or dead animals and animals sacrificed at termination were autopsied and examined for the development of tumours in organs and tissues. No treatment-related differences in growth or survival rates were noted. No

dose-related effects were found either in the incidence or induction time of tumours in the various organs and tissues except in the pituitary gland of males of the top-dose group in which the incidence of tumours was significantly higher than that in controls. However, pituitary tumours are among the most common spontaneous tumours in F344 rats that occur with variable incidence; most of the tumours were microscopic and there were no significant differences in their induction times compared with controls. The authors concluded that the higher incidence of pituitary tumours was not related to caramel administration, but could be explained by the variability of spontaneous tumour incidence (Maekawa et al., 1983)."

"Four groups of 48 male and 48 female Wistar rats were given diets containing 0, 1, 3, or 6% caramel colour III (ammonia catalysed "half open-half closed pan" caramel; no indication about the presence of 4-methylimidazole was given) for 2 years. Food and water intake, growth, mortality, and organ weights were measured; haematological examinations, urinalyses, kidney function tests, and histopathological examinations also were carried out. A decrease in growth, which was significant in the males, was observed at all dose levels. This effect was accompanied by a reduction in the cumulative food intake. A significant reduction in white cell number (in the 6% group) was associated in the early part of the study with a lymphocytopenia, which was present until week 80 in the male rats fed a diet containing 3 or 6% caramel. In the female animals the lymphocytopenia was present until week 52 in these 2 groups (the 1% group was not tested). Spleen weights were reduced in a dose-related manner. The relative weights of the (full) caeca were clearly increased at all dose levels. No changes were found in the pancreata of the control animals, while in the test groups (not dose-related) a total of 10 hyperplastic changes were found. However, the number of tumours of the pancreas showed no relation to the administration of caramel. There was no evidence of a carcinogenic effect. The authors concluded that a no-observed-effect level could not be established for the caramel used in this particular study (Evans et al., 1976)."

"A carcinogenicity study of caramel colour IV was performed on B6C3F1 mice using 5 groups of 50 male and 50 female mice in each group. Two groups of each sex served as controls and the 3 treatment groups received caramel colour IV in the drinking water at dose levels of 2.5, 5.0, or 10.0 g/kg b.w./day for 104 weeks. All animals were observed twice daily and palpated for tissue masses weekly from week 27. Body weights and food intake were recorded weekly, and fluid intake was measured on the first, third, fifth, and seventh days of the first 12 weeks and over a 48-hour period each week thereafter. Although during the course of this study there were sporadic significant differences in mean body weights of treated male and female groups compared to controls, caramel-colour feeding did not have consistent effects on body weights or body-weight gains in either sex. Males of the 10 g/kg b.w.-dose group had lower mean food consumption than the control groups for 67 of the 104 weeks and males receiving 2.5 g/kg b.w. had lower mean food consumption for 21 of the 104 weeks. There were no consistent differences in food intake of males at the 5 g/kg b.w. dose level nor of any of the females. Decreased fluid intake was noted, particularly in males at the higher dose levels; mean fluid intakes of treated females were usually equal to or only slightly lower than controls. There were no treatment-related differences in survival rates, which at termination were 65-75% for the male groups and 67-79% for the female groups. At necropsy, there were dose- and/or treatment-related effects on the gastrointestinal tract and mesenteric lymph nodes, including dark gastrointestinal contents, staining of the mucosa, and diffusely red and congested mesenteric lymph nodes. These changes were not considered to be toxicologically important and there were no other changes of toxicological significance. There was no evidence of treatment-related neoplastic lesions in any organs (MacKenzie, 1985b)."

"A long-term toxicity and carcinogenicity study of caramel colour IV was conducted on F344 rats. In the carcinogenicity portion of the study, 5 groups of 50 male and 50 female weanling rats were selected randomly; 2 groups of each sex served as controls and the 3 treatment groups received caramel colour IV in drinking water at dose levels of 2.5, 5.0, or 10 g/kg b.w./day for 24 months. In

the chronic toxicity portion of the study, groups of 25 male and 25 female rats received caramel colour IV in drinking water at doses of 0, 2.5, 5.0, 7.5, or 10 g/kg b.w./day for 1 year. The following parameters were monitored: mortality, clinical observations including ophthalmic changes, body weight, and food and fluid consumption. In the chronic toxicity portion clinical chemistry and haematological studies were done at intervals of 6 and 12 months and necropsies were performed at 12 months. Clinical chemistry and haematological studies at 6 and 12 months in the chronic toxicity study did not reveal changes of toxicological concern. At 6 months, serum concentrations of blood urea nitrogen and creatinine were reduced in male groups treated with 5.0, 7.5, and 10 g/kg b.w. and in female groups treated with 7.5 and 10 g/kg b.w. caramel colour. Similar changes were noted at 12 months. Creatinine levels were within the normal range for the F344 rat, whereas blood urea nitrogen levels were slightly outside this range. Decreased levels of serum total protein, albumin, and globulin were also noted at the 6-month sampling, particularly in male rats. These changes, which were less marked at 12 months, were not accompanied by any pathology in the liver or kidneys and were not considered to be of toxicological importance. In the carcinogenicity portion of the study, the observations were generally similar to those in the chronic portion. Survival at 24 months ranged from 64-68% for males and 82-92% for females. Random variations in both benign and malignant neoplasms typical of the F344 strain and this age of animal were observed; however, there were no treatment-related differences. The authors concluded that the feeding of caramel colour IV at doses up to 10 g/kg b.w. for 24 months did not induce neoplastic changes or non-neoplastic changes of toxicological importance (MacKenzie, 1985a)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

"The potential carcinogenicity of 4-methylimidazole (4-MEI) was evaluated in a National Toxicology Program (NTP) rodent cancer bioassay in Fischer 344 rats and B6C3F1 mice (NTP, 2007; Chan et al., 2008). The NTP concluded that there was "clear evidence of carcinogenic activity" in male and female mice, based on an increased incidence of lung tumors. The "category of evidence" that the NTP assigns to a rodent cancer bioassay outcome can have significant regulatory implications. This is especially important for 4-MEI, which forms in caramel colorings and other foods during cooking, with potential widespread human exposure in a broad spectrum of food and beverage products. A detailed analysis of all NTP mouse-lung-tumor-only carcinogens reveals that the proper call for lung tumors in the 4-MEI study should have been "some evidence" rather than "clear evidence" of carcinogenic activity for both male and female mice in order to be consistent with the NTP's interpretation of other mouse lung carcinogens showing a similar strength of response. Suggestions are given as to measures the NTP should consider in the preparation of some or all future Technical Reports in order to enhance consistency of interpretation of experimental results." As taken from Haseman JK. 2013. Regul. Toxicol. Pharmacol. 66(1), 109-15. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23545072>

Species	Test conditions	Evidence of carcinogenicity	Reference
Rats, groups of 50/sex per dose level except 100/sex in control group	Caramel IV given in drinking water for at least 2 years. Equivalent to 0, 2.5, 5 and 10 g/kg bw/day. A comprehensive range of tissues were examined microscopically. Good quality study	None	MacKenzie et al. 1992a
Rats, groups of 40/sex, except 80/sex in	Six caramel IV samples were tested. Three (low intensity colours) were given in the diet for 2 years at 0, 5, 10 and 15%, while three others (high intensity colours) were given at	None	Sinkeldam et al. 1976

control group	0, 2, 4 or 6%. In each case, the animals were the offspring of rats treated similarly during pregnancy, and thus would have had in utero exposure. Histopathology was described as “extensive”.		
Rats, groups of 50/sex per dose level	0, 1 or 4% caramel class III in the drinking water for 104 weeks, then 9 weeks untreated, wide range of organs and tissues examined microscopically	None (pituitary tumours were more common in top-dose males, but these are very common in old rats of this strain and were not believed to be related to treatment)	JECFA, 1987 (citing Maekawa et al. 1983)
Rats, groups of 48/sex per dose level	0, 1, 3 or 6% caramel class III in the diet for 2 years, wide range of organs and tissues examined microscopically	None	Evans et al. 1977
Mice, groups of 50/sex per dose level except 100/sex in control group	Caramel IV given in drinking water for at least 2 years. Equivalent to 0, 2.5, 5 and 10 g/kg bw/day. A comprehensive range of tissues were examined microscopically	None	MacKenzie et al. 1992a
Mice, groups of 50/sex per dose level	0, 1.25 or 5% caramel class III in the drinking water for 96 weeks, then 8 weeks untreated, wide range of organs and tissues examined microscopically	None	JECFA, 1987 (citing Hagiwara et al. 1983)

5.7. Irritation/immunotoxicity

EFSA (2011) considered that, due to a lack of data, no definite conclusion could be drawn with respect to intolerance and allergenicity to the four classes of caramel colours, although it noted that no cases have been reported in the published literature.

In relation to the potential haematotoxicity/immunotoxicity of caramel colours, EFSA (2011) reported that lymphocytopenic and immunomodulatory effects have been identified in a number of studies with Class III Ammonia Caramel, but not with the other classes of caramel colours. These effects are attributed to 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI), a constituent in Class III Ammonia Caramel, combined with pyridoxine (vitamin B6) deficiency. These effects were (apparently) transient in nature, disappearing in the later stages of longer-term studies with Class III Ammonia Caramel. In the pivotal 90-day study of MacKenzie et al. on Class III Ammonia Caramel, in which rats were dosed with up to 20 g/kg bw/day caramel colour, containing either 15 mg THI/kg caramel or 295 mg THI/kg caramel (on a solids basis), no dose-related lymphocytopenia occurred at the lower THI level, which is higher than the current maximum level for THI laid down in the specifications for Class III Ammonia Caramel. At a dose level of 20 g/kg bw/day, the ammonia caramel with the higher level of THI induced a statistically significant decrease in lymphocyte counts in both sexes at 2 weeks and, in males only, at 6 weeks. All lymphocyte values in these groups were normal at the termination of the study. The Panel noted however that the results of a short-term oral study carried out by Thuvander and Oskarssen indicated that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B6 status.

“The Panel noted that, in contrast to the findings in rats and mice, in the available short-term human studies consumption of Class III Ammonia Caramel had no effects on total or specified

white blood cell counts at dose levels of up to 200 mg/kg bw/day (with THI levels almost 20 times above those permitted according to current specifications) albeit following short term exposure periods. The study of Houben et al. included subjects with (mild) pyridoxine deficiency, and the authors, in comparing the results obtained in this study with data in rats maintained on normal and pyridoxine-reduced diets, suggested that, with regard to oral intake of THI, humans are less sensitive to Class III Ammonia Caramel-induced lymphocytopenia than are rats. The Panel agreed with this interpretation, based on the available data" (EFSA, 2011).

Studies in the 1970s in rats and mice demonstrated that ingestion of caramel colour III can cause a reduction in total white blood cell counts, especially when the diets had relatively low vitamin B6 contents. Comparative studies indicated that 2-acetyl-4(5)tetrahydroxybutyl-imidazole (THI) was responsible for this lymphocyte depression (Houben & Pennicks, 1994; JECFA, 1987). JECFA noted that there was no such effect in a 90-day study in rats given 20 g/kg bw/day of caramel III containing THI at 10 ppm (15 ppm on a solids basis), and this study was used to derive the ADI of 0-200 mg/kg bw/day for caramel III (JECFA, 1987).

No effects on the number of white blood cells were reported in 24 marginally vitamin B6-deficient human volunteers (aged 65 years or older) who consumed caramel colour III at the acceptable daily intake level (ADI of 200 mg/kg bw/day), containing up to 143 ppm (28.6 ug/kg bw/day) THI, for 7 days (Houben et al. 1992), or in a pilot study where 9 volunteers ingested 1.5 g of caramel colour III per day for 21 days (BIBRA, 1976).

"The lymphocyte depression caused by caramel colour III has been shown to be due largely, if not solely, to this minor component."

"The effects of vitamin E, folic acid, pyridoxine and choline on the capacity of caramel colour III to reduce total lymphocytes in the blood of rats fed caramel colour III were examined. Four groups of 10 male weanling rats were fed Spratt's diet containing 8% caramel colour III and supplemented with vitamin E (100 mg/kg), folic acid (10 mg/kg), pyridoxine hydrochloride (10 mg/kg), or choline chloride (1000 mg/kg). Groups on Spratt's diet alone or on Spratt's diet with 8% caramel colour III without any supplement were used as controls. After 12 days, there was a marked reduction in total white blood cells and lymphocytes in rats fed the diet containing 8% caramel colour III and neutrophil counts were increased. Dietary supplementation with vitamin E, folic acid, or choline did not noticeably affect lymphocyte counts. However, rats receiving the diet supplemented with pyridoxine had white blood cells and lymphocytes in numbers similar to those fed the basal diet without caramel colour III. The basal diet was found to contain 2.3 mg/kg pyridoxine. In a further study using CIVO stock diet (pyridoxine concentration 3 mg/kg), rats receiving 8% caramel colour III in the diet showed a reduction in total white blood cell and lymphocyte numbers; these changes were ameliorated by the addition of 10 mg/kg pyridoxine in the diet. In this study, neutrophils were not affected by the caramel colour III treatment, but the plasma pyridoxal phosphate levels were reduced by treatment (Sinkeldam et al., 1984).

"In order to quantify the relationship between dietary pyridoxine and reduction of lymphocyte counts by caramel colour III, and to study the effects of age, two 14-day studies were performed, one in weanling, and the second in mature, Wistar rats; in other respects the protocols were identical. Groups of 10 male animals were fed diets containing approximately 2.5, 6, 12, or 24 ppm of pyridoxine. At each dietary level groups were given caramel colour III in the drinking water at levels of 0, 1, 4, or 8%. A clear inverse dose relationship between the severity of lymphocyte depression and the pyridoxine level of the diet was observed. In weanling rats fed a diet containing 2.5 ppm pyridoxine, statistically significant lymphocyte reduction occurred at all caramel colour III levels on days 6 and 13. Groups fed diets containing 6, 12, or 24 ppm pyridoxine did not show statistically significant reductions in lymphocyte counts on day 6. However, on day 13, groups receiving 4 or 8% caramel colour III and 6 ppm pyridoxine in the diet had a statistically significant

reduction in lymphocytes. On day 13 at a dietary level of 12 ppm pyridoxine only the 8% caramel colour III group had a statistically significant reduction in lymphocytes. There were no significant reductions in lymphocyte counts in animals fed 24 ppm pyridoxine at any level of caramel colour III intake. In mature rats fed 4 and 8% caramel colour III a statistically significant reduction in lymphocytes occurred at dietary pyridoxine levels of 12 ppm and lower on day 6. On day 13 lymphocytes were significantly decreased at caramel colour III doses 1, 4, and 8% in animals fed a diet containing 2.5 ppm pyridoxine. There were no significant decreases in lymphocytes at these dose levels of caramel colour III in animals fed 6, 12, or 24 ppm pyridoxine (Sinkeldam, 1981; 1982a)."

"An isolation procedure was developed and a fraction of caramel colour III that contained the lymphocyte-depressing activity was isolated. The single component of this fraction that was responsible for the activity was identified as 2-acetyl-4(5)-tetrahydroxybutylimidazole (Kroplien et al., 1984)."

"THI was administered to groups of 10 male weanling Wistar rats at levels of 0, 2, 5, or 20 ppm in drinking water for 7 days; a fifth group received a 1% solution of caramel colour III in drinking water as a positive control. THI produced a marked depression of lymphocyte counts at all dose levels and a dose-dependent increase in neutrophils. The lymphocyte-depressing potency of 2 ppm THI was comparable to that of 1% caramel colour III in the drinking water, indicating a level of approximately 200 mg/kg THI in the caramel sample. This compares with the result of chemical analysis of a sample of this batch of caramel colour III, which indicated a value of 204 mg/kg THI on an 'as is' basis (Sinkeldam, 1982b; Kroplien, 1984)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

"Administration of Caramel Colour III is associated with lymphopenia in laboratory animals, especially if the animals are fed a vitamin B6-deficient diet. Recently, functional immunological alterations in rats exposed to Caramel Colour III have been reported. The component of Caramel Colour III that is responsible for the immunological effects has been shown to be 2-acetyl-4-tetrahydroxybutyl imidazole (THI). In the present study, female Balb/c mice fed a diet with a relatively high vitamin B6 content were exposed to 2 or 10% of a commercial Caramel Colour III preparation with a low THI content (less than 25 ppm) in the drinking water for 9 wk. Although this treatment did not induce a lymphopenia in the exposed mice, flow cytometric analysis of lymphocyte subpopulations demonstrated reductions in the CD4+ and CD8+ cell populations. In addition, the proliferative response of spleen cells to B and T cell mitogens was significantly reduced in the mice exposed to 2% Caramel Colour III. No changes were observed in natural killer cell activity or in the humoral antibody response to a viral antigen. The results indicate that Caramel Colour III that meets the specified limit of less than 25 mg THI/kg may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B6 status." As taken from Thuvander A and Oskarsson A. Food Chem Toxicol. 1994 Jan; 32(1):7-13. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8132168&query hl=33&itool=pubmed_docsum

No decrease in the number of blood lymphocytes occurred in marginally vitamin B6-deficient humans who consumed caramel colour III at the acceptable daily intake level (200 mg/kg body weight) for seven days (Houben et al 1992).

Caramel produced with sodium hydroxide as the catalyst was fed to groups of 15 rats of each sex as dietary levels of 4, 8 or 16% for 13 weeks with 25 rats of each sex as controls. There were no effects on the serum levels of transaminases and urea, on renal function tests or on histology. Caecal weights were increased at all dietary levels, but this effect was not thought to be of

toxicological significance. The haemoglobin concentration and erythrocyte counts were slightly reduced at week 6 but no effect was apparent at week 13. Lower lymphocyte counts were found in males given 8 or 16% caramel for 13 weeks and in females given 8% for 6 weeks. The no-untoward-effect level is considered to be 4% of the diet, providing an intake of approximately 3 g/kg bw/day (Gaunt et al 1977).

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Caramel Color E150D was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Caramel Color E150D when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	299	JTI KB Study Report(s)
In vitro cytotoxicity	299	JTI KB Study Report(s)

“Since the National Toxicology Program (NTP) identified 4(5)-methylimidazole [4(5)-MI] as a cancer causing chemical in 2007 and the State of California added it to the Proposition 65 list of compounds as a carcinogen on January 7, 2011, many researchers and regulatory agencies have become focused on the presence of 4(5)-MI in foods and beverages. 4(5)-MI has been known to form in the Maillard reaction system consisting of a sugar and ammonia—a typical caramel-color preparation method for beverages. 4(5)-MI is identified in various beverages and sauces, which are colored with caramel, as well as in caramel color itself....Various studies indicate that caramel colors and carbonated beverages contain 4(5)-MI in levels ranging from 0 to around 1000 ppm and from 0 to about 500 ppm, respectively. Reports of the toxicity of 4(5)-MI at relatively high levels suggest that it may cause some adverse effects on human consumers.” As taken from Hengel M & Shibamoto T. 2013. J. Agric. Food Chem. 61(4), 780-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23294412?dopt=AbstractPlus>

“Caramel, defined as coloring agent and as an antioxidant, is being used in several kinds of food products. It has been classified into 4 classes to satisfy the requirement of several food and beverage systems. The variation in its consistency owing to its basic content of milk solids, sugars, and fat has been studied. Several methods have been found to estimate the amount of color provided by caramel in food products. Various formulations have been cited for the production of caramel by eradicating the frequent areas of problems during its processing. Caramel has been used as a synthetic colorant replacer in the baking and beverage industries. Researchers have aimed to ascertain the contribution to the antioxidant activity of some caramel-containing soft drinks. This paper is an overview of the classification, physicochemical nature, formulations, coloring properties, antioxidant properties, and toxicity of caramel in different food systems.” As taken from Sengar G & Sharma HK. 2014. J. Food. Sci. Technol. 51(9), 1686-1696. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25190825>

Record for caramel (color) (CAS RN 8028-89-5):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo -	In vitro	Mouse -	865.7	In Vitro	TIVIEQ Toxicology In Vitro.

Inhibitor Concentration Low		fibroblast	mg/L/1H	Toxicity Studies - phototoxicity in vitro	(Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 25,1242,2011
ICLo - Inhibitor Concentration Low	In vitro	Mouse - fibroblast	2.2 mg/L/6H	In Vitro Toxicity Studies - apoptosis in vitro	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 25,1242,2011
ICLo - Inhibitor Concentration Low	In vitro	Mouse - fibroblast	48.7 mg/L/6H	In Vitro Toxicity Studies - phototoxicity in vitro	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 25,1242,2011

As taken from RTECS, 2019a

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

"A study was conducted in rats to determine whether haematological changes were associated with the feeding of high dietary concentrations of caramel colour IV. Sixty-six male rats, mean initial body weights approximately 173 g, were fed caramel colour IV in the diet for 28 days. Sixteen rats were assigned to a control group and 10 rats/group were assigned to levels of 16 or 22% caramel colour IV (high colour intensity) and 34 or 47% caramel colour IV (low colour intensity). Caramel colour III was fed at a 4% level to a positive control group. The parameters observed were body weight, food consumption, haematology (total and differential leucocyte counts), and terminal necropsy with organ weights (caecum - full and empty). Rats were bled from the retro-orbital sinus on days 16, 9, and 2 before treatment and again on days 7, 14, and 28 during treatment. Feeding of these high dietary levels of caramel colour IV led to reduced rates of body-weight gain. The animals fed caramel colour III gained weight at a rate similar to the controls. Animals receiving caramel colour III had progressively lower relative lymphocyte counts commencing 1 week after treatment and increasing in severity with duration of treatment. This pattern of decreased relative lymphocyte counts was not seen with either sample of caramel colour IV and no significant decreases in lymphocyte counts were observed at any dose level of this caramel colour. Caecal weights were increased in all treatment groups (BIBRA, 1978)."

As taken from WHO Food Additives Series 20, available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

"BACKGROUND: Atherosclerosis is a major health burden. Metabolic disorders had been associated with large consumption of soft drinks. The rising incidence of atherosclerosis and metabolic alterations warrants the study of long-term soft drink consumption' effects on metabolism

and atherosclerosis in genetic deficiency of apolipoprotein E which typically develops spontaneous atherosclerosis and metabolic alterations. METHODS: ApoE^{-/-} mice were randomized in 3 groups accordingly with free access to: water (W), regular cola (C) or light cola (L). After 8 weeks, 50% of the animals in each group were euthanized (Treatment: W8, C8, L8). The remaining mice (all groups) drank water for 8 weeks and were euthanized (Washout: W16, C16, L16). Body weight and food and drink consumption were periodically measured. Blood was collected (biochemistry). At autopsy, transverse aortic sinus sections were serially cut and stained (histomorphometry); livers and kidneys were processed (microscopy). MANOVA (identification of variance factors) was followed by ANOVA and LSD tests (within-factor differences between levels). Conventionally a $P < 0.05$ was considered significant. RESULTS: Treatment increased drinking volumes (vs W8: 4 fold C8, $P < 0.0001$; +47% L8, $P < 0.02$). Only C reduced eating amounts (-54%, $P < 0.05$ vs W8). I). Compared with W8: C8 developed hyperglycemia (+43%, $P < 0.03$) and increased non-HDL cholesterol (+54%, $P < 0.05$); L8 showed decreased glycemia (-15%, $P < 0.05$ vs W8) and increased creatinine (2.5 fold, $P < 0.04$), urea (+74, $P < 0.03$) and aspartate-aminotransferase (2.8 fold, $P < 0.05$). Hypercreatininemia was observed in L16 (2.7 fold vs W16, $P < 0.05$). Hypertriglyceridemia (+91%, $P < 0.008$) and hyperuremia (+68%, $P < 0.03$) developed over time of study (age). II). Treatment caused plaque area increase (vs W8: 28% C8, $P < 0.02$ and 50% L8, $P < 0.01$; vs W16: 43% C16, $P < 0.05$ and 68% L16, $P < 0.02$) and stenosis (vs W8: 38% C8, $P < 0.04$ and 57% L8, $P < 0.01$; vs W16: 71% C16, $P < 0.01$ and 46% L16, $P < 0.04$). Age also caused plaque area increase (56%, $P < 0.04$). Treatment- and age-effects on plaque enlargement were additive. CONCLUSION: Cola beverages caused atherosclerotic lesions' enlargement with metabolic (C) or non metabolic disturbances (L). ApoE^{-/-} mice were particularly sensitive to L treatment. These findings may likely relate to caramel colorant and non-nutritive sweeteners in cola drinks and have potential implications in particularly sensitive individuals." As taken from Otero-Losada ME et al. 2013. Cardiovasc. Diabetol. 12, 57. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23547749>

6.3. Nervous system

"4-MEI has been shown to be the most likely toxic component in ammoniated molasses (caramel color III), its being a convulsant to rabbits, mice, and chicks at oral doses of 360 mg/kg b.w. (Nishie et al., 1969)." As taken from WHO Food Additives Series 20.

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

No data available to us at this time.

7. Addiction

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

8. Burnt ingredient toxicity

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

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

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	500	Carmines, 2002 & Rustemeier et al., 2002
	16,000	Baker et al., 2004a
	700 700 (No CAS) 7540 (Cigar)	JTI KB Study Report(s)
	228	Roemer et al., 2014
In vitro genotoxicity	500	Carmines, 2002 & Roemer et al., 2002
	16,000	Baker et al., 2004c
	700 700 (No CAS) 7540 (Cigar)	JTI KB Study Report(s)
	6,840	fGLH Study Report (2010)
	228	Roemer et al., 2014
In vitro cytotoxicity	500	Carmines, 2002 & Roemer et al., 2002
	16,000	Baker et al., 2004c
	700 700 (No CAS) 7540 (Cigar)	JTI KB Study Report(s)
	6,840	fGLH Study Report (2010)
	228	Roemer et al., 2014
Inhalation study	1,617	Gaworski et al., 1998
	500	Carmines, 2002 & Vanscheeuwijck et al., 2002
	16,000	Baker et al., 2004c
	700 700 (No CAS)	JTI KB Study Report(s)
	228	Schramke et al., 2014
Skin painting	5	Gaworski et al., 1999
	700	JTI KB Study Report(s)
In vivo genotoxicity	228 7540 (cigar)	Schramke et al., 2014 JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Caramel Color E150D was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Caramel Color E150D when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	299	JTI KB Study Report(s)
In vitro cytotoxicity	299	JTI KB Study Report(s)

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

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

Endpoint	Tested level	Reference
Aerosol chemistry	0.0004 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro genotoxicity	0.0004 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro cytotoxicity	0.0004 mg/(tobacco portion; 310 mg)	Logic (2019)
In vivo genotoxicity	0.0004 mg/(tobacco portion; 310 mg)	Logic (2019)
Inhalation study	0.0004 mg/(tobacco portion; 310 mg)	Logic (2019)

Aerosol from heated tobacco stick(s) containing Caramel and/or caramel color 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	0.16	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	0.16	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	0.16	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that caramel (color) (CAS RN 8028-89-5) is of “uncertain” persistence in the environment.

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

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that caramel (color) (CAS RN 8028-89-5) is not inherently toxic to aquatic organisms.

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

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

No data available to us at this time.

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that caramel (color) (CAS RN 8028-89-5) is of “uncertain” bioaccumulative potential in the environment.

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

11. References

- Adams K et al. (1992). Assessment of the genotoxic potential of Caramel Colour I in four short-term tests. *Food Chem. Toxicol.* 30(5): 397-402.
- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health record for caramel, color (CAS RN 8028-89-5). Dated 10 March 2017. Available at <https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber=8028-89-5>
- AICIS (undated). Australian Government Department of Health. Australian Inventory of Industrial Chemicals. Record for CAS RN 8028-89-5. Available at <https://www.industrialchemicals.gov.au/chemicals/caramel-color>
- Allen JA et al. (1992). Absence of mutagenic activity in Salmonella and of clastogenic activity in CHO cells of Caramel Colours I, II, III and IV. *Food Chem. Toxicol.* 30(5): 389-395.
- Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. *J. Anal. Appl. Pyrolysis* 74, 145–170.
- 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.
- Bonin and Baker (1980). Mutagenicity testing of some approved food additives with the Salmonella/microsome assay. *Food. Technology in Australia.* 32: 608-611.
- Brusick DJ et al. (1992). Genotoxicity hazard assessment of Caramel Colours III and IV. *Food Chem. Toxicol.* 30(5): 403-410.
- Burdock GA (2010). *Fenaroli's Handbook of Flavor Ingredients*. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.
- 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.

- CCRIS (2002). Record for caramel color A (CAS RN 8028-89-5). CCRIS record no. 8705. Last revision date 6 February 2002 (records no longer being updated after 2011). Available at <https://www.toxinfo.io/>
- CosIng. Cosmetic substances and ingredients database. Record for caramel. Undated Available at <https://ec.europa.eu/growth/tools-databases/cosing/>
- Cosmetics Bench Reference (1996). Published by Cosmetics and Toiletries. ISBN 0-931710-51-0.
- COSMOS Database (undated). Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety. Record for caramel (CAS RN 8028-89-5). Available at <https://ng.cosmosdb.eu/>
- CPID (undated). Consumer Product Information Database. Record for caramel (CAS RN 8028-89-5). Available at <https://www.whatsinproducts.com/>
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/thy03c00>
- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/wzp67e00>
- ECHA (2023). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 12 June 2023. Available at: <https://echa.europa.eu/information-on-chemicals/cl-inventory-database>
- ECHA (undated). European Chemicals Agency. Information on Chemicals. Available at:
- EFSA (2011). Scientific opinion on the re-evaluation of caramel colours (E 150 a,b,c,d) as food additives. EFSA Journal 9(3), 2004 (103 pp.). Available at <https://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2004/epdf>
- European Commission (undated). Food additives database. Available at: Evans JG et al. (1977). Long-term Toxicity Study in the Rat on a Caramel Produced by the "Half Open-half Closed Pan" Ammonia Process. Food Cosmetics Toxicology, 15: 523. <https://ec.europa.eu/food/food-feed-portal/screen/food-additives/search>
- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 13 October 2022. Available at: <https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances>
- FDA (2023a). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Title 21. Last updated 24 May 2023. Available at <https://ecfr.federalregister.gov/>
- FDA (2023b). US Food and Drug Administration. Inactive Ingredient Database. Data through 1 January 2023. Available at <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>
- fGLH Study report (2010).
- Folmer DE et al. (2018). A U.S. population dietary exposure assessment for 4-methylimidazole (4-MEI) from foods containing caramel colour and from formation of 4-MEI through the thermal treatment of food. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 35(10), 1890-1910. DOI: 10.1080/19440049.2018.1508892. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/30130454>
- Gaunt I et al. (1977). Toxicological Investigations of Caramels. II A Short-term Study in the Rat on Sodium Hydroxide-catalysed Caramel. BIBRA, Research Report No. 1.
- Gaworski CL et al. (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposures in rats. Inhalation Toxicology, 10:357-381.
- Gaworski CL et al. (1999). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology 139 (1999) 1-17.
- Haldi J & Wynn W (1951). A study to determine whether or not caramel has any harmful physiological effect. I. Unpublished report from Emory University, Atlanta, GA, USA (cited in JECFA, 1987).

- Hall RL and Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. III. GRAS substances. Food Technology, 19(2), 151-197. Available at https://www.femaflavor.org/sites/default/files/3. GRAS Substances%282001-3124%29_0.pdf
- Harigaea Y et al. (2000). Mutagenicity studies of Caramel I. Nippon Shokuhin Kagaku Gakkaishi 7(1):28-34 (cited in CCRIS, 2002).
- Haseman JK (2013). Evaluating consistency in the interpretation of NTP rodent cancer bioassays: an examination of mouse lung tumor effects in the 4-MEI study. Regul. Toxicol. Pharmacol. 66(1), 109-15. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23545072>
- Health Canada (2022). Drugs and Health Products. Natural Health Products Ingredients Database. Records for caramel (CAS RN 8028-89-5) and caramel flavour (no CAS RN listed). Last updated 9April 2022. . Available at <http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredsReq.do?srchRchTxt=caramel&srchRchRole=-1&mthd=Search&lang=eng>
- Hengel M & Shibamoto T (2013). Carcinogenic 4(5)-methylimidazole found in beverages, sauces, and caramel colors: chemical properties, analysis, and biological activities. J. Agric. Food Chem. 61(4), 780-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23294412?dopt=AbstractPlus>
- Houben GF and Penninks AH. (1994). Immunotoxicity of the colour additive caramel colour III; a review on complicated issues in the safety evaluation of a food additive. Toxicology. 1994 Aug 12; 91(3):289-302. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8079366&query=hl=30&itool=pubmed_docsum
- Houben GF et al. (1992). Effects of Caramel Colour III on the Number of Blood Lymphocytes: A Human Study on Caramel Colour III Immunotoxicity and a Comparison for the Results with Data from Rat Studies. Food and Chemical Toxicology, 5, 427.
- HSDB (2002). Record for caramel color (CAS RN 8028-89-5). Hazardous Substances Databank Number: 1920. Last Revision Date: 13 May 2002. Available at <https://www.toxinfo.io/>
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Available at <https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list>
- IUCLID Dataset (2000). Caramel (color) (8028-89-5)JECFA (1987). Toxicological evaluation of certain food additives and contaminants. WHO Food Additive Series 20. Twenty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives.
- JECFA (2001). Evaluation of certain food additives and contaminants. WHO tech. Report 901. Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives.
- JTI KB Study Report (s).
- JTI Study Reports (s).
- Kawachi T et al. (1980). Results of recent studies on the relevance of various short-term screening tests in Japan. Applied Methods in Oncology, 3, 253-267.
- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey.
- 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. (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).
- Liang J et al. (2019). Dietary intake assessment of caramel colours and their processing by-products 4-methylimidazole and 2-acetyl-4-tetrahydroxy-butylimidazole for the Chinese population. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 36(7), 1009-1019. DOI: 10.1080/19440049.2019.1615137. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31100047/>
- Lloyd R. A. et al.(1976). Flue-cured tobacco flavour. 1. Essence and oil components. Tobacco Science, 20, 40.
- Logic (2019). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- MacKenzie KM et al. (1992a). Toxicity and Carcinogenicity studies of Caramel IV in F344 Rats and B6C3F1 mice. Food and Chemical Toxicology 30, 431-443.
- Martindale (1993). The Extra Pharmacopoeia. Edited by J. E. F. Reynolds. Thirteenth edition. The Pharmaceutical Press. ISBN 0-85369-300-5.
- 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.
- Morgareidge K (1974). Teratologic evaluation of FDA 71-82 (caramel, bakers and confectioners) in mice, rats and rabbits. Unpublished, Food and Drug Research Lab. Inc. report No. PB 234-870 (cited in JECFA, 1987).
- OECD. Organisation for Economic Co-operation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Caramel (color) (CAS RN 8028-89-5). Accessed March 2017. Available at: <http://webnet.oecd.org/CCRWeb/Search.aspx>
- Otero-Losada ME et al.(2013). Metabolic disturbances and worsening of atherosclerotic lesions in ApoE-/- mice after cola beverages drinking. Cardiovasc. Diabetol. 12, 57. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23547749>
- 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.
- RTECS (2019a). Registry of Toxic Effects of Chemical Substances. Record for caramel (color) (CAS RN 8028-89-5). Last updated March 2019.
- RTECS (2019b). Registry of Toxic Effects of Chemical Substances. Record for caramel A (no CAS RN given). Last updated March 2019.
- RTECS (2019c). Registry of Toxic Effects of Chemical Substances. Record for caramel C (no CAS RN given). Last updated March 2019.
- 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.
- Selim S et al. (1992). Absorption, distribution and excretion of the colour fraction of Caramel Colour IV in the rat. Food Chem Toxicol. 1992 May; 30(5):445-51. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=1644386&query_hl=35&itool=pubmed_docsum
- Sengar G & Sharma HK (2014). Food caramels: a review. J. Food. Sci. Technol. 51(9), 1686-1696. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25190825>

- Sinkeldam E J et al.(1976). Chronic (two year) feeding study in rats with six different ammonia caramels. Unpublished report No. R 4961 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands (cited in JECFA, 1987).
- Sprong C et al. (2014). Refined exposure assessment of E150 food colours with use levels provided by the industry. RIVM Letter report 050015001. Available at <http://www.rivm.nl/bibliotheek/rapporten/050015001.pdf>
- Stedman, R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207
- Stich H. F. et al (1981).Clastogenic activity of caramel and caramelised sugars. Mutation Res., 91: 129.
- Sugimura et al (1979). Mutagenic factors in cooked foods. CRC Crit. Rev. Toxicol., 6(3): 189-209.
- Thuvander A and Oskarsson A. (1994). Effects of subchronic exposure to Caramel Colour III on the immune system in mice. Food Chem Toxicol. 1994 Jan; 32(1):7-13. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8132168&query hl=33&itool=pubmed_docsum
- Tierney W J (1980). An in-utero range-finding study of caramel colour no. 3, sample 3-1, in rats. Unpublished project no. 79-2405 from Bio/dynamics Inc. East Millstone, NJ, USA (cited in JECFA, 1987).
- Til H P & Spanjers M (1973). Reproduction study in rats with six different ammonia caramels. Unpublished report no. R 4068 from CIVO/TNO, Zeist, The Netherlands (cited in JECFA, 1987).
- Tomankova K et al. (2011). In vitro cytotoxicity and phototoxicity study of cosmetics colorants. Toxicology In Vitro. 25(6):1242-50. PubMed abstract, available at <http://www.ncbi.nlm.nih.gov/pubmed/21570462>
- US EPA (2023a). Safer Chemical Ingredients List. Last updated 27 March 2023. Available at <https://www.epa.gov/saferchoice/safer-ingredients>
- US EPA InertFinder Database (2023). Last updated 24 May 2023. Available at <https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1>
- US EPA Substance Registry Services (SRS) – TSCA and CDR lists. Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.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 (2002) 113-131.
- WHO Food Additives Series 20. Caramel colours, classes I,II,III, and IV. 20-JECFA 29/99. Available at <http://www.inchem.org/documents/jecfa/jecmono/v20je11.htm>

12. Other information

- Klus H et al. (2009). Smokeless Tobacco - An Overview. Beiträge zur Tabakforschung 23(5), 248–276. Available at <http://www.degruyter.com/view/j/cttr.2009.23.issue-5/cttr-2013-0865/cttr-2013-0865.xml?rskey=LEa1cP&result=13>
- Rodgman A (2004). Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. III. Ingredients Reportedly Used in Various Commercial Cigarette Products in the USA and Elsewhere. Beiträge zur Tabakforschung 21(2), 47–104. Available at <http://www.degruyter.com/view/j/cttr.2004.21.issue-2/cttr-2013-0771/cttr-2013-0771.xml?rskey=O0glOm&result=9>

13. Last audited

June 2023

SCIENTIFIC OPINION

on the re-evaluation of caramel colours (E 150 a,b,c,d) as food additives¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The ANS Panel provides a scientific opinion re-evaluating the safety of the caramel colours (E150a (Class I), E150b (Class II), E150c (Class III), E150d (Class IV)) used as food additives. The caramel colours are a complex mixture of compounds produced by heating carbohydrates under controlled heat and chemical processing conditions; they are divided into four classes according to the manufacturing reactants used. The caramel colours were previously evaluated by the SCF and by JECFA, which concluded that a numerical ADI was not necessary for Class I, but established ADIs for the other classes of caramels, ranging from 160-200 mg/kg bw/day. Given the consistency in the toxicological database, the Panel establishes a group ADI of 300 mg/kg bw/day for the caramel colours, by applying an uncertainty factor of 100 to a NOAEL of 30 g/kg bw/day (highest dose tested) identified in 13-week rat studies with Class IV and a similar NOAEL identified in a rat reproductive toxicity study, also with Class IV. Comparable NOAELs for Classes II, III and IV were reported in the SCF and JECFA evaluations. Within this group ADI, the Panel establishes an individual ADI of 100 mg/kg bw/day for Class III due to new information regarding the immunotoxicity of THI. The Panel concludes that the anticipated dietary exposure of child and adult populations may exceed the ADIs for Classes I, III and IV caramels, but exposure estimates to Class II were below the ADI. Exposure estimates for the caramel constituents THI, 4-MEI and SO₂ are not of concern, but the Panel welcomes additional studies to clarify remaining uncertainties regarding effects of THI on the immune system. The Panel notes that other constituents of caramel colours including 5-HMF and furan may be present at levels that may be of concern, and considers that the specifications should include maximum levels for these constituents.

© European Food Safety Authority, 2011

KEY WORDS

Plain Caramel, Caustic Caramel, caramel colour I, E 150a, Caustic Sulphite Caramel, caramel colour II, E 150b, Ammonia Caramel, caramel colour III, E 150c, Sulphite Ammonia Caramel, caramel colour IV, E 150d, CAS Registry Number 8028-89-5, food colouring substance.

1 On request from the European Commission, Question No EFSA-Q-2008-237; EFSA-Q-2008-238; EFSA-Q-2008-239; EFSA-Q-2008-240, adopted on 3 February 2011.

2 Panel members: F. Aguilar, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Gürtler, J. König, C. Lambré, J.-C. Larsen, J.-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R.A. Woutersen. Correspondence: ans@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Group A on Food Additives and Nutrient Sources: F. Aguilar, N. Bemrah, P. Galtier, J. Gilbert, S. Grilli, R. Gürtler, C. Lambré, J.C. Larsen, J.-C. Leblanc, A. Mortensen, I. Pratt, Ch. Tlustos, I. Stankovic, and EFSA staff: A Rincon for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the re-evaluation of caramel colours (E 150a,b,c,d) as food additives. EFSA Journal 2011;9(3):2004 [103 pp.]. doi:10.2903/j.efsa.2011.2004. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of caramel colours (E 150a,b,c,d) when used as food colouring substances.

Caramel colours are colouring substances authorised as food additives in the EU, and are classified according to the reactants used in their manufacture as follows: Class I Plain Caramel or Caustic Caramel (E 150a); Class II Caustic Sulphite Caramel (E 150b); Class III Ammonia Caramel (E 150c) and Class IV Sulphite Ammonia Caramel (E 150d).

The four classes of caramel colours have been previously evaluated by the EU Scientific Committee for Food (SCF), by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by the Nordic Council of Ministers (TemaNord). Both JECFA and the SCF concluded that a numerical Acceptable Daily Intake (ADI) was not necessary for Class I Plain Caramel, considering that it contains no added ammonia or sulphite and that it is likely to be produced in normal cooking processes. For Class II Caustic Sulphite Caramel, JECFA established an ADI of 0-160 mg/kg bw/day, while the SCF included Class II Caustic Sulphite Caramel within the ADI of 200 mg/kg bw/day that it had already established for Class IV Sulphite Ammonia Caramel, based on information indicating that its chemical composition was similar to and intermediate between that of Class I Plain Caramel and Class IV Sulphite Ammonia Caramel. For Class III Ammonia Caramel, the SCF allocated an ADI of 200 mg/kg bw/day with the proviso that the content of the constituent 2-acetyl-4-tetrahydroxy-butyylimidazole (THI) should not exceed 10 mg/kg colour on a colour intensity basis. JECFA has also allocated an ADI of 0-200 mg/kg bw/day to this colour, together with a specification for a maximum level for THI of 25 mg/kg caramel colour. For Class IV Sulphite Ammonia Caramel, both the SCF and JECFA have established an ADI of 200 mg/kg bw/day.

Specifications for the four classes of caramel colours have been defined in Commission Directive 2008/128/EC⁴ and by JECFA (2006). The different classes of caramel colours are variously defined (in addition to their method of production) by the degree of binding to Diethylamino Ethyl (DEAE) cellulose and to phosphoryl cellulose (for Classes I Plain Caramel and Class III Ammonia Caramel), by the absorbance ratio (Classes II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel) and by their colour intensity. The solids content for the different classes range from 62-77% (Class I Plain Caramel), 65-72% (Class II Caustic Sulphite Caramel), 53-83% (Class III Ammonia Caramel) or 40-75% (Class IV Sulphite Ammonia Caramel). The maximum level of the constituent 4-methylimidazole (4-MEI), found in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel only, is restricted to ≤ 250 mg/kg caramel on a colour intensity basis under Commission Directive 2008/128/EC, while the constituent THI, found in Class III Ammonia Caramel only, is restricted to ≤ 10 mg/kg caramel on a colour intensity basis.

The Panel noted that the caramel colours are poorly characterised, and it is not clear whether the controls on manufacturing processes are sufficient to minimise batch-to-batch variability, particularly with respect to levels of individual Low Molecular Weight (LMW) constituents. The wide range of starting materials and reactants that may be used for the production of caramel colours may result in a variety of end products, with different physical, chemical and toxicological properties. The Panel noted that concerns about e.g. chemical composition, purity and similarity of various caramel colours have also been raised in the past by the SCF. The Panel also noted that a number of the identified or theoretical LMW constituents of caramel colours are genotoxic under certain experimental conditions and in some cases have carcinogenic potential, e.g. furan and 5-hydroxymethyl-2-furfural (5-HMF),

⁴ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities, L 6, 10.1.2009, p.6.

which may be relevant to the toxicological profile of the caramel colours. The Panel considered that the toxicological studies carried out on specific caramel colours would have involved exposure to these compounds, and therefore the anticipated toxicological effect should have been detected in these studies, as exemplified by the toxicological profiles of Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel due to the presence of the imidazoles THI and 4-MEI

According to information from industry, slight variation of manufacturing process parameters (starting material, temperature and time) allows the production of a large range of different qualities of product within each caramel category and also results in differences in chemical composition and physical properties. This is further evidenced by variation in THI and/or 4-MEI concentrations in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, respectively. The Panel noted that there was limited information about the relationship between processing parameters for the caramel colours and the formation and nature of heat-derived constituents of these colours.

The Panel noted that data on the toxicokinetics of the caramel colours are very limited, but indicate little uptake of the high molecular weight fraction of the colours from the gastrointestinal tract, with the bulk of the material being excreted in the faeces. Specific data showed that the small fraction of Class IV Sulphite Ammonia Caramel that is absorbed has been shown to be distributed to lymphoreticular tissue, and eventually excreted in the urine. The Panel considered that individual constituents of the LMW fraction of caramel colours (e.g. MW less than 500 g/mol) are likely to be absorbed, although little information is available to confirm this assumption. The caramel colours are of low toxicity both in short-term tests and in chronic toxicity/carcinogenicity studies. The available short-term studies on Class I Plain Caramel, Class II Caustic Sulphite Caramel, Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, employing generally high dose levels in drinking water, show some dose-related effects, including reduced body weight gain associated with reduced food and fluid consumption, pigmentation of mesenteric lymph nodes, enlargement of the caecum, reduced urinary output associated with increases in specific gravity of the urine, diarrhoea and increases in caecal and kidney weights, unaccompanied by any histopathological change. Additionally, animal studies on Class III Ammonia Caramel have shown evidence of lymphocyte depression and other evidence of immunotoxicity, which are considered to be due to the presence of THI, a potent immunosuppressant, in this caramel.

The Panel concluded that the effects on body-weight were in part due to the reduced water intake caused by poor palatability of the drinking water rather than toxic effects of the caramel colours per se, and that the other effects seen were secondary both to the reduced fluid intake and the intake of large quantities of osmotically-active caramel material. The Panel considered that these effects are not of toxicological significance in establishing the safety of the caramel colours.

Caramel colours have been extensively tested for genotoxic potential in a variety of assays *in vitro* and *in vivo*. The results in *in vitro* systems were generally negative, with a few marginally positive findings, and no positive findings have been reported in *in vivo* assays. Overall the Panel concluded that there were no concerns regarding the genotoxic potential of caramel colours.

The findings in the long-term toxicity studies carried out with Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel were similar to, and did not reveal any pattern of toxicity not already seen in, the 90-day oral toxicity studies carried out with these caramel colours. No evidence of carcinogenicity was seen in 2-year studies in rats on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. In a parallel study in mice, there was similarly no evidence for a carcinogenic potential of Class IV Sulphite Ammonia Caramel. The Panel noted that no long-term toxicity or carcinogenicity data were available for Class I Plain Caramel and Class II Caustic Sulphite Caramel. The Panel considered however that given the fact that Class I Plain Caramel is likely to be produced in normal cooking processes, also considering the long-term toxicity and carcinogenicity data available on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, and the rather similar toxicological profile of all the caramel colours, there are no concerns regarding the long-term

toxicity or carcinogenicity of Class I Plain Caramel or Class II Caustic Sulphite Caramel, nor regarding the carcinogenicity of Class III Ammonia Caramel or Class IV Sulphite Ammonia Caramel.

In relation to the reproductive and developmental toxicity of the caramel colours, the Panel noted that no data were available for Class I Plain Caramel and Class II Caustic Sulphite Caramel. However given the data available on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel and the toxicological similarities between all four classes of caramel colours, the Panel concluded that there are no concerns regarding reproductive and or developmental toxicity of Class I Plain Caramel or Class II Caustic Sulphite Caramel. There were no indications that either Class III Ammonia Caramel or Class IV Sulphite Ammonia Caramel can induce reproductive and or developmental toxicity in mice, rats, or rabbits following gavage administration at levels of up to 1600 mg/kg bw/day. In a reproductive toxicity study with Class IV Sulphite Ammonia Caramel in rats, at the top dose of 25% in the diet, equivalent to approximately 28 g/kg bw/day, pups showed a higher incidence of alopecia compared with pups in the control group and a generalized poor condition during the last 7 days of suckling. The number of implantation sites, litter size and of live pups at days 0, 4, and 21 of lactation in the 20%-dose group were significantly lower than control values, the Panel noted however that there was no dose-related trend, since in the 25% dose group these parameters were not statistically different from controls. The Panel considered that a No-Observed-Adverse-Effect-Level (NOAEL) of 25-30 g/kg bw/day for female rats could be identified in this study.

The Panel noted that no multigeneration study is available on any of the four classes of caramel colours.

Due to a lack of data, no definite conclusion can be drawn with respect to intolerance and allergenicity to the four classes of caramel colours under evaluation. The Panel noted, however that no cases of intolerance and allergenicity intolerance and allergenicity have been reported in published literature.

In relation to the potential haematotoxicity/immunotoxicity of caramel colours, effects have been identified in animal studies with Class III Ammonia Caramel, but not with the other classes of caramel colours. The Panel noted that lymphocytopenic and immunomodulatory effects have been seen in a number of studies with Class III Ammonia Caramel, and that the overall conclusion to be drawn from these studies is that THI, a constituent in Class III Ammonia Caramel, together with deficiency of pyridoxine (vitamin B₆), as a dietary influence, was primarily responsible for these effects. The Panel also noted that these effects were (apparently) transient in nature, disappearing in the later stages of longer-term studies with Class III Ammonia Caramel. The Panel noted that in the pivotal 90-day study of MacKenzie et al. on Class III Ammonia Caramel, in which rats were dosed with up to 20 g/kg bw/day caramel colour, containing either 15 mg THI/kg caramel or 295 mg THI/kg caramel, on a solids basis, no dose-related lymphocytopenia occurred in the animals fed caramel containing approximately 15 mg THI/kg. The latter level is higher than the current maximum level for THI laid down in the specifications for Class III Ammonia Caramel. Class III Ammonia Caramel containing 295 mg THI/kg, at a dose level of 20 g/kg bw/day, induced a statistically significant decrease in lymphocyte counts in both sexes at 2 weeks and only in male rats at 6 weeks. All lymphocyte values in these groups were normal at the termination of the study. The Panel noted however that the results of a short-term oral study carried out by Thuvander and Oskarssen indicated that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B₆ status.

The 90-day oral toxicity study on Class III Ammonia Caramel in rats carried out by Mackenzie et al. included an evaluation of the toxicity of THI after a 4-week dosing period. In rats maintained on a normal, pyridoxine-replete diet, the short-term NOAEL of THI for the reduction of total lymphocytes was determined to be 120 µg/kg bw/day in female rats, and 380 µg/kg bw/day in male rats. Houben et al. reported intakes of THI alone or Class III Ammonia Caramel containing THI that, in combination with manipulation of dietary pyridoxine levels, resulted in lymphocytopenia and could therefore be regarded as Low-Observed-Adverse-Effect-Levels (LOAELs) for this effect. These ranged from 57.2

µg THI/kg bw/day (provided by a level of 0.4% Class III Ammonia Caramel in drinking water, in rats maintained on a low-pyridoxine diet, 2-3 mg/kg diet) to levels of 200 µg THI/kg bw/day or higher. Sinkeldam and co-workers reported decreases in lymphocyte counts in rats receiving 0.1% Caramel Colour (III) in drinking water, equivalent to 20 µg THI/ kg bw/day, for 1 week and maintained on a low-pyridoxine diet (2-3 mg/kg diet). The Panel considered that these findings in pyridoxine-deficient rats may be of limited relevance for human health risk assessment. Overall the Panel concluded from these results that a NOAEL for the lymphocytopenic effects of THI in pyridoxine-replete rats lies in the range of 120-400 µg/kg bw/day as indicated by the studies of MacKenzie et al, Sinkeldam et al and Houben et al.

The Panel noted that, in contrast to the findings in rats and mice, in the available short-term human studies consumption of Class III Ammonia Caramel had no effects on total or specified white blood cell counts at dose levels of up to 200 mg/kg bw/day (with THI levels almost 20 times above those permitted according to current specifications) albeit following short term exposure periods. The study of Houben et al. included subjects with (mild) pyridoxine deficiency, and the authors, in comparing the results obtained in this study with data in rats maintained on normal and pyridoxine-reduced diets, suggested that, with regard to oral intake of THI, humans are less sensitive to Class III Ammonia Caramel-induced lymphocytopenia than are rats. The Panel agreed with this interpretation, based on the available data.

The Panel noted that another imidazole constituent of Class III Ammonia Caramel, 4-MEI, which is also found in Class IV Sulphite Ammonia Caramel, is considered to be responsible for the convulsions observed after administration of high doses of this caramel to a range of species. The Panel considered that the acute toxicity of 4-MEI is not of toxicological concern since the maximum level of 4-MEI is restricted to ≤ 250 mg/kg in these caramel colours under Commission Directive 2008/128/EC. The Panel noted that 4-MEI has been demonstrated to have a carcinogenic potential in mice in a recent National Toxicology Programme (NTP) study. The Panel considered, however, that the carcinogenic effect of 4-MEI seen in mice in this study was thresholded, based on the lack of genotoxicity of 4-MEI, also noting that alveolar/bronchiolar neoplasms occur spontaneously at high incidence in B6C3F1 mice. The Panel concluded therefore that the intermediate dose of 625 mg 4-MEI/kg diet, equivalent to 80 mg 4-MEI/kg bw/day could be considered to be a NOAEL in this study.

The Panel, in evaluating the overall toxicological database on the four classes of caramel colours, considered that while the potential constituents 4-MEI (present in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel) and THI (found in Class III Ammonia Caramel only) must be taken into account in the safety evaluation of these caramel colours as food additives, as discussed above, all four caramel colours are otherwise similar in their toxicological effects. The Panel also noted that the effects produced by the caramel colours can be anticipated to be additive in nature.

The Panel considered that, in spite of the absence of full chemical characterisation of the four classes of caramel colours, given the consistency in the toxicological database, the caramel colours can be considered as a single group in terms of assessing their safety. The Panel considered therefore that a group ADI can be established for the caramel colours. Given, however, concerns regarding the immunotoxicity of THI, present in Class III Ammonia Caramel, the Panel decided to define an individual ADI for this caramel within the overall group ADI based on the currently available database. While the Panel noted the toxicological data gaps for caramel colours, including the generally sparse database on reproductive toxicity for caramel colours as a whole and the absence of long-term toxicity and carcinogenicity studies on Class I Plain Caramel and Class II Sulphite Caramel, the Panel considered that missing data for these classes of caramel colours can be accounted for by data from another class.

The Panel considered that several studies carried out in rats with the different caramel colours are relevant for establishment of a group ADI for the caramel colours. These include the three studies used previously by JECFA and SCF to define the respective ADIs for the individual Classes:

- a 90-day study with Class II Caustic Sulphite Caramel, providing a NOAEL of 16 g/kg bw/day, the highest dose tested,
- a 90-day study with Class III Ammonia Caramel, providing a NOAEL of 20 g/kg bw/day, the highest dose tested,
- a 2-year oral toxicity study in rats with Class IV Sulphite Ammonia Caramel, providing a NOAEL of 10 g/kg bw/day, the highest dose tested.

The Panel considered that several additional toxicological studies should be taken into account when establishing the group ADI, and these include:

- a 13-week toxicity study in rats with Class IV Sulphite Ammonia Caramel in drinking water , providing a NOAEL of 30 g/kg bw/day, the highest dose level tested,
- another 13-week drinking water study in rats with Class IV Sulphite Ammonia Caramel providing a NOAEL of 30 g/kg bw/day, the highest dose level tested,
- a 90-day study in Beagle dogs with Class IV Sulphite Ammonia Caramel providing a NOAEL of 6.25 g/kg bw/day, the highest dose tested,
- a 96-week drinking water study in mice with Class III Ammonia Caramel, providing a NOAEL of 8.4 g/kg bw/day, the highest dose tested,
- a 2-year dietary study in rats with Class III Ammonia Caramel providing a NOAEL of 3 g/kg bw/day, the highest dose tested,
- a 104-week study in rats with Class III Ammonia Caramel, providing a NOAEL of 2 g/kg bw/day, the highest dose tested,
- a reproductive toxicity study in rats with Class IV Sulphite Ammonia Caramel providing in the dams a NOAEL of 25-30 g/kg bw/day.

Given that:

- the NOAELs in all these studies were the highest dose levels tested,
- the effects of the caramel colours in 90-day studies were generally similar to those reported in the long-term studies,
- available reproduction and developmental toxicity studies, although limited, do not reveal any effects of concern,
- the studies reveal no effects on the reproductive organs,
- the effect of most concern, i.e. lymphocytopenia, can, as also stated by JECFA, best be evaluated from short-term studies, and
- the long-term studies support the conclusion that the caramel colours are not carcinogenic,

the Panel decided to use the highest NOAEL of 30 g/kg bw/day reported in several of these studies, still the highest dose level tested, as the basis to derive a group ADI for the caramel colours. The Panel

noted that whilst there were arguments for increasing the default uncertainty factor of 100, to compensate for limitations in the toxicological databases on reproductive toxicity, equally compelling arguments could be advanced for deriving a chemical-specific adjustment factor below the default uncertainty factor. The Panel therefore applied an uncertainty factor of 100 to the NOAEL of 30 g/kg bw/day to derive a group ADI of 300 mg/kg bw/day. Overall, based on the available database, the Panel considered that this would provide a sufficient margin of safety.

In relation to Class III Ammonia Caramel, the Panel considered the available data on the immunotoxicity of THI, a constituent of this caramel class only. The Panel noted that no dose-related effects on haematological parameters were reported in the 90-day study of MacKenzie, using a Class III Ammonia Caramel containing a THI level of 15 mg/kg, while with a Class III Ammonia Caramel containing a much higher level of THI of 295 mg/kg, only transient effects on lymphocytes were seen. The study of MacKenzie provided a NOAEL of 20 g/kg bw/day for Class III Ammonia Caramel, the highest dose tested. The Panel also noted, however, the results of the study of Thuvander and Oskarssen, indicating that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B₆ status. While the Panel considered that this study should not be used as a pivotal study for the purposes of risk assessment without further substantiation, given a number of studies in rats showing no effect on haematological parameters over longer periods and at higher dose levels than those used in the study of Thuvander and Oskarssen, the Panel considered that it should be taken into account in establishing an ADI for Class III Ammonia Caramel. The Panel applied an additional uncertainty factor of 2 together with the default uncertainty factor of 100 to the NOAEL of 20 g/kg bw/day identified from the MacKenzie study. The Panel therefore establishes, within the group ADI for all caramel colours and based on the currently available database, an ADI of 100 mg/kg bw/day for Class III Ammonia Caramel.

The Panel noted that this means that within the group ADI of 300 mg/kg bw/day established for the four caramel colours, only 100 mg/kg bw/day of this 300 mg/kg bw/day can be made up by Class III Ammonia Caramel.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers. As caramel colours Class I, II, III and IV are authorised *quantum satis* in almost all categories, the refined exposure estimates have been performed only for Tier 3 using the maximum reported use levels or when no usages were reported to EFSA, values defined by decision rules for *quantum satis* usages were used.

Exposure estimates for children (1-14 years old) have been done by the Panel for 11 European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland, Germany, Denmark, Cyprus, Greece) based on detailed individual food consumption data provided by the EXPOCHI consortium. As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5- 4.5 years) were made by the Panel with the use of detailed individual food consumption data available from the Union of European Beverage Associations (UNESDA) report. For the adult population, the Panel has selected the UK population as representative of the EU consumers for estimates of exposure.

The mean dietary exposure of European children including UK pre-school children ranged from 76.9 to 427.2 mg/kg bw/day for Class I Plain Caramel, from 8.7 to 34.6 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 21.7 to 302.4 mg/kg bw/day for Class III Ammonia Caramel, and from 23.2 to 506.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel. At the 95th or 97.5th percentile, estimates ranged from 179.6 to 882.2 mg/kg bw/day for Class I Plain Caramel, from 18.5 to 117.3 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 107.9 to 757.3 mg/kg bw/day for Class III Ammonia Caramel, and from 129.7 to 1480.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors (>10% in all or several countries) to the total anticipated exposure of children were for Class I Plain Caramel: non alcoholic flavoured drinks (12% to 55%), fine bakery wares (15% to 32%), desserts including flavoured milk products (11% to 48%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 56%), soups (11% to 32%) and malt bread (16% to 49%). For Class II Caustic Sulphite Caramel the main contributors were fine bakery wares (12% to 53%), desserts including flavoured milk products (11% to 41%), edibles ices (11% to 22%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 45%), soups (18% to 54%) and malt bread (19% to 55%). For Class III Ammonia Caramel the main contributors were fine bakery wares (13% to 45%), desserts including flavoured milk products (12% to 44%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 79%), and vinegar (12% to 45%), while in one country non alcoholic flavoured drink, malt bread confectionery, and sausages, pates and terrines contributed 29%, 15%, 13% and 10%, respectively. For Class IV Sulphite Ammonia Caramel the main contributors were non alcoholic flavoured drinks (13% to 51%), confectionery (20% to 81%), fine bakery wares (10% to 29%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (10% to 24%), and malt bread (10% to 34%).

The anticipated dietary exposure reported for the UK adult population gives a mean of 136.6 mg/kg bw/day and a 97.5th percentile of 429.3 mg/kg bw/day for Class I Plain Caramel; a mean of 21.7 mg/kg bw/day and a 97.5th percentile of 109.5 mg/kg bw/day for Class II Caustic Sulphite Caramel; a mean of 60.8 mg/kg bw/day and a 97.5th percentile of 295.0 mg/kg bw/day for Class III Ammonia Caramel; and a mean of 89.4 mg/kg bw/day and 97.5th percentile of 368.9 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors to the total anticipated exposure of adults were for Class I Plain Caramel non alcoholic flavoured drinks (30%), beer and cidre bouché (27%), soups (16%), and sauces, seasonings (e.g. curry powder, tandoori) and pickles (10%). For Class II Caustic Sulphite Caramel the main contributors were beer and cidre bouché (50%) and soups (20%). For Class III Ammonia Caramel the main contributors were beer and cidre bouché (48%) and sauces, seasonings (e.g. curry powder, tandoori) and pickles (22%). For Class IV Sulphite Ammonia Caramel the main contributors were confectionery (65%) and non alcoholic flavoured drinks (23%).

The Panel also evaluated combined anticipated dietary exposure to all four classes of caramel colours, taking into account the highest maximum reported level for all caramel classes, described in Table 8, from each food category. When considering this scenario, as presented in Table 10, anticipated mean combined dietary exposure reported for European children, including UK pre-school children, ranged from 83.5 to 698.3 mg/kg bw/day. At the 95th/97.5th percentile, estimates ranged from 224.8 to 1672.3 mg/kg bw/day. For the UK adult population this scenario gave a range of exposures of 194.8 and 474.3 mg/kg bw/day for the mean and the 97.5th percentile, respectively.

The main contributors to the total combined anticipated exposure to caramel colours for children were non alcoholic flavoured drinks (11% to 28%), confectionery (19% to 58%), fine bakery wares (15% to 29%), desserts including flavoured milk products (10% to 31%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (14% to 44%), and malt bread (16% to 46%). Soups were estimated to contribute from 25% to 28% in two countries and vinegar was estimated to contribute 20% in one country. For the adult population the main contributors (>10%) were confectionery (30%), non alcoholic flavoured drinks (21%), beer, cidre bouché (19%) and soups (11%).

The Panel noted that the anticipated dietary exposure of the adult population at the 97.5th percentile to Class I Plain Caramel exceeds the group ADI of 300 mg/kg bw/day proposed for the caramel colours. Similarly, the anticipated dietary exposure of the adult population at the 97.5th percentile to Class IV Ammonia Caramel exceeds this group ADI. For children, the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for Class I Plain Caramel exceed the group ADI of 300 mg/kg bw/day. Similarly, for children, the upper end of both the mean intake ranges and also the

95th/97.5th percentile intakes for IV Sulphite Ammonia Caramel exceed the group ADI of 300 mg/kg bw/day.

The anticipated dietary exposure to Class II Sulphite Caramel for both adults and children was below the group ADI of 300 mg/kg bw/day.

For Class III Ammonia Caramel the upper end of the mean intake range for children exceeds the individual ADI of 100 mg/kg bw/day established for this colour within the group ADI, while the 97.5th percentile anticipated dietary exposures of both the child and adult populations are above this ADI of 100 mg/kg bw/day.

The Panel noted that anticipated combined dietary exposures of both adults and children to all caramel colours exceed the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile, while the ADI is also exceeded by the combined mean intake for children. In the case of children, this exceedance applies to the upper end of the exposure range only.

Reflecting the concerns regarding the immunotoxicity seen in a number of studies with either Class III Ammonia Caramel or with THI alone, found as a constituent in Class III Ammonia Caramel, and the carcinogenicity of 4-MEI, found as a constituent in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, the Panel has estimated exposure to THI and 4-MEI as a result of consumption of these caramel colours in the diet. Additionally the Panel has also estimated exposure to sulphur dioxide, present in Class II and Class IV caramel colours as a result of the production method. The Panel concludes overall that the exposure estimates for THI, 4-MEI or sulphur dioxide are not of concern, but notes remaining uncertainties regarding the effects of THI on the immune system. The Panel would welcome additional studies to clarify these effects.

The Panel notes that variations in the manufacturing processes of the caramel colours may result in a wide variability in the nature and levels of the various constituents, including constituents of toxicological concern such as 5-HMF and furan. Given this likely variability, the Panel considers that in order to further guarantee the safety of caramel colours with respect to their minor constituents, such as THI, 4-MEI, 5-HMF and furan, it would be prudent to reduce their levels as much as technologically feasible. The Panel considers therefore that the specifications for the caramel colours should be updated and extended to also include maximum levels for constituents of possible concern not yet included in the specifications, such as for example 5-HMF and furan.

The Panel additionally concludes that there is limited information about the relationship between processing parameters for the caramel colours and the formation and nature of heat-derived constituents, which is also relevant for the control of manufacturing processes. Future research work is recommended in this respect.

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	10
Background as provided by the European Commission.....	11
Terms of reference as provided by the European Commission	11
Assessment	12
1. Introduction	12
2. Technical data	12
2.1. Identity of the substances.....	12
2.2. Specifications	15
2.3. Manufacturing process	18
2.4. Methods of analysis in food	19
2.5. Reaction and fate in food	19
2.6. Case of need and proposed uses	19
2.6.1. Actual levels of use of caramel colours Class I, II, III and IV.....	20
2.7. Information on existing authorisations and evaluations.....	33
2.8. Exposure	35
2.8.1. Exposure assessment	35
3. Biological and toxicological data	39
3.1. Absorption, distribution, metabolism and excretion	39
3.2. Toxicological data.....	40
3.2.1. Acute oral toxicity	40
3.2.2. Short-term and subchronic toxicity	40
3.2.3. Genotoxicity	45
3.2.4. Chronic toxicity and carcinogenicity.....	49
3.2.5. Reproductive and developmental toxicity	52
3.2.6. Hypersensitivity (intolerance/allergenicity)	55
3.2.7. Haematotoxicity and immunotoxicity	55
3.2.8. Human studies	56
3.2.9. Studies on 4-methylimidazole (4-MEI) and 2-acetyl-4-tetrahydroxy-butylimidazole (THI)	57
3.2.10. Overview of the toxicological profiles of furan and 5-HMF, LMW constituents of the caramel colours	60
4. Discussion.....	61
Conclusions	71
Documentation provided to EFSA	72
References	74
Annex A.	86
Annex B.....	88
Annex C.....	93
Annex D.	95
Glossary and/or abbreviations	101

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the Framework Directive 89/107/EEC⁵ on food additives, the Scientific Committee for Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor the European Food Safety Authority (EFSA).

Directive 89/107/EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives⁶ which applied as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

In accordance with Regulation (EC) No 1333/2008, the Commission should, after consultation with EFSA, set up by 20 January 2010 an evaluation programme for EFSA to re-evaluate the safety of the permitted food additives. That programme will define the needs and the order of priorities according to which the approved food additives are to be examined.

Food colours were among the first additives to be evaluated, therefore many of the evaluations are old. For some of these colours new studies have become available and the results of these studies should be included in the evaluation. Therefore, food colours should be evaluated with priority. The order of priorities for the re-evaluation of the remaining permitted food additives will be set in the Regulation for the re-evaluation program.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to start a systematic re-evaluation of all authorised food additives and to issue scientific opinions on these additives, taking into account that colours as a group should be given the highest priority for re-evaluation for the reasons outlined above.

⁵ OJ L 40, 11.2.1989, p. 27

⁶ OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. INTRODUCTION

The present opinion deals with the re-evaluation of the safety of caramel colours (E 150a,b,c,d) when used as food colouring substances.

Caramel colours are colouring substances authorised as food additives in the EU. The caramel colours are divided into four classes, Class I, Class II, Class III, and Class IV, according to the reactants used in their manufacture. The four classes of caramel colours have been previously evaluated by the EU Scientific Committee for Food (SCF) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Nordic Council of Ministers have reviewed the caramel colours in a report which takes into account the literature published on these colouring substances up to the year 2000 (TemaNord, 2002).

The Panel was not provided with newly submitted dossiers on the caramel colours and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. TECHNICAL DATA

2.1. Identity of the substances

The caramel colours are a complex mixture of compounds produced by heating carbohydrates under controlled heat and chemical processing conditions (JECFA, 2006). They are dark brown to black liquids or solids having an odour of burnt sugar and a pleasant, somewhat bitter taste (Kamuf et al, 2003; JECFA, 2006). They are miscible with water and contain colloidal aggregates that account for most of their colouring properties and characteristic behaviour towards acids, electrolytes and tannins (Kamuf et al., 2003; JECFA, 2006).

The caramel colours are covered by the general CAS Registry number of 8028-89-5 and EINECS number 232-435-9. They have no specific chemical name, structural or chemical formula. The caramel colours are grouped into four classes according to the reactants used in their manufacture as described in section 2.3: Manufacturing Process, and synonyms used for the four classes of caramel colours are as follows:

- Class I Plain Caramel (E 150a): caramel colour I, caustic caramel and spirit caramel.
- Class II Caustic Sulphite Caramel (E 150b): caramel colour II and caustic sulphite process.
- Class III Ammonia Caramel (E 150c): caramel colour III, ammonia process caramel, closed-pan ammonia process caramel, open-pan ammonia process caramel, bakers' caramel, confectioners' caramel, and beer caramel.
- Class IV Sulphite Ammonia Caramel (E 150d): caramel colour IV, Sulphite Ammonia process caramel, sulphite ammonia process, acid-proof caramel, beverage caramel, and soft-drink caramel.

Each caramel colour carries an isoelectric charge which is different for each class (Licht et al., 1992a).

Dross and Baltes (1989), Licht et al. (1992a) and others describe caramel colours as complex mixtures with a composition approximating that shown below:

Mass Range (g/mol)	Composition
0-1000	56-60%
1000-10 000	ca. 20%
>10 000	20-30%

In the analysis of the Low Molecular Weight (LMW) fraction (constituents with MW below 1000 g/mol) of Caramel Class IV, five of the major constituents have been identified as disaccharides, glucose, 1,6-anhydroglucose, laevulinic acid and 5-hydroxymethyl-2-furfural (5-HMF) (Licht et al., 1992c). 5-HMF is used as a marker substance of the LMW fractions (Licht et al., 1992 b-c). Licht and co-workers found 5-HMF in all 157 samples of Caramel Class I, II, III and IV analysed. For Class I, levels of 5-HMF ranged between 700 and 27 300 mg/kg on an as is basis (9 samples analysed); for Class II, levels were between 3300 and 33 700 mg 5-HMF/kg (18 samples analysed); for Class III, between 10 and 3900 mg 5-HMF/kg reported; Class IV, between 4900 and 21 400 mg 5-HMF/kg reported (90 samples analysed) (Licht et al., 1992b-c; EUTECA, 2011a,b). Another study indicated that caramel products may contain 110-9500 mg 5-HMF/kg (Murcovic and Pichler, 2006).

The Panel noted that extensive research has been carried out into the chemistry of the browning process of complex foods (e.g. the Maillard reaction (O'Brien et al., 1998)), and also into the chemistry of caramelisation of sugars such as sucrose (Kitts et al., 2006), with identification of LMW substances such as acrylamide, 5-HMF, 2-furfyl methyl ketone and 5-methyl-2-furfyl methyl ketone, which have either also been detected in the caramel colours or are theoretical LMW constituents of these mixtures. LMW constituents of caramel colours produced with ammonia include *N*-heterocyclic compounds such as pyridines, pyrazines, pyrroles and imidazoles (Patey et al., 1987; Myers and Howell, 1992) although no quantitative data are available (EUTECA, 2011b). The occurrence of furanoid compounds has also been reported (Meyers and Howell, 1992), with furan being specifically identified in some caramel colours e.g. Class IV Sulphite Ammonia Caramel (Bononi and Tateo, 2007). Industry has reported that levels of furan up to 0.18 mg/kg caramel colour have been detected (Class I (1 sample): 0.15 mg/kg; Class II (1 sample): 0.05 mg/kg; Class III (1 sample): 0.18 mg/kg; Class IV (28 samples): average: 0.02 mg/kg, maximum: 0.06 mg/kg) (EUTECA, 2010b; EUTECA, 2011).

Industry has reported that acrylamide was not detectable in 16 commercial samples of Caramel Class IV at a limit of detection of 1 µg/kg caramel colour (EUTECA, 2011a,b). Caramel Classes I, II and III have not been investigated in this study, the formation of acrylamide not being expected in caramel classes I or II due to the lack of a nitrogen source in their manufacturing process (EUTECA, 2011a). In a single sample of Caramel Class III analysed, acrylamide was not detectable at a limit of detection of 100 µg/kg caramel colour (EUTECA, 2011b).

Furthermore industry presented recent analytical data of caramel colours manufactured in the years between 2008 and 2010 on levels of 2-acetyl-4-tetrahydroxy-butylimidazole (THI) (Class III (45 samples): mean: 7.7 mg/kg, range: 2.4–10 mg/kg) and 4-methylimidazole (4-MEI) (Class III (103 samples): mean: 41.6 mg/kg, range: 5–140 mg/kg; Class IV single strength (35 samples): mean: 102.4 mg/kg, range: 48–183 mg/kg; Class IV double strength (152 samples): mean: 88.3mg/kg, range: 22.7–147 mg/kg). The data on the occurrence of heat-derived constituents in caramel colours which have been made available by industry are compiled in Table 1.

Table 1: Occurrence of heat-derived constituents in Caramel Classes I, II, III and IV according to data provided by industry (EUTECA, 2011)

	Class I	Class II	Class III	Class IV
5-HMF	700 – 27 300 mg/kg (n = 9)	3300 – 33 700 mg/kg (n = 18)	10 – 3900 mg/kg (n = 40)	4900– 21 400 mg/kg (n = 90)
Furan	151 µg/kg (n = 1)	52 µg/kg (n= 1)	177 µg/kg (n = 1)	Maximum: 59 µg/kg (n = 28)
THI	No data	No data	2.4 – 10 mg/kg (n = 45)	No data
4-MEI	No data	No data	5 - 140 mg/kg (n = 103)	Single strength: 48 -183 mg/kg (n = 35) Double strength: 23-147 mg/kg (n = 152)
Acrylamide	No data	No data	Not detected (limit of detection = 100 µg/kg) (n = 1)	Not detected (limit of detection = 1 µg/kg) (n = 16)
Otherfuran-, pyridines-, pyrazines- and imidazole- derivatives	No data	No data	No data	No data

The Panel noted that the complexity of caramel colours means that they are rather poorly characterised, and it is not clear whether manufacturing processes are sufficiently controlled to guarantee limited batch-to-batch variability of both LMW and higher molecular weight constituents. The wide range of starting materials and reactants that may be used for the production of caramel colours may result in a variation of end products, with different physical and chemical properties. Concerns about e.g. chemical composition, purity and similarity of various caramel colours have also been raised in the past by the SCF although overall the SCF noted that “*although the caramelisation reaction is a complex one, and many different compounds are formed, especially in the presence of ammonia, within a given class there is a broad similarity in qualitative chemical composition*” (SCF, 1989).

According to information from industry, slight changes in time and temperatures of the reaction, or in starting materials, can produce changes in the chemical composition and physical properties of the final product (EUTECA, 2010b; EUTECA, 2011a). Furthermore industry indicated that variation of manufacturing process parameters allows the production of a large range of different qualities of products within each caramel class. Concerning Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel there is also a wide range of products made by caramel manufacturers, their differences in chemical composition including some variation in THI and/or 4-MEI concentrations, respectively (EUTECA, 2011a).

The Panel noted that there was limited information about the detailed relationship between processing parameters for the caramel colours and the formation and nature of heat-derived constituents. The Panel also noted that a number of the identified or theoretical constituents of caramel colours, e.g. furan, acrylamide and 5-HMF, are genotoxic under certain experimental conditions and in some cases have carcinogenic potential, which may be relevant to the toxicological profile of the caramel colours. The Panel considered however that the toxicological studies carried out on the caramel colours would

have involved exposure to these compounds, and therefore the anticipated toxicological effect due to the LMW fraction constituents should have been detected in these studies.

2.2. Specifications

Specifications for the four classes of caramel colours have been defined in Commission Directive 2008/128/EC⁷ and by JECFA (2006) (Tables 2-5). The different classes of caramel colours are variously defined (in addition to their method of production) by the degree of binding to Diethylamino Ethyl (DEAE) cellulose in an acidic solution (0.025 N HCl) (Licht et al., 1992a; JECFA, 2006; Directive 2008/128/EC). Under these conditions Class II Sulphite Caramel and Class IV Sulphite Ammonia Caramel are negatively charged and bind to the positively charged DEAE cellulose, while Class III Ammonia Caramel, being positively charged, and Class I Plain Caramel, carrying no net charge, do not bind to DEAE cellulose (Licht et al., 1992a).

Classes I Plain Caramel and Class III Ammonia Caramel are differentiated from each other by binding to negatively charged phosphoryl cellulose, the positively charged Class III Ammonia Caramel showing binding, while Class I Plain Caramel shows little or no binding (Licht et al., 1992a). The absorbance ratio (A₂₈₀/560) is used to further distinguish between Classes II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel, while the absorbance ratio of the coloured constituents of the various caramel colours (other than Class I Plain Caramel) bound either to DEAE cellulose or to phosphoryl cellulose provides further differentiation between the classes (Licht et al., 1992a). The solids content for the different classes range from 62-77% (Class I Plain Caramel), 65-72% (Class II Caustic Sulphite Caramel), 53-83% (Class III Ammonia Caramel) or 40-75% (Class IV) (JECFA, 2006).

The caramel colours are also defined in terms of their colour intensity, colour intensity being expressed as the absorbance of a 0.1% (w/v) solution of caramel colour solids in water in a 1 cm cell at 610 nm (Licht et al., 1992a; JECFA, 2006; Directive 2008/128/EC). The colour intensities of the various classes are specified as follows: Class I Plain Caramel: 0.01-0.12; Class II Caustic Sulphite Caramel 0.06-0.10; Class III Ammonia Caramel: 0.08-0.36; and Class IV Sulphite Ammonia Caramel: 0.10-0.60 (JECFA, 2006). The Panel noted that the specifications for colour intensity laid down in Directive 2008/128/EC are identical to those of JECFA (2006), except for those for Class II Sulphite Caramel, for which Directive 2008/128/EC specifies a range of 0.05 – 0.13. The latter range is also proposed for Class II Sulphite Caramel by Licht et al. (1992a).

Table 2: Specifications for Class I Plain Caramel according to Commission Directive 2008/128/EC and JECFA (2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006) ⁽²⁾
Colour bound by DEAE cellulose	≤ 50 %	≤ 50 %
Colour bound by phosphoryl cellulose	≤ 50 %	≤ 50 %
Solid content	-	62 – 77 %
Colour intensity	0.01 - 0.12 ⁽¹⁾	0.01 – 0.12
Total nitrogen	≤ 0.1 %	≤ 0.1 %
Total sulphur	≤ 0.2 %	≤ 0.3 %
Arsenic	≤ 1 mg/kg	≤ 1 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb) ⁽³⁾	≤ 25 mg/kg	-

⁷ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities, L 6, 10.1.2009, p.6.

- (¹) Colour intensity is defined as the absorbance of a 0.1 % (w/v) solution of caramel colour solids in water in a 1 cm cell at 610 nm.
- (²) Arsenic and lead metals limits are expressed on the basis of the product as is; other limits and ranges are, unless otherwise stated, expressed on a solids basis.
- (³) The specifications for total heavy metals (as Pb), of < 25 mg/kg will be deleted from the specifications for caramel colours in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008 and repealing Directives: 2008/128/EC (purity criteria concerning colours), 2008/84/EC (purity criteria on food additives other than colours and sweeteners) as amended and 2008/60/EC (purity criteria concerning sweeteners), currently under discussion.

Table 3: Specifications for Class II Caustic Sulphite Caramel according to Commission Directive 2008/128/EC and JECFA (2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006) (³)
Colour bound by DEAE cellulose	> 50%	> 50%
Solid content	-	65 – 72 %
Colour intensity	0.05 – 0.13 ⁽¹⁾	0.06 – 0.10
Total nitrogen	≤ 0.3 % (²)	≤ 0.2 %
Sulphur dioxide	≤ 0.2 % (²)	≤ 0.2 %
Total sulphur	0.3 – 3.5 % (²)	1.3 – 2.5 %
Sulphur bound by DEAE cellulose	> 40 %	-
Absorbance ratio of colour bound by DEAE cellulose	19 – 34	-
Absorbance ratio (A 280/560)	> 50	> 50
Arsenic	≤ 1 mg/kg	≤ 1 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb) ⁽⁴⁾	≤ 25 mg/kg	-

(¹) Colour intensity is defined as the absorbance of a 0.1 % (w/v) solution of caramel colour solids in water in a 1 cm cell at 610 nm.

(²) Expressed on equivalent colour basis, i.e. expressed in terms of a product having a colour intensity of 0.1 absorbance units.

(³) Arsenic and lead metals limits are expressed on the basis of the product as is; other limits and ranges are, unless otherwise stated, expressed on a solids basis.

(⁴) The specifications for total heavy metals (as Pb), of < 25 mg/kg will be deleted from the specifications for caramel colours in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008 and repealing Directives: 2008/128/EC (purity criteria concerning colours), 2008/84/EC (purity criteria on food additives other than colours and sweeteners) as amended and 2008/60/EC (purity criteria concerning sweeteners), currently under discussion.

Table 4: Specifications for Class III Ammonia Caramel according to Commission Directive 2008/128/EC and JECFA (2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006) (³)
Colour bound by DEAE cellulose	≤ 50 %	≤ 50 %
Colour bound by phosphoryl cellulose	> 50 %	> 50 %
Solid content	-	53 – 83 %
Colour intensity	0.08 – 0.36 ⁽¹⁾	0.08 – 0.36
Ammoniacal nitrogen	≤ 0.3 % (²)	≤ 0.4 % (²)
4-Methylimidazole (4-MEI)	≤ 250 mg/kg (²)	≤ 300 mg/kg (≤ 200 mg/kg (²))
2-Acetyl-4-tetrahydroxy-butylimidazole (THI)	≤ 10 mg/kg (²)	≤ 40 mg/kg (≤ 25 mg/kg (²))
Total sulphur	≤ 0.2 % (²)	≤ 0.3 % (²)
Total nitrogen	0.7 – 3.3 % (²)	1.3 – 6.8 % (²)
Absorbance ratio of colour bound by phosphoryl cellulose	13 - 35	-
Arsenic	≤ 1 mg/kg	≤ 1 mg/kg

Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb) ⁽⁴⁾	≤ 25 mg/kg	-

⁽¹⁾ Colour intensity is defined as the absorbance of a 0.1 % (w/v) solution of caramel colour solids in water in a 1 cm cell at 610 nm.

⁽²⁾ Expressed on an equivalent colour basis, i.e. expressed in terms of a product having a colour intensity of 0.1 absorbance units.

⁽³⁾ Arsenic and lead metals limits are expressed on the basis of the product as is; other limits and ranges are, unless otherwise stated, expressed on a solids basis.

⁽⁴⁾ The specifications for total heavy metals (as Pb), of < 25 mg/kg will be deleted from the specifications for caramel colours in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008 and repealing Directives: 2008/128/EC (purity criteria concerning colours), 2008/84/EC (purity criteria on food additives other than colours and sweeteners) as amended and 2008/60/EC (purity criteria concerning sweeteners), currently under discussion.

Table 5: Specifications for Class IV Sulphite Ammonia Caramel according to Commission Directive 2008/128/EC and JECFA (2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006) ⁽⁴⁾
Colour bound by DEAE cellulose	> 50 %	> 50 %
Solid content	-	40 - 75 %
Colour intensity	0.10 - 0.60 ⁽¹⁾	0.10 - 0.60
Ammoniacal nitrogen	≤ 0.6 % ⁽²⁾	≤ 2.8 %
Sulphur dioxide	≤ 0.2 % ⁽²⁾	≤ 0.5 %
4-methylimidazole (4-MEI)	≤ 250 mg/kg ⁽²⁾	≤ 1000 mg/kg (≤ 250 mg/kg ⁽²⁾)
Total nitrogen	0.3 - 1.7 % ⁽²⁾	0.5 - 7.5 %
Total sulphur	0.8 - 2.5 % ⁽²⁾	1.4 - 10.0 %
Nitrogen/sulphur ratio of alcohol precipitate	0.7 - 2.7	-
Absorbance ratio of alcohol precipitate	8 - 14 ⁽³⁾	-
Absorbance ratio (A _{280/560})	≤ 50	≤ 50
Arsenic	≤ 1 mg/kg	≤ 1 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb) ⁽⁵⁾	≤ 25 mg/kg	-

⁽¹⁾ Colour intensity is defined as the absorbance of a 0.1 % (w/v) solution of caramel colour solids in water in a 1 cm cell at 610 nm.

⁽²⁾ Expressed on an equivalent colour basis, i.e. expressed in terms of a product having a colour intensity of 0.1 absorbance units.

⁽³⁾ Absorbance ratio of alcohol precipitate is defined as the absorbance of the precipitate at 280 nm divided by the absorbance at 560 nm (1 cm cell).

⁽⁴⁾ Arsenic and lead metals limits are expressed on the basis of the product as is; other limits and ranges are, unless otherwise stated, expressed on a solids basis.

⁽⁵⁾ The specifications for total heavy metals (as Pb), of < 25 mg/kg will be deleted from the specifications for caramel colours in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008 and repealing Directives: 2008/128/EC (purity criteria concerning colours), 2008/84/EC (purity criteria on food additives other than colours and sweeteners) as amended and 2008/60/EC (purity criteria concerning sweeteners), currently under discussion.

The constituents 4-MEI, found in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, and THI, found only in Class III Ammonia Caramel, are of particular relevance in relation to the toxicological profile of these classes of caramel colours. 4-MEI is limited to a maximum level of 250 mg/kg⁸ caramel colour, expressed on an equivalent colour basis (as defined in the footnotes to

⁸ The limit for 4-MEI in Class III and Class IV caramels is proposed to be 200 mg/kg in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008 and repealing Directives: 2008/128/EC (purity criteria concerning colours), 2008/84/EC (purity criteria on food additives other than colours and sweeteners) as amended and 2008/60/EC (purity criteria concerning sweeteners), currently under discussion.

Tables 4 and 5), under Commission Directive 2008/128/EC and to a maximum level of 200 mg/kg caramel colour according to the JECFA specifications. Analysis of food grade caramel colours at the time of the initial evaluations by JECFA (JECFA, 1972, 1977) indicated that commercial Class III Ammonia Caramel of undefined origin contained 50-500 mg 4-MEI/kg (Heyns, 1971), while other examinations have shown ranges of 100-700 mg 4-MEI/kg (Battelle Memorial Institute, 1971). 4-MEI is formed by interaction of ammonia with reducing sugars (Morgan and Edwards, 1986), and it has been shown that the yields of imidazole compounds increased linearly with the increment of molar ratio of ammonia to glucose (Komoto, 1962).

The residual levels for THI are < 10 mg/kg Class III Ammonia Caramel under Commission Directive 2008/128/EC and < 25 mg/kg according to the JECFA specifications. The absence of THI in Caramel Classes I and II is due to the fact that there is no nitrogen source present that would allow its formation (EUTECA, 2010a). The presence of THI in Class IV Sulphite Ammonia Caramel has not been demonstrated by current analytical methodologies, and it is suggested by the industry that the presence of sulphite/sulphur dioxide may interfere with or inhibits the formation of THI (EUTECA, 2010a).

2.3. Manufacturing process

All four caramel classes are prepared by the heat treatment (caramelisation) of carbohydrates. According to the European Technical Committee on Caramels (EUTECA) website, the reaction is controlled with respect to temperature and pressure, but no information was provided about the actual temperature or pressure used. In all cases the carbohydrate raw materials are commercially available food grade nutritive sweeteners consisting of glucose, fructose, invert sugar and/or polymers thereof (e.g. glucose syrups, sucrose or invert sugars, and dextrose). To promote caramelisation and hence produce greater colour intensity, food-grade acids, alkalis or salts may be used in amounts consistent with Good Manufacturing Practices (GMP) (Kamuf et al, 2003; Emerton, 2008).

The acids used are food-grade sulphuric or citric acids, although phosphoric or carbonic acids may also be used; the alkalis used are ammonium, sodium, potassium or calcium hydroxides or mixtures thereof; the salts used are carbonate, hydrogen carbonates, sulphates, ammonium, sodium, potassium or calcium phosphate (EUTECA, 2007a).

According to JECFA (JECFA, 2006) and EUTECA (2007a) the four classes into which the caramel colours are divided depend on the reagent used in their manufacture:

Class I Plain Caramel. No reagent (no sulphite and/or ammonium compounds).

Class II Caustic Sulphite Caramel. Reagent: sulphite compounds (no ammonium compounds).

Class III Ammonia Caramel. Reagent: ammonium compounds (no sulphite compounds).

Class IV Sulphite Ammonia Caramel. Reagent: sulphite and ammonium compounds.

The ammonium compounds used are one or any of the following: ammonium hydroxide, ammonium carbonate and ammonium hydrogen carbonate, ammonium phosphate, ammonium sulphate, ammonium sulphite and ammonium hydrogen sulphite. Where sulphite compounds are used, they are one or any of the following: sulphurous acid, potassium, sodium and ammonium sulphites and hydrogen sulphites (JECFA, 2006).

Food-grade polyglycerol esters of fatty acids may be used as processing aids (antifoam) in amounts not greater than those required to produce the intended effect. Caramel colour is Polymerase Chain Reaction (PCR) negative, because any trace of protein in corn syrup raw material is denatured under the high temperatures used in caramel manufacturing (Kamuf et al., 2003).

The shelf life for a caramel colour under ambient storage conditions will normally be listed as either one or two years depending on the class. The caramelisation reaction continues at a slow rate during

ambient temperature storage, with both colour and viscosity increasing with time. For example, a class IV double-strength liquid stored at ambient temperature increases in colour intensity from 0.235 to 0.282 over 33 months (Kamuf et al., 2003).

2.4. Methods of analysis in food

Licht et al. (1992b, 1992c) have characterised caramel colour classes, looking for consistency within a class, regardless of manufacturer and intended end-use. Specifications for caramel colours were proposed to ensure comparability within classes (Licht et al., 1992a). Licht et al. (1992a) indicated that methods used for the characterisation of the classes include: ultrafiltration, cellulose chromatography, High Performance Liquid Chromatography (HPLC), size-exclusion HPLC, chromatographic profiling using refractive index and ultraviolet detection, HPLC combined with carbohydrate analysis and Gas Chromatography/Mass Spectrometry (GC/MS). Patey et al. (1985) used Gas Chromatography (GC) and GC/MS to analyse trimethylsilyl derivatives of LMW fractions from caramel colours which revealed as many as 147 individual compounds, with considerable variability in patterns and levels. The technique allowed identification of the manufacturer of UK-produced Class III Ammonia Caramel but not of class type. UK-produced Class I Plain Caramel and Class IV Sulphite Ammonia Caramel and Canadian and Japanese-produced Class II Caustic Sulphite Caramel each had a distinctive fingerprint unlike UK-produced Class III Ammonia Caramel.

Each caramel molecule carries a net electrical (colloidal) charge formed during processing. Class I Plain Caramel carries a slightly negative charge, Class II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel are strongly negative and Class III Ammonia Caramel is strongly positive. These electrical properties can be used in caramel analysis particularly using Capillary Electrophoresis (CE). Royle and Radcliffe (1999) used CE and ultrafiltration to distinguish caramel colours, and later extended this work to distinguish malts from caramel colours (Royle et al., 2002).

The complex nature and limited knowledge of chemical composition of caramel colours makes their identification in food products a significant analytical challenge. However, Ciolino (1998) used a combination of marker compounds and HPLC-UV profiling to detect caramel in adulterated acerloa juice. Sádecká and Tóthová (2010) have used synchronous fluorescence spectroscopy to measure caramel content in brandy and mixed wine spirits at levels above 4 mg/l. There are no recent publications attempting the analysis of caramel colours in more complex foods, although modern instrumental methods and the use of chemometrics for fingerprinting do hold some promise.

2.5. Reaction and fate in food

The complexity of caramel colours makes it almost impossible to study the reaction and fate of individual caramel colours in foods. This can only be addressed by analysing for each of the individual constituents, which to date has not been pursued. However, in general the caramel colours are all heat- and light-stable, although acid stability varies considerably, the negatively-charged caramel colours generally being more stable under acid conditions (Emerton, 2008).

2.6. Case of need and proposed uses

Caramel colours are permitted *quantum satis*⁹ in all foodstuffs except those listed in Annexes II and III of Directive 94/36/EC¹⁰ on colours for use in foodstuffs, in which the use of colours is prohibited or

⁹ Article 2 (7) of Directive 94/36/EC states: 'In the Annexes to this Directive '*quantum satis*' means that no maximum level is specified. However, coloring matters shall be used according to good manufacturing practice at a level not higher than is necessary to achieve the intended purpose and provided that they do not mislead the customer'.

¹⁰ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Communities, L 237, 10.9.94.

restricted to certain food colours. The main use of the caramel colours is to impart a brown colour and associated characteristic caramel flavour to a range of foodstuffs including, in particular, non-alcoholic (cola-type) drinks, beers and ciders, meat/fish analogues and soups and gravies (Tennant, 2007). The relative proportions of the four classes of caramel colours used in Europe are as follows: 1 %: Class I Plain Caramel, 2 %: Class II Caustic Sulphite Caramel, 25-27 %: Class III Ammonia Caramel and 70-72 %: Class IV Sulphite Ammonia Caramel (EUTECA, 2007b). Use of Class IV Sulphite Ammonia Caramel is mostly associated with cola-flavoured drinks.

2.6.1. Actual levels of use of caramel colours Class I, II, III and IV

The current use levels of the caramel colours was made available to the Panel for several food categories for finished products by EUTECA (Tennant, 2007,) and by the Confederation of the Food and Drink Industries of the EU (CIAA) (CIAA, 2009). These data are presented in Tables 6 and 7.

EUTECA carried out a survey of usage as part of a wider survey by the Natural Food Colours Association (NATCOL) in 2006-2007 (Tennant, 2007). All manufacturers of caramel colours who are members of EUTECA were asked at the time of the survey to provide information about the formulation of the products in combination with the amounts of the formulation used in each food. No usage data were made available as a result of this survey for Class I Plain Caramel (E150a) or Class II Caustic Sulphite Caramel (E150b). However new information on usage data was made available to the Panel from EUTECA, based on a study implemented on February 2010 by the SFFCC (Syndicat des Fabricants Français de Caramels Colorants) (EUTECA 2010c). The Panel noted that in comparison to the data provided in the Tennant report (2007) based on a food additive usage survey, the new data provided by EUTECA in 2010 contain levels of use for Class I Plain Caramel or Class II Caustic Sulphite Caramel, as well as some new levels of use for Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel in foodstuffs that had not been mentioned in the previous report.

2.6.1.1. Beverages

Class I Plain Caramel:

For non-alcoholic flavoured drinks, the CIAA reported typical use levels ranging from 13 to 1500 mg/l and an extreme use level of 2000 mg/l (CIAA, 2009). EUTECA (2010c) provided use levels ranging from 500-10 000 mg/l with a typical use level of 4000 mg/l.

For beer, the CIAA (2009) provided values ranging from 0 to 6000 mg/l with a typical use level of 3000 mg/l. EUTECA (2010c) provided values for beer and cidre bouché ranging from 2000-10 000 mg/l with a typical use level of 6000 mg/l for beer and 2000 mg/l for cidre bouché.

For whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit (other than Tsikoudia and Tsipouro and eau de vie de marc marque nation), the CIAA (2009) provided typical values ranging from 0 to 2000 mg/l with a extreme use level of 15000 mg/l. EUTECA (2010c) provided a typical use level of 2000 mg/l.

For aromatised wines-based drinks (except bitter soda), americano, liqueur wines and quality liqueur wines produced in specific regions, and spirit drinks, EUTECA (2010c) provided a typical use level of 5000 mg/l.

For aromatised wines, aromatised wines-based drinks and aromatised wine-products cocktails, EUTECA (2010c) provided a typical use level of 10 000 mg/l. For fruit wine, EUTECA (2010c) provided a typical use level of 6000 mg/l.

For liquid food supplements/dietary integrators, EUTECA (2010c) provided a typical value of 15 000 mg/l.

Class II Caustic Sulphite Caramel:

For non-alcoholic flavoured drinks, the CIAA reported typical use levels ranging from 0 to 30 mg/l (CIAA, 2009).

For whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit (other than Tsikoudia and Tsipouro and eau de vie de marc marque nation), EUTECA (2010c) provided a typical use level of 2000 mg/l. For cidre bouché, EUTECA (2010c) provided a typical use level of 3000 mg/l.

For aromatised wines-based drinks (except bitter soda), americano, liqueur wines and quality liqueur wines produced in specific regions, spirit drinks, and aromatised wines, aromatised wines-based drinks and aromatised wine-products cocktails, EUTECA (2010c) provided a typical use level of 5000 mg/l. For fruit wine, EUTECA (2010c) provided a typical use level of 3000 mg/l.

For liquid food supplements/dietary integrators, the CIAA (2009) provided a typical value of 620 mg/l. EUTECA (2010c) provided a typical use level of 15 000 mg/l.

Class III Ammonia Caramel:

For non-alcoholic flavoured drinks, the CIAA reported typical use levels ranging from 55 to 100 mg/l and extreme use levels ranging from 55 to 500 mg/l (CIAA, 2009). Tennant (2007) and EUTECA (2010c) reported usage data range from 340 to 1360 mg/l. Tennant (2007) reported a typical use level of 1000 mg/l, whilst EUTECA (2010c) indicated typical use levels 55-100 mg/l for “carbonate” category.

For spirituous beverages, aromatized wines and fruit wines, Tennant (2007) and EUTECA (2010c) provided values ranging from 136 to 680 mg/l with a typical use level of 600 mg/l. For fruit wines category, CIAA (2009) also provided extreme values ranging from 30 to 60 mg/l with a typical use level of 40 mg/l.

For beer, the CIAA (2009) provided typical values ranging from 0 to 7500 mg/l with extreme use levels ranging from 0 to 8000 mg/l. Tennant (2007) and EUTECA (2010c) provided data for the same food category. The use levels range from 340 to 2040 mg/l with a typical value of 2000 mg/l. The same data were reported by Tennant (2007) and EUTECA (2010c) for cidre bouché.

Class IV Sulphite Ammonia Caramel:

For non-alcoholic flavoured drinks, the CIAA reported typical use levels ranging from 0 to 1600 mg/l and extreme values ranging from 0 to 2800 mg/l. Tennant (2007) and EUTECA (2010c) reported usage data range from 3000 to 4000 mg/l. Tennant (2007) reported a typical use level of 1600 mg/l,

whilst EUTECA (2010c) indicated a typical use level of 5000 mg/l for “carbonate” category. UNESDA reported an actual maximum use level of 1600 mg/l (UNESDA, 2006).

For cidre bouché, EUTECA (2010c) provided a typical use level of 500 mg/l. For spirituous beverages, Tennant (2007) and EUTECA (2010c) provided values ranging from 4000 to 5000 mg/l with a typical use level of 5000 mg/l.

For fruit wines, CIAA (2009) provided extreme values ranging from 30 to 560 mg/l with typical use levels ranging from 70 to 330 mg/l. EUTECA (2010c) provided a typical use level of 500 mg/l.

For Americano and aromatised wines, Tennant (2007) and EUTECA (2010c) provided a typical use level of 1000 mg/l and 5000 mg/l, respectively. For whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit (other than Tsikoudia and Tsipouro and eau de vie de marc marque nation), Tennant (2007) and EUTECA (2010c) provided values ranging from 4000 to 5000 mg/l with a typical use level of 5000 mg/l.

For liquid food supplements and dietary integrators, the CIAA (2009) reported a range of typical data ranging from 25 to 615 mg/l.

Table 6: Data reviewed on use levels of caramel in beverages

	Beverages	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/l)	Reported maximum use levels (mg/l)
Class I Plain Caramel	Non-alcoholic flavoured drinks	CIAA (2009)	13- 1500	2000
		EUTECA, 2010c	4000	10 000
	Beer, cidre bouché	CIAA (2009)	3000	6000
		EUTECA, 2010c	6000 Cidre: 2000	10 000
	Whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit	CIAA (2009)	0 -2000	15 000
		EUTECA, 2010c	2000	
	Aromatised wines-based drinks (except bitter soda)	EUTECA, 2010c	5000	
	Americano	EUTECA, 2010c	5000	
	Liqueur wines and quality liqueur wines produced in specific regions	EUTECA, 2010c	5000	
	Spirit drinks, (including products less than 15% alcohol by volume) ¹	EUTECA, 2010c	5000	
	Aromatized wines, aromatised wine-based drinks and aromatized wine-products cocktails as mentioned in Regulation 1601/91 ¹	EUTECA, 2010c	10 000	
	Fruit wine (still or sparkling)	EUTECA, 2010c	6000	

	Beverages	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/l)	Reported maximum use levels (mg/l)
	Liquid food supplements/dietary integrators	EUTECA, 2010c	15 000	
Class II Caustic Sulphite Caramel	Non-alcoholic flavoured drinks	CIAA (2009)	0- 30	
	Cidre bouché	EUTECA, 2010c	3000	
	Whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit	EUTECA, 2010c	2000	
	Aromatised wines-based drinks (except bitter soda)	EUTECA, 2010c	5000	
	Americano	EUTECA, 2010c	5000	
	Liqueur wines and quality liqueur wines produced in specific regions	EUTECA, 2010c	5000	
	Spirit drinks, (including products less than 15% alcohol by volume) ¹		5000	
	Aromatized wines, aromatised wine-based drinks and aromatized wine-products cocktails as mentioned in Regulation 1601/91 ¹	EUTECA, 2010c	5000	
	Fruit wine (still or sparkling)	EUTECA, 2010c	3000	
	Liquid food supplements/dietary integrators	CIAA (2009) EUTECA, 2010c	620 15 000	
Class III Ammonia Caramel	Non-alcoholic flavoured drinks	CIAA (2009)	55- 100	500
		Tennant (2007)	1000	1360
		EUTECA, 2010c	55-100 ³	
	Spirit drinks, aromatized wines and fruit wines	Tennant (2007); EUTECA, 2010c	600	680
		CIAA (2009) ²	40	60
	Beer	CIAA (2009)	0-7500	8000
		Tennant (2007); EUTECA, 2010c	2000	2040
	Cidre bouché	Tennant (2007); EUTECA, 2010c	2000	2040
Class IV Sulphite Ammonia Caramel	Non-alcoholic flavoured drinks	UNESDA (2006)		1600
		CIAA (2009)	0-1600	2800
		Tennant (2007)	1600	4000
		EUTECA, 2010c	5000 ³	
	Spirit drinks (including products less than 15% alcohol by volume) ¹	Tennant (2007); EUTECA, 2010c	5000	5000
	Cidre bouché	EUTECA, 2010c	500	

	Beverages	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/l)	Reported maximum use levels (mg/l)
	Fruit wines (still or sparkling)	CIAA (2009)	70-330	560
		EUTECA, 2010c	500	
	Aromatised wines wine-based drinks (except bitter soda)	Tennant (2007); EUTECA, 2010c	5000	5000
	Americano	Tennant (2007); EUTECA, 2010c	1000	1000
	Whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit	Tennant (2007); EUTECA, 2010c	5000	5000
	Liquid food supplements/dietary integrators	CIAA (2009)	25-615	

¹ Except those mentioned in Annex II and III of Directive 94/36/EC

² Only for fruit wines

³ In the “Carbonates” category

2.6.1.2. Foodstuffs

Class I Plain Caramel

For confectionery products, the Panel was provided with data from the CIAA which reported typical use levels ranging from 25 to 9800 mg/kg and extreme use levels from 2 to 3800 mg/kg (CIAA, 2009). EUTECA (2010c) provided use levels ranging from 500-10 000 mg/kg with a typical use level of 2500 mg/kg.

For decorations and coatings, the CIAA (2009) reported a typical use level of 4 mg/kg and extreme values of 40 mg/kg.

For fine bakery wares, the CIAA (2009) reported extreme values ranging from 25 to 4900 mg/kg with typical values ranging from 25 to 800 mg/kg. EUTECA (2010c) provided use levels ranging from 1000-15 000 mg/kg with a typical use level of 3000 mg/kg.

For edible ices, the Panel was provided with data from the CIAA which reported extreme use levels ranging from 1.3 to 1990 mg/kg, with typical values ranging from 1.3 to 900 mg/kg (CIAA, 2009). EUTECA (2010c) provided a typical use level of 5000 mg/kg.

For desserts, including flavoured milk products, the CIAA (2009) reported typical and extreme use levels of 2774 mg/kg. EUTECA (2010c) provided use levels ranging from 1000-15 000 mg/kg with a typical use level of 5000 mg/kg.

Use level data have been reported for sauces and seasonings by the CIAA (2009) with a range of typical use levels from 0.5 to 13000 mg/kg and extreme use levels ranging from 0.5 to 21 000 mg/kg.

EUTECA (2010c) provided use levels ranging from 1000-100 000 mg/kg with a typical use level of 5000 mg/kg.

For snacks (dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products) as well as for other savoury snack products and savoury peanuts, nuts or hazelnuts, typical and extreme use levels have been reported by the CIAA (2009) and range from 200 to 1600 mg/kg. EUTECA (2010c) provided a typical use level of 3000 mg/kg.

For dietetic formulae for weight control intended to replace total daily food intake or an individual meal, the CIAA (2009) reported a typical use level of 1800 and maximum usage of 2500 mg/kg.

Data for soups have been provided by CIAA (2009) which reported typical levels ranging from 50 to 300 mg/kg and maximum levels of 50 to 1332 mg/kg. EUTECA (2010c) provided use levels ranging from 2000-50 000 mg/kg with a typical use level of 10 000 mg/kg.

For meat and fish analogues based on vegetable proteins, the CIAA (2009) provided a typical use level of 16 000 mg/kg. EUTECA (2010c) provided use levels ranging from 1000-40 000 mg/kg with a typical use level of 10 000 mg/kg.

For the category extruded, puffed and/or fruit-flavoured breakfast cereals, CIAA (2009) provided a typical use level of 5610 mg/kg and a maximum value of 10 010 mg/kg.

For jams, jellies and marmalades, the Panel was provided with data from the CIAA which reported a typical use level of 1000 mg/kg and a maximum value of 4000 mg/kg (CIAA, 2009).

For malt bread, EUTECA (2010c) provided use levels ranging from 1000-30 000 mg/kg with a typical use level of 1000 mg/kg.

For sausages, pâtés and terrines, EUTECA (2010c) provided use levels ranging from 1000-8000 mg/kg with a typical use level of 2000 mg/kg.

For vinegar and smoked fish, EUTECA (2010c) provided a typical use level of 6000 and 10 000 mg/kg, respectively.

For complete formulae for weight control, EUTECA (2010c) provided a typical use level of 7000 mg/kg.

Class II Sulphite Caramel

For confectionery products, the Panel was provided with data from the CIAA which reported no use in such products (CIAA, 2009).

For fine bakery wares, the CIAA (2009) reported extreme values ranging from 14 to 3000 mg/kg with typical values ranging from 14 to 1500 mg/kg. EUTECA (2010c) provided a typical use level of 1000 mg/kg.

For edible ices, the Panel was provided with data from the CIAA which reported extreme use levels ranging from 120 to 7620 mg/kg, with typical values ranging from 2840 to 4000 mg/kg (CIAA, 2009). EUTECA (2010c) provided a typical use level of 3000 mg/kg.

For flavoured processed cheese, the CIAA (2009) reported no use in such products.

Use level data have been reported for sauces and seasonings by the CIAA (2009) with a range of typical use levels from 0 to 2000 mg/kg. EUTECA (2010c) provided a typical use level of 6000 mg/kg.

For sausages, pâtés and terrines, EUTECA (2010c) provided a typical use level of 1000 mg/kg. For malt bread, vinegar and smoked fish, EUTECA (2010c) provided a typical use level of 3000, 5000 and 10 000 mg/kg, respectively.

For desserts, EUTECA (2010c) provided a typical use level of 1000 mg/kg.

For snacks (dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products) as well as for other savoury snack products and savoury peanuts, nuts or hazelnuts, EUTECA (2010c) provided a typical use level of 1000 mg/kg.

For complete formulae for weight control, meat and fish, and soups EUTECA (2010c) provided a typical use level of 5000, 5000 and 10 000 mg/kg, respectively.

Class III Ammonia Caramel

For confectionery products, the Panel was provided with data from the CIAA which reported use levels ranging from 0 to 8000 mg/kg with typical values ranging from 0 to 5000 mg/kg (CIAA, 2009). Tennant (2007) and EUTECA (2010c) reported use levels ranging from 136 to 680 mg/kg with a typical value of 600 mg/kg.

For candied fruit, vegetables, mustarda di frutta, Tennant (2007) and EUTECA (2010c) provided use level data ranging from 340 to 2040 mg/kg with a typical level of 2000 mg/kg.

For decorations and coatings, Tennant (2007) and EUTECA (2010c) provided use level data ranging from 2040 to 3400 mg/kg with a typical level of 3000 mg/kg.

For fine bakery wares, the CIAA (2009) reported extreme values ranging from 40 to 11500 mg/kg with typical values ranging from 70 to 11500 mg/kg. Tennant (2007) and EUTECA (2010c) reported values ranging from 340 to 1360 mg/kg and a typical use level of 1000 mg/kg.

For edible ices, Tennant (2007) and EUTECA (2010c) reported values ranging from 340 to 1360 mg/kg and a typical use level of 1000 mg/kg.

For flavoured processed cheese, the CIAA (2009) reported no use in such products.

For desserts, including flavoured milk products, the CIAA (2009) reported typical use levels of 5120 mg/kg, while Tennant (2007) and EUTECA (2010c) reported values ranging from 340 to 1360 mg/kg and a typical use level of 1000 mg/kg.

Use level data have been reported for sauces and seasonings by the CIAA (2009) with a range of extreme use levels from 0 to 25000 mg/kg and typical use levels ranging from 600 to 3200 mg/kg. Tennant (2007) and EUTECA (2010c) provided data ranging from 10 000 to 100 000 mg/kg, high use levels relating to concentrated products.

For snacks (dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products) as well as for other savoury snack products and savoury peanuts, nuts or hazelnuts, data have been reported by Tennant (2007) and EUTECA (2010c) and range from 340 to 2040 mg/kg with a typical value of 2000 mg/kg.

For complete formulae and nutritional supplements for use under medical supervision, the CIAA (2009) reported a typical use level of 9500 mg/kg.

Data for soups have been provided by CIAA (2009) which reported extreme levels ranging from 30 to 3000 mg/kg and typical levels of 100 to 2000 mg/kg. EUTECA (2010c) provided a typical use level of 2000 mg/kg.

For noodles, CIAA (2009) reported a typical use level of 510 mg/kg.

For meat and fish analogues based on vegetable proteins, Tennant (2007) and EUTECA (2010c) reported use levels of 10 000 mg/kg. The CIAA (2009) reported extreme use levels ranging from 100 to 5000 mg/kg and typical use levels ranging from 200 to 5000 mg/kg

For the category extruded, puffed and/or fruit-flavoured breakfast cereals and malt bread, Tennant (2007) and EUTECA (2010c) provided usage data ranging from 340 to 2040 mg/kg with a typical use level of 2000 mg/kg.

For malt bread, Tennant (2007) and EUTECA (2010c) provided use levels ranging from 340-2040 mg/kg with a typical use level of 2000 mg/kg.

For sausages, pâtés and terrines, EUTECA (2010c) provided use levels ranging from 1000-5000 mg/kg with a typical use level of 1000 mg/kg.

For vinegar and mustard, EUTECA (2010c) provided a typical use level of 2000 mg/kg.

Class IV Sulphite Ammonia Caramel

For confectionery products, the Panel was provided with data from the CIAA which reported extreme use levels ranging from 0 to 8000 mg/kg with typical values ranging from 0 to 5000 mg/kg (CIAA, 2009). Tennant (2007) and EUTECA (2010c) reported use levels ranging from 1000 to 300 000 mg/kg (high use levels relating to concentrated products) with a typical value of 1000 mg/kg.

For fine bakery wares, the CIAA (2009) reported extreme values ranging from 30 to 1700 mg/kg with typical values ranging of 250 mg/kg. Tennant (2007) and EUTECA (2010c) reported extreme values ranging from 3000 to 5000 mg/kg and a typical use level of 5000 mg/kg.

For edible ices, the CIAA (2009) reported extreme values ranging from 100 to 5200 mg/kg and typical use levels ranging from 400 to 3500 mg/kg. EUTECA (2010c) provided a typical use level of 3000 mg/kg.

For flavoured processed cheese, the CIAA (2009) reported no use in such products.

Use level data have been reported for sauces and seasonings by the CIAA (2009) with a range of extreme use levels from 0 to 10 000 mg/kg and typical use levels ranging from 0 to 2000 mg/kg. Tennant (2007) and EUTECA (2010c) provided data ranging from 20 000 to 100 000 mg/kg (high use levels relates to concentrated products) with typical values of 20 000 mg/kg.

For meat and fish analogues based on vegetable proteins, Tennant (2007) and EUTECA (2010c) reported a use level of 1000 mg/kg. The CIAA (2009) provided extreme use levels ranging from 0 to 2000 mg/kg and typical use levels ranging from 280 to 2000 mg/kg

Data for soups have been provided by Tennant (2007) and EUTECA (2010c) which reported a typical level of 4000 mg/kg.

For malt bread, Tennant (2007) and EUTECA (2010c) reported use level data of 5000 mg/kg.

For vinegar, the typical use level reported by Tennant (2007) and EUTECA (2010c) was 2000 mg/kg.

For sausages, pâtés and terrines, EUTECA (2010c) provided use levels ranging from 1000-5000 mg/kg with a typical use level of 1000 mg/kg.

For desserts and snacks (dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products) as well as for other savoury snack products and savoury peanuts, nuts or hazelnuts, EUTECA (2010c) provided a typical use level of 1000 mg/kg.

Table 7. Data reviewed on use levels of caramel in foodstuff.

	Foodstuff	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/kg)	Reported maximum use levels (mg/kg)
Class I Plain Caramel	Confectionery product	CIAA (2009)	25-9800	9800
		EUTECA, 2010c	2500	10 000
	Decorations and coatings	CIAA (2009)	4	40
	Fine bakery wares	CIAA (2009)	25-800	4900
		EUTECA, 2010c	3000	15 000
	Edible ices	CIAA (2009)	1.3-900	1990
		EUTECA, 2010c	5000	
	Dessert (including flavoured milk products)	CIAA (2009)	2774	2774
		EUTECA, 2010c	5000	15 000
	Sauces and seasonings	CIAA (2009)	0.5-13 000	21 000
		EUTECA, 2010c	5000	100 000
	Snacks, savoury peanuts, nuts and hazelnuts	CIAA (2009)	200-1600	1600
		EUTECA, 2010c	3000	
	Soups	CIAA (2009)	50-300	1332
		EUTECA, 2010c	10 000	50 000
	Meat and fish analogues based on vegetable proteins	CIAA (2009)	16 000	
		EUTECA, 2010c	10 000	40 000
	Extruded, puffed and/or fruit flavoured breakfast cereals	CIAA (2009)	5610	10 010
	Jam, jellies and marmelades	CIAA (2009)	1000	4000
	Malt bread	EUTECA, 2010c	10 000	30 000
	Vinegar	EUTECA, 2010c	6000	
	Sausages, pâtés and terrines	EUTECA, 2010c	2000	8000
	Dietetic formulae for weight control	CIAA (2009)	1800	2500
		EUTECA, 2010c	7000	
	Smoked fish	EUTECA, 2010c	10 000	
Class II Caustic Sulphite	Fine bakery wares	CIAA (2009)	14-1500	3000
		EUTECA, 2010c	1000	
	Edible ices	CIAA (2009)	2840-4000	7620
		EUTECA, 2010c	3000	
	Sauces and seasonings	CIAA (2009)	0-2000	
		EUTECA, 2010c	6000	
	Malt bread	EUTECA, 2010c	3000	
	Vinegar	EUTECA, 2010c	5000	
	Sausages, pâtés and terrines	EUTECA, 2010c	1000	

	Foodstuff	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/kg)	Reported maximum use levels (mg/kg)
Caramel	Dessert	EUTECA, 2010c	1000	
	Smoked fish	EUTECA, 2010c	10 000	
	Snacks, savoury peanuts, nuts and hazelnuts	EUTECA, 2010c	1000	
	Complete formulae for weight control	EUTECA, 2010c	5000	
	Soups	EUTECA, 2010c	10 000	
	Meat and fish analogues based on vegetable proteins	EUTECA, 2010c	5000	
Class III Ammonia Caramel	Confectionery product	CIAA (2009)	0-5000	8000
		Tennant (2007); EUTECA, 2010c	600	680
	Candied fruit, vegetables, mustarda di fruta	Tennant (2007); EUTECA, 2010c	2000	2040
	Decorations and coatings	Tennant (2007); EUTECA, 2010c	3000	3400
	Fine bakery wares	CIAA (2009)	70-11 500	11500
		Tennant (2007); EUTECA, 2010c	1000	1360
	Edible ices	Tennant (2007); EUTECA, 2010c	1000	1360
	Desserts (including flavoured milk products)	CIAA (2009)	5120	
		Tennant (2007); EUTECA, 2010c	1000	1360
	Sauces and seasonings	CIAA (2009)	600-3200	25 000
		Tennant (2007); EUTECA, 2010c		100 000*
	Snacks, savoury peanuts, nuts and hazelnuts	Tennant (2007); EUTECA, 2010c	2000	2040
	Complete formulae and nutritional supplements	CIAA (2009)	9500	
	Soups	CIAA (2009)	100-2000	3000
		EUTECA, 2010c	2000	
	Noodles	CIAA (2009)	510	
	Meat and fish analogues based on vegetable proteins	Tennant (2007); EUTECA, 2010c	10 000	10 000
		CIAA (2009)	200-5000	5000
	Extruded, puffed and/or fruit –flavoured breakfast cereals and malt bread	Tennant (2007); EUTECA, 2010c	2000	2040
	Malt bread	Tennant (2007); EUTECA, 2010c	2000	2040
	Vinegar	EUTECA, 2010c	2000 (balsamic: 100 g/l)	
	Sausages, pâtés and terrines	EUTECA, 2010c	1000	5000
	Mustard	EUTECA, 2010c	2000	

	Foodstuff	Data provided by	Reported typical use levels (range lowest-highest or maximum) (mg/kg)	Reported maximum use levels (mg/kg)
Class IV Sulphite Ammonia Caramel	Confectionery products	CIAA (2009)	0-5000	8000
		Tennant (2007); EUTECA, 2010c	1000	300 000*
	Fine bakery wares	CIAA (2009)	250	1700
		Tennant (2007); EUTECA, 2010c	5000	5000
	Edible ices	CIAA (2009)	400-3500	5200
		EUTECA, 2010c	3000	
	Sauces and seasonings	CIAA (2009)	0-2000	10 000
		Tennant (2007); EUTECA, 2010c	20 000	20 000
	Soups	Tennant (2007); EUTECA, 2010c	4000	4000
	Meat and fish analogues based on vegetable proteins	Tennant (2007); EUTECA, 2010c	1000	1000
		CIAA (2009)	280-2000	2000
	Malt bread	Tennant (2007); EUTECA, 2010c	5000	5000
	Vinegar	Tennant (2007); EUTECA, 2010c	2000 (balsamic: 100 g/l)	2000
	Sausages, pâtés and terrines	EUTECA, 2010c	1000	5000

* High use levels related to concentrated products

In order to refine the exposure assessment for children and adults to food colours, the Panel has defined some rules to identify maximum reported use levels based on maximum actual usage or maximum analytical data. The rules followed in order to deal with *quantum satis* authorisation, with use levels data or observed analytical data, for all regulated colours re-evaluated by the Panel, are given in Annex A. Table 8 summarises the maximum reported use levels for caramel colours Class I, II, III and IV in beverages and foodstuffs used for the refined exposure assessment. They have been defined by applying the rules reported in Annex A to the data available to EFSA.

Table 8: Maximum Permitted Levels of use for caramel colours Class I, II, III and IV in beverages and foodstuffs according to European Parliament and Council Directive 94/36/EC and maximum reported use levels of caramel colours Class I, II, III and IV in beverages and foodstuffs used for the refined exposure assessment (Annex A)

	Maximum Permitted Level (mg/l)	Maximum Reported Use Level (mg/l)			
		Class I	Class II	Class III	Class IV
Beverages					
Non-alcoholic flavoured drinks	<i>quantum satis</i>	10 000 ¹	30 ¹	1360 ¹	5000 ¹

Beverages	Maximum Permitted Level (mg/l)	Maximum Reported Use Level (mg/l)			
		Class I	Class II	Class III	Class IV
Bitter soda, bitter vino as mentioned in Reg (EEC) 1601/91	<i>quantum satis</i>	100 ²	100 ²	100 ²	100 ²
Cider and perry	<i>quantum satis</i>	6000 ¹	3000 ¹	680 ¹	600 ^{1**}
cidre bouché	<i>quantum satis</i>	2000 ¹	3000 ¹	2040 ¹	500 ¹
Americano	<i>quantum satis</i>	5000 ¹	5000 ¹	100 ²	1000 ¹
Aperitif wines, spirit drinks including products with less than 15 % alcohol by volume	<i>quantum satis</i>	30 ²	30 ²	30 ²	30 ²
Aromatised wine, aromatised wine-based drinks and aromatised wine product cocktails as mentioned in Reg (EEC) 1601/91, except those mentioned in Annex II or IIIs	<i>quantum satis</i>	10000 ¹	5000 ¹	680 ¹	200 ²
Aromatised wine-based drinks (except bitter soda) and aromatised wines as mentioned in Reg (EEC) 1601/91	<i>quantum satis</i>	5000 ¹	5000 ¹	680 ¹	5000 ¹
Spirit drinks, (including products less than 15 % alcohol by volume) except those mentioned in Annex II or III	<i>quantum satis</i>	5000 ¹	5000 ¹	680 ¹	5000 ¹
Beer	<i>quantum satis</i>	10 000 ¹	3000 ²	8000 ¹	500 ²
Whisky, whiskey, grain spirit (other than Korn or Kornbrand or eau de vie de seigle marque nationale luxembourgeoise), wine spirit, rum brandy, weinbrand, grape marc, grape marc spirit (other than Tsikoudia and Tsipouro and eau de vie de marc marque nation	<i>quantum satis</i>	15 000 ¹	2000 ¹	200 ²	5000 ¹
Fruit wines (still or sparkling)	<i>quantum satis</i>	6000 ¹	3000 ¹	680 ¹	560 ¹
Liqueur wines and quality liqueur wines produced in specified regions	<i>quantum satis</i>	5000 ¹	5000 ¹	200 ²	200 ²
Liqueurs, including fortified beverages with less than 15 % alcohol by volume	<i>quantum satis</i>	200 ²	200 ²	200 ²	200 ²
Liquid food supplements/dietary integrators	<i>quantum satis</i>	15 000 ¹	15 000 ¹	100 ²	620 ^{1**}
Bigarreaux cherries in syrup and in cocktails	<i>quantum satis</i>	150 ²	150 ²	150 ²	150 ²

Foodstuffs	Maximum Permitted Level (mg/kg)	Maximum reported use level (mg/kg)			
		Class I	Class II	Class III	Class IV
Breakfast sausages with a minimum cereal content of 6 %	<i>quantum satis</i>	100 ²	100 ²	100 ²	100 ²

Foodstuffs	Maximum Permitted Level (mg/kg)	Maximum reported use level (mg/kg)			
		Class I	Class II	Class III	Class IV
Burger meat with a minimum vegetable and/or cereal content of 4 %	<i>quantum satis</i>	100 ²	100 ²	100 ²	100 ²
Candied fruits and vegetables, Mostarda di frutta	<i>quantum satis</i>	200 ²	200 ²	2050 ^{1**}	200 ²
cocktail cherries and candies cherries	<i>quantum satis</i>	200 ²	200 ²	200 ²	200 ²
Complete formulae and nutritional supplements for use under medical supervision	<i>quantum satis</i>	50 ²	50 ²	9500 ¹	1000 ²
Confectionery	<i>quantum satis</i>	10 000 ¹	-*	8000 ¹	300 000 ¹
Decoration of chocolates	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Decorations and coatings	<i>quantum satis</i>	40 ¹	500 ²	3400 ¹	500 ²
Desserts including flavoured milk products	<i>quantum satis</i>	15 000 ¹	1000 ¹	5120 ¹	1000 ¹
Dietetic formulae for weight control intended to replace total daily food intake or an individual meal	<i>quantum satis</i>	7000 ¹	5000 ¹	50 ²	50 ²
Edible casings	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Edible cheese rind	<i>quantum satis</i>	100 ²	100 ²	100 ²	100 ²
Edible ices	<i>quantum satis</i>	5000 ¹	8000 ^{1**}	1360 ¹	5200 ¹
External coating of confectionery	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
External coating of sugar confectionery for the decoration of cake and pastries	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Extruded, puffed and/or fruit-flavoured breakfast cereals	<i>quantum satis</i>	10 010 ¹	500 ²	2040 ¹	500 ²
Fine bakery wares	<i>quantum satis</i>	15 000 ¹	3000 ¹	11500 ¹	5000 ¹
fish analogues based on vegetable proteins	<i>quantum satis</i>	40 000 ¹	5000 ¹	10 000 ¹	2000 ¹
Fish paste and crustacean paste	<i>quantum satis</i>	100 ²	100 ²	100 ²	100 ²
Fish roe	<i>quantum satis</i>	300 ²	300 ²	300 ²	300 ²
Flavoured processed cheese	<i>quantum satis</i>	100 ²	-*	-*	-*
Jam, jellies and marmalades and other similar fruit preparations including low calorie products	<i>quantum satis</i>	4000 ¹	100 ²	100 ²	100 ²
Kippers	<i>quantum satis</i>	20 ²	20 ²	20 ²	20 ²
Malt bread	<i>quantum satis</i>	30 000 ¹	3000 ¹	2040 ¹	5000 ¹
Meat analogues based on vegetable proteins	<i>quantum satis</i>	40 000 ¹	5000 ¹	10 000 ¹	2000 ¹
Mustard	<i>quantum satis</i>	300 ²	300 ²	2000 ¹	300 ²
Other savoury snack products and savoury peanuts, nuts or hazelnuts	<i>quantum satis</i>	3000 ¹	1000 ¹	2040 ¹	1000 ¹
Pre-cooked crustaceans	<i>quantum satis</i>	250 ²	250 ²	250 ²	250 ²
Preserves of red fruits	<i>quantum satis</i>	200 ²	200 ²	200 ²	200 ²

Foodstuffs	Maximum Permitted Level (mg/kg)	Maximum reported use level (mg/kg)			
		Class I	Class II	Class III	Class IV
Salmon substitutes	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Sauces, seasonings (for example curry powder, tandoori), pickles, relishes, chutney and piccalilli	<i>quantum satis</i>	100 000 ¹	6000 ¹	100 000 ¹	20 000 ¹
Saucisses de Strasbourg	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Sausages, pâtés and terrines	<i>quantum satis</i>	8000 ¹	1000 ¹	5000 ¹	5000 ¹
Smoked fish	<i>quantum satis</i>	10 000 ¹	10 000 ¹	100 ²	100 ²
Snacks: dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products	<i>quantum satis</i>	3000 ¹	1000 ¹	2040 ¹	1000 ¹
Solid food supplements/dietary integrators	<i>quantum satis</i>	300 ²	300 ²	300 ²	300 ²
Soups	<i>quantum satis</i>	50 000 ¹	10 000 ¹	3000 ¹	4000 ¹
Surimi	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Vegetables in vinegar, brine or oil (excluding olives)	<i>quantum satis</i>	500 ²	500 ²	500 ²	500 ²
Vinegar	<i>quantum satis</i>	6000 ¹	5000 ¹	100 000 ¹	100 000 ¹

¹ Maximum reported use level or maximum level determined by analysis.

² *quantum satis* rules data.

* No usage reported

**Rounded value

2.7. Information on existing authorisations and evaluations

The caramel colours have previously been evaluated by the SCF and by JECFA. They have additionally been reviewed by TemaNord (TemaNord, 2002).

Evaluations of Class I Plain Caramel

The JECFA evaluation of caramel colours in 1969 concluded that caramel colour (Class I Plain Caramel) could be considered as a natural constituent of the diet, and that a toxicological discrimination between caramel produced by cooking or heating sugars (burnt sugar caramel) and caramel colours commercially produced by processes not using ammonia or ammonium salts was unwarranted (JECFA, 1970). JECFA allocated an ADI "not limited" to Class I Plain Caramel at their fifteenth meeting (JECFA, 1972a), and reconfirmed this ADI in 1985 (JECFA, 1986). In an opinion published in 1975, SCF concurred with the JECFA position on Class I Plain Caramel, and following further discussions in 1983 and 1987, the SCF saw no reason to change its previous opinion on Class I Plain Caramel. A numerical ADI was thus found not necessary (SCF, 1975, 1984 and 1989).

Evaluations of Class II Caustic Sulphite Caramel

The SCF in 1987 concluded that Class II Caustic Sulphite Caramel was temporarily acceptable pending provision of further information on the composition and homogeneity of caramel colours falling within this class (SCF, 1989). Following provision of additional information indicating that the composition of Caustic Sulphite Caramel is intermediate between that of Class I Plain Caramel and that of Class IV Sulphite Ammonia Caramel, SCF considered, at its 76th meeting in 1990, that the existing toxicity data on Class II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel

provided adequate documentation for the safety in use for both these classes of caramel and it therefore included Class II Caustic Sulphite Caramel within the ADI of 200 mg/kg bw/day it had already established for Class IV Sulphite Ammonia Caramel.

In 1985, JECFA concluded that Class II Caustic Sulphite Caramel was sufficiently different from other classes of caramel colours to warrant a separate evaluation but that there were insufficient data to do so. No ADI was established (JECFA, 1986). At its 55th meeting, the Committee re-evaluated the data and established an ADI of 160 mg/kg bw/day for Class II Caustic Sulphite Caramel, based on a No-Observed-Adverse-Effect-Level (NOAEL) of 16 g/kg bw/day identified in a 90-day study in rats (JECFA, 2001).

Evaluations of Class III Ammonia Caramel

The SCF in 1975 established an ADI of 100 mg/kg bw/day for Class III Ammonia Caramel SCF, 1975). In 1989 the ADI was revised and the SCF allocated a value of 200 mg/kg bw/day, possibly based on the 90-day rat study by MacKenzie (1985b; subsequently published in the open literature as MacKenzie et al., 1992b). The ADI was made temporary pending a review of further studies and especially of the attempts to reduce the content of THI in Class III Ammonia Caramel (SCF, 1989). Finally, at the latest SCF evaluation of 1997, on the basis of the data supplied to the SCF, the Committee decided to allocate a full ADI of 200 mg/kg bw/day to Class III Ammonia Caramel with the provision that the THI content should not exceed 10 mg/kg colour (SCF, 1997).

JECFA in 1972 first established a temporary ADI of 100 mg/kg bw/day for caramel colours prepared by processes using ammonia or ammonium salts (Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel) (JECFA, 1972b). In 1977 JECFA noted that the specifications for Class III Ammonia Caramel were ambiguous since they also covered caramel colours manufactured by the Class IV Sulphite Ammonia Caramel process. Separate specifications were prepared for Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. It was at that time determined that the principal toxic effect of Class III Ammonia Caramel was depression of circulating lymphocytes and leucocytes, an effect for which a clear no-effect level could not be determined, based on the available studies. Accordingly, the temporary ADI was revoked (JECFA, 1977, 1978). JECFA at its 29th meeting (JECFA, 1986, 1987) carried out a detailed evaluation of all available data on Class III Ammonia Caramel and concluded that it could also allocate an ADI of 200 mg/kg bw/day for this caramel colour. JECFA concluded that the effect of most concern, i.e. lymphocytopenia, could best be evaluated from short-term studies, and based its ADI on the no-effect level of 20 g/kg bw/day in a 90-day study in rats using Class III Ammonia Caramel which contained approximately 15 mg THI/kg on a solids basis.

Evaluations of Class IV Sulphite Ammonia Caramel

In 1975 the SCF established a temporary group ADI of 100 mg/kg bw/day for Class IV Sulphite Ammonia Caramel and Class III Ammonia Caramel, because of the inadequacy of the available chronic studies. In 1983 the SCF noted that the genotoxicity data on Class IV Sulphite Ammonia Caramel did not indicate mutagenic potential and that subchronic studies did not show haematological effects (as were observed in the studies with Class III Ammonia Caramel). The available subchronic studies, including a one generation study in rats, indicated a NOAEL for Class IV Sulphite Ammonia Caramel of 10% in the diet. The SCF concluded that continued use of Class IV Sulphite Ammonia Caramel was justified until the results of long-term studies and adequate specifications were prepared (SCF, 1984). In 1987, the SCF concluded that the available long-term studies using Class IV Sulphite Ammonia Caramel on rats and mice showed no significant effects and considered that Class IV Sulphite Ammonia Caramel was toxicologically acceptable. An ADI of 200 mg/kg bw/day was established (SCF, 1989), based on a NOAEL for Class IV Sulphite Ammonia Caramel of 10% in the diet, however, it is not clear which uncertainty factor was used to reach this ADI.

JECFA in 1972 first established a temporary ADI of 100 mg/kg bw/day for caramel colours prepared by processes using ammonia or ammonium salts (Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel) (JECFA, 1972). Separate specifications were subsequently prepared for Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, and at the 21st meeting of JECFA (JECFA, 1978) the temporary ADI of 100 mg/kg bw/day was retained only for Class IV Sulphite Ammonia Caramel. The temporary ADI for Class III Ammonia Caramel was revoked at that time.

JECFA had drawn attention to the need for adequate specifications for Class IV Sulphite Ammonia Caramel and for a long-term study of carcinogenicity (JECFA, 1980). The temporary ADI of 100 mg/kg bw/day was extended pending the results of long-term toxicity studies. At its 29th meeting (JECFA, 1986) the JECFA based its evaluation on the no-effect level of 10 g/kg bw/day in the then provided long-term/carcinogenicity study in the rat (MacKenzie, 1985c, now published as MacKenzie et al. 1992c), to which (in view of the ancillary human data in which no adverse effects other than laxation were observed) a uncertainty factor of 50 was applied. This resulted in the allocation of an ADI of 200 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

Caramel colour is listed as generally recognized as safe (GRAS) at defined use levels as a general-purpose food additive in CFR 21, Section 182.1235 (CFR, 2006).

2.8. Exposure

2.8.1. Exposure assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate the intake of food additives. For each successive Tier, this involves a further refinement of intakes assessment. The approach goes from the conservative estimates that form the first Tier (Tier 1) of screening, to progressively more realistic estimates that form the Second (Tier 2) and Third (Tier 3) steps.

2.8.1.1. Crude estimates (Budget Method)

In the case of caramel colours Class I, II, III and IV, as these colours are permitted at *quantum satis* in all foodstuffs, the Panel could not estimate the dietary exposure using the Budget Method (Tier 1).

2.8.1.2. Refined estimates

As caramel colours Class I, II, III and IV are authorised *quantum satis* in almost all categories, the refined exposure estimates have been performed only for Tier 3 using the maximum reported use levels or when no maximum usages were reported to EFSA, maximum values defined by decision rules for *quantum satis* usages were used (Table 8).

Exposure estimates for children (1-14 years old) have been done by the Panel for 11 European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland, Germany, Denmark, Cyprus, Greece) based on detailed individual food consumption data provided by the EXPOCHI consortium (Huybrechts et al., 2010). As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5- 4.5 years old) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2008).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and also because estimates are provided on more refined adult food consumption data rather than those available to the Panel (e.g. EFSA concise food consumption database, which gives access to aggregate

food categories consumed by 15 European countries), the Panel decided to select the UK population as representative of the EU consumers for estimates of dietary exposure to all 4 classes of caramel colours. Estimates of exposure to each of the classes of caramel colours, caramels Class I, II, III and IV, for the UK adult population (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 2000-2001) available from the UNESDA report (Tennant, 2008) .

Table 9 summarises the anticipated exposure of children and adults to each of the classes of caramel colours, caramels Class I, II, III and IV

When considering the maximum reported use levels from Table 8, the mean dietary exposure of European children (aged 1-10 years and weighing 15-29 kg, except for Cypriot children where the reported body weight was 54 kg for 11-14 years old), including UK pre-school children, ranged from 76.9 to 427.2 mg/kg bw/day for Class I Plain Caramel, from 8.7 to 34.6 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 21.7 to 302.4 mg/kg bw/day for Class III Ammonia Caramel, and from 23.2 to 506.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel. At the 95th or 97.5th percentile, estimates ranged from 179.6 to 882.2 mg/kg bw/day for Class I Plain Caramel, from 18.5 to 117.3 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 107.9 to 757.3 mg/kg bw/day for Class III Ammonia Caramel, and from 129.7 to 1480.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors (>10% in all or several countries) to the total anticipated exposure of children were for Class I Plain Caramel: non alcoholic flavoured drinks (12% to 55%), fine bakery wares (15% to 32%), desserts including flavoured milk products (11% to 48%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 56%), soups (11% to 32%) and malt bread (16% to 49%). For Class II Caustic Sulphite Caramel the main contributors were fine bakery wares (12% to 53%), desserts including flavoured milk products (11% to 41%), edibles ices (11% to 22%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 45%), soups (18% to 54%) and malt bread (19% to 55%). For Class III Ammonia Caramel the main contributors were fine bakery wares (13% to 45%), desserts including flavoured milk products (12% to 44%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 79%), and vinegar (12% to 45%), while in one country non alcoholic flavoured drink, malt bread confectionery, and sausages, pates and terrines contributed 29%, 15%, 13% and 10%, respectively. For Class IV Sulphite Ammonia Caramel the main contributors were non alcoholic flavoured drinks (13% to 51%), confectionery (20% to 81%), fine bakery wares (10% to 29%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (10% to 24%), and malt bread (10% to 34%).

When considering the maximum reported use levels from Table 8, anticipated dietary exposure reported for the UK adult population give a mean of 136.6 mg/kg bw/day and a 97.5th percentile of 429.3 mg/kg bw/day for Class I Plain Caramel; a mean of 21.7 mg/kg bw/day and a 97.5th percentile of 109.5 mg/kg bw/day for Class II Caustic Sulphite Caramel; a mean of 60.8 mg/kg bw/day and a 97.5th percentile of 295.0 mg/kg bw/day for Class III Ammonia Caramel; and a mean of 89.4 mg/kg bw/day and 97.5th percentile of 368.9 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors (>10% in all or several countries) to the total anticipated exposure of adults were for Class I Plain Caramel non alcoholic flavoured drinks (30%), beer and cidre bouché (27%), soups (16%), and sauces, seasonings (e.g. curry powder, tandoori) and pickles (10%). For Class II Caustic Sulphite Caramel the main contributors were beer and cidre bouché (50%) and soups (20%). For Class III Ammonia Caramel the main contributors were beer and cidre bouché (48%) and sauces, seasonings (e.g. curry powder, tandoori) and pickles (22%). For Class IV Sulphite Ammonia Caramel the main contributors were confectionery (65%) and non alcoholic flavoured drinks (23%).

Table 9: Summary of anticipated exposure to caramel colours Class I, II, III and IV using the tiered approach (EC, 2001) in children and adult populations

Tier 3. Maximum reported use levels	Adult (>18 years old) ²	Children (1-10 years old, 15-29 kg body weight) ¹

	Mean Exposure (mg/kg bw/day)	Exposure 97.5th-percentile (mg/kg bw/day)	Mean exposure (mg/kg bw/day)	Exposure 95th(³) or 97.5th(²) percentile (mg/kg bw/day)
Class I	136.6	429.3	76.9 -427.2	179.6-882.2
Class II	21.7	109.5	8.7-34.6	18.5-117.3
Class III	60.8	295	21.7-302.4	107.9-757.3
Class IV	89.4	368.9	23.2-506.2	129.7-1480.2

¹ Except for Cypriot children where the reported body weight was 54 kg for 11-14 years old.

² For the UK population, estimates are based on the UNESDA report which gives the 97.5th percentile intake (Tennant, 2008).

³ For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.

2.8.1.3. Combined exposure

The Panel also evaluated combined anticipated dietary exposure to all four classes of caramel colours, taking into account the highest maximum reported level for all caramel classes, described in Table 8, from each food category. When considering this scenario, as presented in Table 10, anticipated mean combined dietary exposure reported for European children, including UK pre-school children, ranged from 83.5 to 698.3 mg/kg bw/day. At the 95th/97.5th percentile, estimates ranged from 224.8 to 1672.3 mg/kg bw/day. For the UK adult population this scenario gave a range of exposures of 194.8 and 474.3 mg/kg bw/day for the mean and the 97.5th percentile, respectively.

The main contributors (>10% in several countries) to the total combined anticipated exposure to caramel colours for children were non alcoholic flavoured drinks (11% to 28%), confectionery (19% to 58%), fine bakery wares (15% to 29%), desserts including flavoured milk products (10% to 31%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (14% to 44%), and malt bread (16% to 46%). Soups were estimated to contribute from 25% to 28% in two countries and vinegar was estimated to contribute 20% in one country.

For the adult population the main contributors (>10%) were confectionery (30%), non alcoholic flavoured drinks (21%), beer and cidre bouché (19%), and soups (11%).

Table 10: Summary of anticipated combined exposure to all caramel colours in children and adult populations

Tier 3. Maximum reported use levels	Adult (>18 years old) ²		Children (1-10 years old, 15-29 kg body weight) ¹	
	Mean Exposure (mg/kg bw/day)	Exposure 97.5th percentile (mg/kg bw/day)	Mean exposure (mg/kg bw/day)	Exposure 95th(³) or 97.5th(²) percentile (mg/kg bw/day)
All caramel colours	194.8	474.3	83.5 - 698.3	224.8 - 1672.3

¹ Except for Cypriot children where the reported body weight was 54 kg for 11-14 years old.

² For the UK population, estimates are based on the UNESDA report which gives the 97.5th percentile intake (Tennant, 2008).

³ For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.

2.8.1.4. Dietary exposure to 4-MEI, present in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel; THI, present in Class III Ammonia Caramel and sulphur dioxide (SO₂), present in Class II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel.

Maximum Levels (ML) for the constituents 4-MEI (< 250 mg/kg caramel colour) found in Class III Ammonia caramel and Class IV Sulphite Ammonia Caramel; THI (< 10 mg/kg caramel colour) found in Class III Ammonia caramel and sulphur dioxide (<0.2% caramel colour) found in Class II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel have been specified under Commission Directive 2008/128/EC. These constituents are of particular relevance in relation to the toxicological profile of these classes of caramel colours.

Maximum Reported Levels (MRL) have been provided by the Industry (EUTECA, 2011) for 4-MEI, being 140 mg/kg for Class III Ammonia Caramel and 183 mg/kg for Class IV Sulphite Ammonia Caramel. The MRL for THI (EUTECA, 2011) are the same as the maximum levels specified in Commission Directive 2008/128/EC (10 mg/kg) for Class III Ammonia Caramel. Using the maximum levels defined for these constituents in the specifications for caramel colours and the MRL from EUTECA (2011), the Panel derived anticipated exposure to 4-MEI, THI and sulphur dioxide arising from the anticipated exposure from the diet to caramel colours for children and adults, as estimated by the Panel and presented in Table 9.

Table 11 summarises the anticipated exposure of children and adults to 4-MEI, THI and sulphur dioxide.

Table 11: Summary of anticipated exposure to 4-MEI, THI and sulphur dioxide in child and adult populations from uses of caramel colours Class II, Class III and Class IV.

Constituents	MLs or MRLs ⁴	Adult (>18 years old) ²		Children (1-10 years old, 15-29 ¹ kg body weight) ³	
		Mean Exposure (mg/kg bw/day)	Exposure 97.5 th percentile (mg/kg bw/day)	Mean exposure (range lowest-highest) (mg/kg bw/day)	Exposure 95 th or 97.5 th percentile ² (range lowest-highest) (mg/kg bw/day)
Sulphur dioxide	0.2%*				
Class II		0.043	0.219	0.017-0.069	0.037-0.235
Class IV		0.179	0.738	0.046-1.012	0.259-2.960
4-MEI					
Tier 2 : MLs (EC)	0.025%*				
Class III		0.015	0.074	0.005-0.076	0.027-0.189
Class IV		0.022	0.092	0.006-0.127	0.032-0.370
Tier 3: MRLs (EUTECA, 2011a,b)					
Class III	0.014%**	0.009	0.041	0.003-0.042	0.015-0.106
Class IV	0.0183%**	0.016	0.068	0.004-0.093	0.024-0.271
THI	0.001%*				
Class III		0.0006	0.003	0.0002-0.003	0.0011 - 0.0076

¹ Except for Cypriot children where the reported body weight was 54 kg for 11-14 years old.

² For the UK population, estimates are based on the UNESDA report which gives the 97.5th percentile intake (Tennant, 2008).

³ For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.

⁴ ML: Maximum Levels from the Commission Directive 2008/128/EC; MRL: Maximum Reported Levels from the industry (EUTECA, 2011a,b)

*ML from the Commission Directive 2008/128/EC

**MRL from the industry (EUTECA, 2011a,b)

Based on the MLs defined for these constituents in the specifications for caramel colours and on the MRLs from industry (EUTeca, 2011a,b), anticipated dietary exposure calculated for the UK adult population gives the lowest means and highest 97.5th percentiles of 0.04 and 0.74 mg/kg bw/day for sulphur dioxide; of 0.009 and 0.092 mg/kg bw/day for 4-MEI; and of 0.0006 and 0.003 mg/kg bw/day for THI.

In children, anticipated dietary exposure gives the lowest means and highest 95th/97.5th percentiles of 0.017 and 2.96 mg/kg bw/day for sulphur dioxide; of 0.003 and 0.370 mg/kg bw/day for 4-MEI; and of 0.0002 and 0.008 mg/kg bw/day for THI.

3. BIOLOGICAL AND TOXICOLOGICAL DATA

In considering the biological and toxicological data available on the four classes of caramel colours, the Panel noted, as described in more detail in Section 2.1, that the wide range of starting materials and reactants used for the production of caramel colours may result in variability in chemical composition and purity, and that information on the composition of the materials tested is generally lacking, other than on the broad class of caramel that was tested together with information on some key constituents such as THI and 4-MEI. As already outlined in section 2.2, the Panel noted that LMW constituents of caramel colours produced with ammonia (Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel) include N-heterocyclic compounds such as pyridines, pyrazines, pyrroles and imidazoles. The occurrence of furanoid compounds in caramel colours is also reported, with furan being specifically identified in some caramel colours e.g. Class IV Sulphite Ammonia Caramel. The presence of acrylamide has also been reported.

The Panel also noted that a number of the identified or theoretical constituents of caramel colours, e.g. furan, acrylamide and 5-HMF, have genotoxic and in some cases carcinogenic potential, which may be relevant to the toxicological profile of the caramel colours. The Panel considered that the toxicological studies carried out on the caramel colours will have involved exposure to these compounds, in addition to the constituents THI and 4-MEI, and any anticipated toxicological effect due to the LMW fraction constituents of caramel colours should have been detected in these studies. A brief overview of the toxicological profiles of THI, 4-MEI, furan and 5-HMF is however provided in Sections 3.2.9 and 3.2.10 of this opinion. Acrylamide has not been included in this overview since the data provided by industry, as reported in Section 2.1, indicate that acrylamide was not detectable (limit of detection 1 µg/kg) in Class IV Sulphite Ammonia Caramel and Class III Ammonia Caramel (limit of detection 100 µg/kg), and its presence in Classes I and II caramels is not anticipated (EUTeca, 2011a,b).

3.1. Absorption, distribution, metabolism and excretion

In a study on the toxicokinetic behaviour of Class III Ammonia Caramel reported by JECFA (JECFA, 1987), groups of rats (2-4) received 10 or 20% caramel solutions (volume not defined) for 100 days. Examination of the faecal contents revealed that about one-third of the colour-giving constituents (not defined) appeared to be absorbed. According to the authors, no conclusions could be drawn regarding the absorption of colourless constituents (Haldi and Wynn, 1951). The Panel considered that few conclusions regarding the toxicokinetics of the caramel colours could be drawn from this very limited study.

JECFA also described a more comprehensive study on the toxicokinetics of Class IV Sulphite Ammonia Caramel in male F344 rats (Selim et al., 1992). In this study Caramel Colour IV (Class IV Sulphite Ammonia Caramel) prepared from uniformly-labelled [U-¹⁴C]-glucose was ultrafiltered in order to isolate the High Molecular weight Colour Fraction (HMcF). The colour fraction that was non-permeable to a 10 000-Da porosity membrane contained 84% of the colour, 22% of the solids and 24% of the radioactivity of the ¹⁴C-Caramel Colour IV. The Panel noted that no further characterisation of the HMcF was carried out, and hence no information is available on its composition. The absorption, distribution and excretion of ¹⁴C-HMcF was evaluated after administration of single or multiple oral doses of the material at a dose level of 2.5 g/kg bw. Following

administration of a single oral dose of ^{14}C -labelled material, groups of 4 rats were killed at intervals of 4, 8, 12, 24, and 96 hours after dosing, and radioactivity was measured in the following tissues/organs; blood, brain, heart, lungs, liver, kidneys, spleen, thymus, mesenteric and cervical lymph nodes, gastrointestinal tract (contents and tissues), and carcass. Most of the radioactivity was located in the gastrointestinal tract, and only low levels of radioactivity were found in blood and tissues. The specific radioactivity in the thymus, mesenteric lymph nodes, spleen, kidneys, and liver exceeded that in blood but, with the exception of the mesenteric lymph nodes, the radioactivity was cleared rapidly over the 96-hour study period. With the exception of the gastrointestinal tract, the highest tissue levels attained, in the liver and kidneys, never exceeded 0.02 % and 0.01 % respectively of the administered dose.

Rats on the multiple oral dosage regimen were given unlabelled HMCF in their drinking water for 13 days before the administration of a bolus dose of ^{14}C -HMCF at a dose level of 2.5 g/kg bw on day 14. On both dosage regimes, the predominant route of excretion was in the faeces. Only 1-2 % of the administered radioactivity was excreted in the urine and only a negligible amount was found in the expired air. More than 99% of the administered radioactivity was excreted in the faeces within 96 hours. The principal tissues in which radioactivity were found were the mesenteric lymph nodes, liver, kidney and tissues of the gastro-intestinal tract. No major differences were observed in the absorption, distribution or excretion patterns between the single and multiple oral dose regimens. The authors concluded that after administration of large doses of the coloured constituents of Class IV Sulphite Ammonia Caramel, only a small fraction was absorbed, distributed in lymphoreticular tissue, and eventually excreted in the urine; uptake by the mesenteric lymph nodes appeared to account for the pigmentation observed in this tissue (Selim et al., 1992).

The Panel noted that, except for the study on HCMF of Class IV Ammonium Sulphite Caramel described above, data on the toxicokinetics of the caramel colours are lacking. The Panel considered that individual constituents of the low molecular weight fraction of caramel colours (e.g. MW less than 500) are likely to be absorbed, although little information is available to confirm this.

3.2. Toxicological data

3.2.1. Acute oral toxicity

No data are available on the acute oral toxicity of Class I Plain Caramel, Class II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel. As reported by JECFA (JECFA, 1987), the oral LD_{50} of Class III Ammonia Caramel in rats was reported to be higher than 17 500 mg/kg bw (Chacharonis, 1960, 1963). In mice and rabbits Class III Ammonia Caramel administered at single doses of up to 10 000 and 15 000 mg/kg bw respectively did not result in treatment-related effects (Sharratt, 1971). In earlier JECFA evaluations (JECFA, 1972, 1977), it was reported that convulsions were observed after administration of Class III Ammonia Caramel (conceivably at higher doses than those used by Sharratt, 1971) to a range of species and by different routes. These convulsions were however considered to be attributable to the presence of 4-MEI in the Class III Ammonia Caramel product. Additional information on the toxicological profile of 4-MEI is provided in Section 3.2.9.

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Class I Plain Caramel

One short-term study on Class I Plain Caramel is described by JECFA and TemaNord. Class I Plain Caramel was administered to groups of 20 weanling female Wistar rats at levels of 0, 15, or 30% in the diet (highest dose equivalent to 30 g/kg bw/day) for 8 weeks, followed by a 4-week recovery period. Dose-related diarrhoea was observed in the treated animals and food efficiency was decreased (dose not specified), but the growth rate was normal. Haematological indices, in particular leucocyte counts, were normal throughout the study. The relative caecal weights were increased after eight weeks, but

returned to normal by the end of the 4-week recovery period. Discolouration of the mesenteric lymph nodes was observed in animals of both treatment groups after eight weeks, but the discolouration diminished during the recovery period. No other gross or microscopic pathological changes were reported (Sinkeldam and van der Heyden, 1976a).

In an additional study published subsequent to the JECFA and TemaNord reports, Caramel A (described by the authors as being in accordance with the definition, properties, identity test results and purity test results stipulated for Class I Plain Caramel) was orally administered at dosage levels of 0.5, 1, and 2 ml/kg bw/day to 5 Crj:CD (SD) IGS strain rats of each sex for 28 consecutive days (Harigae et al., 2000a). The control group received water alone. No deaths and no clinical signs of toxicity were noted in either sex of any group, and no treatment-related effects were reported on body weight, food consumption, urinalysis, ophthalmology, blood chemical analysis, organ weights or on histopathological findings. Females in all caramel colour A groups showed significant decreases in Activated Partial Thromboplastin Time (APTT) in comparison with the control group, and females receiving 0.5 ml/kg bw/day or 2 ml/kg bw/day showed significant increases in the lymphocyte fraction of total white blood cells and significant decreases in the neutrophil fraction. However all values were within the historical control range, and the authors concluded that these findings occurred by chance, and were not due to caramel colour A. Occasional histopathological findings including vitreous inclusions in proximal renal tubular epithelial cells in rats receiving Caramel A were also concluded by the authors to have occurred by chance, and were not due to caramel colour A. The non-toxic dose of caramel colour A was estimated by the authors to be at least 2 ml/kg bw/day (equivalent to 2.66 g/kg bw/day, sp. gr. 1.33) (Harigae, 2000a). The Panel agreed with this conclusion.

3.2.2.2. Class II Caustic Sulphite Caramel

A 90-day oral toxicity study has been carried out with Class II Caustic Sulphite Caramel in rats. Five groups of 20 male and 20 female weanling Fischer F-344 rats were given Class II Caustic Sulphite Caramel in drinking water at dose levels of 0, 4, 8, 12, or 16 g/kg bw/day for 90 days. Body weights and food and water intakes were recorded weekly. The principal observations 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 bw/day of Class II Caustic Sulphite Caramel, and slight, dose-related increases in absolute and relative kidney weights and full and empty caecum weights, with no evidence of significant histopathological changes in any tissue. Dose-related staining of the gastrointestinal tract and mesenteric lymph nodes was observed, with deposits of yellow pigment observed microscopically in the caecal mucosa and mesenteric lymph nodes (only the highest dose groups were examined microscopically) (MacKenzie, 1985a; MacKenzie et al., 1992a).

JECFA, in evaluating this study, concluded in agreement with the authors of the study that the decreased body-weight gain and increased kidney weight observed in the rats were the consequence of the reduced consumption of food and fluids, probably resulting from the reduced palatability of the drinking-water due to the presence of the caramel (JECFA, 1987). JECFA considered that the pigmentation of mesenteric lymph nodes and enlargement of the caecum were not of toxicological significance and concluded that the NOAEL was the highest dose tested, 16 g/kg bw/day. The Panel agreed with this NOAEL.

3.2.2.3. Class III Ammonia Caramel

The constituents THI and 4-MEI, found in Class III Ammonia Caramel, are of particular relevance in relation to the toxicological profile of this class of caramel.

In previous evaluations of Class III Ammonia Caramel including those of JECFA and SCF, a substantial number of studies on the short-term toxicity of Class III Ammonia Caramel have been described. One of the most notable effects in these studies was a reduction in circulating lymphocytes (lymphocytopenia), measured in haematological investigations. The overall conclusion that was drawn

from these studies at the time is that THI, a constituent in Class III Ammonia Caramel, and pyridoxine (vitamin B₆), a general constituent of the diet, play a major role in Class III Ammonia Caramel-related lymphocyte depression. Regarding the effects on white blood cells, JECFA in its evaluations commented that depression of lymphocyte counts has been shown to be due largely, if not solely, to THI. This conclusion was probably based on the studies of Sinkeldam and co-workers (Sinkeldam et al., 1982b, 1988) and Kroplien (1984, 1985), among others. Due to the large number of studies on Class III Ammonia Caramel, including mechanistic studies on the leucocytopenia observed in studies with this caramel colour, summaries of these studies are provided in Annex B, with overviews of key studies being provided in the body of the opinion. Similarly, summaries of specific studies on THI are provided in Annex D.

In these studies, as the normal proliferative activity of all lymphoid organs was not affected, it was concluded that THI did not induce lymphocytopenia by reduced production of lymphocytes (Houben et al., 1992a; Houben and Penninks, 1994). There were however indications of a diminished migration of mature cells from the thymus to the periphery (Sinkeldam et al., 1988; Houben et al., 1992a; Houben and Penninks, 1994). In addition, splenic cells of rats did not show the expected response upon mitogenic stimulation (Houben et al., 1992a; Houben and Penninks, 1994). More recently it has been shown, in a study in which mice were given THI in drinking water at a level of 50 mg/l for 3 days, that the diminished migration of lymphocytes from lymphoid tissues such as the thymus and spleen induced by THI was related to an increase in sphingosine 1-phosphate (S1P) in these tissues (Schwab et al., 2005). Further discussion of the immunotoxicity of both Class III Ammonia Caramel and THI is provided in sections 3.2.7 and 3.2.9 of this opinion.

The pivotal study used by both the SCF and JECFA to derive an ADI for Class III Ammonia Caramel was the 90-day oral toxicity study in rats carried out by MacKenzie et al. (1985b, 1992b). In this study, groups of weanling F344 rats (20 animals/sex/group) were given 2 different samples of Class III Ammonia Caramel in the drinking water at dose levels of 0, 10, 15, or 20 g/kg bw/day (sample A; containing approximately 15 mg THI/kg caramel on a solids basis;) and 20 g/kg bw/day (sample B; containing 295 mg THI/kg caramel on a solids basis) for 90 days. The diet contained >10 mg pyridoxine/kg. Although the findings were not always consistent, the animals treated with 15 and 20 g/kg bw/day of both sample A and B generally had decreased body weights and body weight gains compared with controls. All treated groups had significantly decreased food and drinking water intake compared with controls. The authors concluded that the effects on body weight gain were in part due to the reduced water intake caused by poor palatability of the drinking water rather than toxic effects of the caramel colour per se. The authors also suggested that a reduction in food intake is an invariable response to either voluntary or forced restriction of water intake by the rat (MacKenzie et al., 1992b).

Both sample A and sample B Class III Ammonia Caramel induced treatment-related increases in the absolute and relative weights of the caecum (full and empty) in both sexes at all dose levels. Absolute and relative kidney weights were also increased in a dose-related manner and were considered by the authors to be a consequence of reduced water intake. No histopathological changes in the kidneys of any of the test groups were observed. A dose-dependent decrease in the absolute weight of the thymus was observed in sample A males and sample B males and females. This effect was however only statistically significant at a dose of 20 g/kg bw/day. Neither sample had an effect on the relative thymus weight, and the Panel concluded overall that the effect on absolute thymus weight was not of toxicological relevance. Other differences in absolute and relative organ weights appeared to be a consequence of the dose-related reduction in body weight.

Sample B Class III Ammonia Caramel (containing 295 mg THI/kg) at a dose level of 20 g/kg bw/day induced a statistically significant decrease in lymphocyte counts in both sexes at 2 weeks and only in male rats at 6 weeks. All lymphocyte values in these groups were normal at the termination of the study. No statistically significant decreases in lymphocyte counts occurred in male or female groups fed batch A Class III Ammonia Caramel (containing approximately 15 mg THI/kg) at any of the dose levels, with the exception of male rats receiving 10 g/kg bw/day (but not at 15 or 20 g/kg bw/day) at 2 weeks only. There were no consistent changes in clinical chemistry values, except for a slight but

statistically significant increase in blood urea nitrogen values in rats of both sexes receiving sample B Class III Ammonia Caramel at 6 and 13 weeks, and in male rats receiving 20 g/kg bw/day of sample A Class III Ammonia Caramel at 13 weeks. Urinalysis revealed decreased urinary volume and increased specific gravity in animals receiving either sample A or sample B Class III Ammonia Caramel at 6 weeks. At termination at 13 weeks, these differences were only significant in males receiving 20 g/kg bw/day of either sample A or sample B Class III Ammonia Caramel. The only treatment-related microscopic changes noted were minimal to moderate accumulation of pigment in the tissues of the intestinal tract and mesenteric lymph nodes without other histopathological evidence of tissue damage.

To determine whether the poor palatability of the drinking water due to the presence of high levels of Class III Ammonia Caramel was the mechanism underlying the decreased body-weight gains frequently noted in toxicity studies with Class III Ammonia Caramel, a paired-feeding study was conducted (Sinkeldam, 1979). In this study a group of 10 male Wistar rats received drinking water containing 12 % Class III Ammonia Caramel (equivalent to 6 g/kg bw/day) whereas controls were permitted food and water intake quantitatively equivalent to that consumed by the exposed group. As similar decreases in body weight gain were noted in both exposed and control animals, it was concluded that growth depression after exposure to Class III Ammonia Caramel is the result of decreased fluid and food intake. Poor palatability of the drinking water containing the colour was the probable cause of these changes (Sinkeldam, 1979), and it has also been suggested that a reduction in food intake is an invariable response to either voluntary or forced restriction of water intake by the rat (MacKenzie et al., 1992b). The Panel agreed with this interpretation.

In a study in female Balb/c mice, the animals received a diet containing 0, 2 or 10% (equivalent to approximately 0, 3.3, or 16.7 g/kg bw/day) of a commercial Class III Ammonia Caramel preparation in the drinking water for 9 weeks. The sample of Class III Ammonia Caramel used contained < 25 mg THI/kg; the diet contained relatively high vitamin B₆ content (not specified). Although a treatment-related lymphocytopenia was not evident, flow cytometric analysis demonstrated reductions in the CD4⁺ and CD8⁺ lymphocyte subpopulations in exposed mice. In addition, the proliferative response of spleen cells to B and T cell mitogens was significantly reduced in the mice exposed to 2% Ammonia Caramel. No changes were observed in natural killer cell activity or in the humoral antibody response to a viral antigen (Thuvander and Oskarsson, 1994).

Overall, although some statistically-significant effects were seen in animals receiving Class III Ammonia Caramel compared with controls in the study of MacKenzie et al, they were not considered by the authors to be toxicologically relevant, and consequently the NOAEL for Class III Ammonia Caramel was considered to be 20 g/kg bw/day (MacKenzie, 1985b; MacKenzie et al., 1992b). JECFA concurred with this NOAEL and consequently allocated an ADI of 200 mg/kg bw/day to the colour (JECFA, 1987). The Panel also agreed that the NOAEL in this study was 20 g/kg bw/day.

3.2.2.4. Class IV Sulphite Ammonia Caramel

JECFA describes a number of short-term and subchronic studies with Class IV Sulphite Ammonia Caramel. Summaries of the more important studies for this opinion are provided in this section, other studies are provided as summaries in Annex C, as background information. The constituent 4-MEI is of particular relevance in relation to the toxicological profile of this class of caramel.

Female Wistar rats (20/group) received stock diet to which 0, 10, or 20% Class IV Sulphite Ammonia Caramel containing 202 mg 4-MEI/kg was added. From week 2-6 the levels of Class IV Sulphite Ammonia Caramel in the diet were increased to 15 and 25% and from week 7-16, the levels were increased to 25 and 30%. A 4-week recovery period followed. No effects were found on food consumption, growth, leucocyte count or relative thymus weight. The relative weights of the caecum, both filled and empty were increased after 4 weeks of feeding Class IV Sulphite Ammonia Caramel, however after the recovery period of 4 weeks the increases had disappeared. Gross examination at autopsy after 16 weeks of feeding Class IV Sulphite Ammonia Caramel revealed a dose-related, brown-greenish coloration of the mesenteric lymph nodes in all test animals at the highest dose level.

Microscopically, the lymph nodes of the test rats showed pigment accumulation. After the recovery period of 4 weeks the colour change and pigmentation were less, but still visible (Sinkeldam and van der Heyden, 1975).

Wistar rats (10 animals/sex/group) were fed diets containing 0, 1.25, 2.5, 5, 10, and 15% of low colour intensity Class IV Sulphite Ammonia Caramel or 0, 0.5, 1, 2, 4, and 6% high colour intensity Class IV Sulphite Ammonia Caramel for 10 weeks. In this study and also those of Procter et al., 1976, BIBRA, 1977, and in the genotoxicity studies on Class IV Sulphite Ammonia Caramel reported by Brusick and co-workers, as reported below, the descriptor low intensity caramel referred to a 'single strength' Class IV caramel (typically colour intensity up to 0.18) and high intensity caramel refers to a 'double strength' Class IV caramel (typically, >0.2 colour intensity). Class IV Sulphite Ammonia Caramel caused loose stools at the highest dose levels, although body weight gains were not affected. Leucocyte counts were not affected. Slight indications of caecal enlargement were observed. Minimal amounts of pigment were observed in the mesenteric lymph nodes of rats fed 2.5% and higher of the low colour intensity sample, and 1% and higher of the high colour intensity sample (Sinkeldam and van der Heyden, 1976b).

Sprague-Dawley rats (15 animals/sex/group) were fed diets containing 1.25, 2.5, 5, 10, and 15% of low colour intensity Class IV Sulphite Ammonia Caramel or 0.5, 1, 2, 4, and 6% high colour intensity Class IV Sulphite Ammonia Caramel for 10 weeks. In the animals fed 15% low colour intensity Class IV Sulphite Ammonia Caramel, the faeces became soft within 2 weeks. The water content of the faeces from the highest dose groups (both high and low intensity) was higher than that of the controls. Body weight gains were slightly decreased in male, but not female, rats fed high and low intensity caramel colour. No effects were found on total white cell or lymphocyte counts or pigmentation of the mesenteric lymph nodes. Caecal weights were generally increased in all test groups fed Class IV Sulphite Ammonia Caramel (Procter et al., 1976).

Weanling Wistar rats (10-60 animals/sex/group) were fed diets containing 0, 0.5, 1.0, 2.0, 4.0, or 16.0% of high or low colour intensity Class IV Sulphite Ammonia Caramel for 10 weeks, followed by a recovery period with basal diet (7 or 28 days). Decreased body weight gains were noted in animals of both sexes fed 16% high colour intensity Class IV Sulphite Ammonia Caramel, but not in the groups fed low colour intensity caramel. Food intake was not consistently altered. There were no consistent or dose-related differences in lymphocyte counts or leucocyte counts. Liver weights were significantly increased in the group fed 16% Class IV Sulphite Ammonia Caramel (high colour intensity). Increased relative kidney weights were observed in the groups fed 2, 4, and 16% Class IV Sulphite Ammonia Caramel (high colour intensity) and 16% Class IV Sulphite Ammonia Caramel (low colour intensity), although no histological changes were observed. Increased caecal weights were seen only at the 16% feeding level for both the low intensity and the high intensity Class IV Sulphite Ammonia Caramel. At necropsy pigmentation of the lymph nodes was seen at the 16% feeding level of both low intensity and high intensity caramel colour. Microscopically, pigmentation was observed in the mesenteric lymph nodes in the males and females in the groups fed 16% Class IV Sulphite Ammonia Caramel (high colour intensity). Relative weights of the liver and kidneys and caecal weights returned to normal during the recovery period (BIBRA, 1977).

Weanling F344 rats (groups of 30 males and 30 females) were given Class IV Sulphite Ammonia Caramel in drinking water at concentrations which provided intakes of 0, 15, 20, 25, or 30 g/kg bw/day for 13 weeks (Heidt and Rao, 1980). All rats given Class IV Sulphite Ammonia Caramel produced dark-coloured, soft or sticky, liquid and/or odorous faeces which stained and caused alopecia of the perianal area, most noticeably at the higher dose levels. Dose-related significant decreases in food intake, water consumption (after correction for caramel content), and body weight gains were observed in both males and females at all dose levels of caramel and were attributed to the poor palatability of the drinking solutions. All treatment groups of both sexes had significantly reduced blood urea nitrogen and alkaline phosphatase levels at both 45 and 90 days. Total serum protein values of both sexes in the treatment groups were lower than controls at 90, but not at 45, days. The authors suggested that these effects may be due to reduced food intake and growth retardation. All treated rats

had reduced urine volume and increased urine specific gravity, protein, ketones, and acidity, which were associated with decreased water consumption. Treatment-related changes were seen in absolute and relative weights of brain, liver, heart, male gonads, thymus, spleen and kidney weights were observed, although no histopathological changes were seen in these organs that correlated with the organ weight changes. Both absolute and relative thymus weights were significantly decreased in all male treatment groups and in females receiving 25 or 30 g/kg bw/day. Absolute spleen weights were significantly decreased in males receiving 25 or 30 g/kg bw/day and in females receiving 20, 25 and 30 g/kg bw/day. Both absolute and relative kidney weights were significantly increased in all treated rats, both males and females. Full and empty caecum weights were also increased in all treatment groups, with macroscopically visible caecal enlargement. Dark staining of the mucosa of the caecum and colon was noted in all treatment groups. The only treatment-related histopathological changes reported were an accumulation of yellowish-tan pigment in macrophages of the mesenteric lymph nodes, together with a thickening of the caecal mucosa and the tunical muscularis of the caecum. The authors considered that these changes in the caecum and mesenteric lymph nodes were nonspecific consequences of administration of large quantities of caramel, as also concluded by JECFA in their 1970 evaluation, and that the observed decreases in organ weights were largely related to the changes in body weight seen in the study (Heidt and Rao, 1980). The Panel concurred with this interpretation and noted that the effects reported in the study have been seen in the majority of other studies in the rat conducted with high dose levels of the caramel colours (see also section 3.2.4.3). The Panel concluded therefore that 30 g/kg bw/day, the highest dose tested, could be considered a NOAEL in this study.

Class IV Sulphite Ammonia Caramel was evaluated in a 13-week drinking water study in F344 rats, as a preliminary study for chronic toxicity and carcinogenicity studies (MacKenzie, 1985b; MacKenzie et al., 1992c). Class IV Sulphite Ammonia Caramel was mixed with demineralised water and the solutions given to the animals ad libitum in the drinking water. Groups of 30 male and 30 female rats were given Class IV Sulphite Ammonia Caramel for 13 weeks at levels of 0, 15, 20, 25 or 30 g/kg bw/day, based on body weight data and drinking water consumption data. Treated rats had dose-related lower water consumption than controls. This was attributed by the authors to poor palatability of the drinking water, and was generally associated with decreased food consumption and body weights, as previously described for the parallel study on Class III Ammonia Caramel (section 3.2.2.3). Rats given Class IV Sulphite Ammonia Caramel often had soft or liquid malodorous faeces. Blood biochemical changes in the rat (i.e. reduced blood urea nitrogen, alkaline phosphatase and total serum protein) appeared to be related to dietary influences and were not considered by the authors to be toxicologically significant. There were no treatment-related alterations in haematological variables (MacKenzie, 1985b, MacKenzie et al., 1992c). The authors noted that *“it is well known that when the water intake of rodents is limited, food intake will be proportionally reduced and there will be a reduced rate of body weight gain. Reduced water intake also affects renal function, reducing urinary output and increasing specific gravity. A compensatory hypertrophy of the kidney, without pathological changes, also occurs”* (MacKenzie et al., 1992c). The authors considered therefore that the NOAEL in this study was 30 g/kg bw/day. The Panel agreed with this conclusion.

As reported by JECFA, adult beagle dogs (groups of 3 males and 3 females) received 0, 6, 12.5, or 25% Class IV Sulphite Ammonia Caramel in their diet (estimated by the Panel to be equivalent to 0, 1.5, 3.1 or 6.25 g/kg bw/day for a 10 kg dog eating 250 g of chow per day) 5 days per week for 90 days. No significant adverse effects on growth, behaviour, food consumption, mortality, liver function, kidney function, haematology, urinalysis, gross pathology, or histopathology were noted (Kay and Calandra, 1962c). Based on this report, the Panel considered that the NOAEL in this study was 6.25 g/kg bw/day, the highest dose tested.

3.2.3. Genotoxicity

Caramel colours have been extensively tested for genotoxic potential in a variety of assays *in vitro*, reflecting the fact that browning and caramelising products formed in food have been suggested or demonstrated to contain potentially genotoxic chemicals. Overall the results in a range of *in vitro*

systems were generally negative, with a few marginally positive findings (e.g. Kawachi et al., 1980; Ishidate and Yoshikawa, 1980; Jensen et al., 1983; Ishidate et al., 1984; Yu et al., 1984; Aeschbacher, 1986). The caramel colours investigated in these publications were generally commercial caramel colours whose nature and classification was not further specified by the authors (JECFA, 1987). On the basis of these marginally inconclusive results, the International Technical Caramel Association (ITCA) in the period 1980–1990 carried out a comprehensive testing programme with samples of well-defined caramel colours to determine the genotoxic potential of the different caramel classes in a battery of genotoxicity tests *in vitro* and *in vivo* (ITCA, 1990). The results of these studies are summarised below for the different caramel classes.

3.2.3.1. Class I Plain Caramel

As described by JECFA, two samples of Class I Plain Caramel with different colour intensities were tested in a bacterial mutagenicity (Ames) assay using *Salmonella typhimurium* (*S. typhimurium*) strains TA98, TA100, TA1535, TA1537, and TA1538. Class I Plain Caramel was neither mutagenic nor cytotoxic, either with or without activation by rat liver S-9 fraction, at up to 20 µl per plate (Richold and Jones, 1980a; 1980b, unpublished study not available to the Panel).

The genotoxicity of Class I Plain Caramel was also investigated in a battery of three valid *in vitro* short-term tests and one *in vivo* (Adams et al., 1992). In this study a total of 15 caramel colours representative of all four caramel classes were examined for genotoxic activity using the *S. typhimurium* plate incorporation assay (Ames test). Five bacterial strains, TA1535, TA1537, TA1538, TA98 and TA100 were used in all the plate incorporation tests, some of the caramel colours being tested both with and without a pre-incubation stage. The results of the bacterial mutation assay with Class I Plain Caramel showed no evidence of genotoxic activity. The Panel noted that it is not clear whether the result reported for Class I Plain Caramel in the publication of Adams et al. (1992) was also that reported in the earlier unpublished reports of Richold and Jones (1980a, 1980b).

In the mammalian cell mouse lymphoma L5178Y assay Class I Plain Caramel produced a statistically significant increase in mutation frequencies, but only at dose levels of 6.5 mg/ml and above in the absence of S-9 metabolic activation system. A cytogenetic assay *in vitro*, using Chinese Hamster Ovarian (CHO) cells, showed evidence of chromosomal damage in the presence of Class I Plain Caramel but only in the absence of S-9 and only at the highest dose level of 10 mg/ml. A negative result for Class I Plain Caramel was obtained in the mouse micronucleus assay *in vivo*, using a single oral dose of 5000 mg/kg bw Class I Plain Caramel with examination of bone marrow from groups of 5 male and 5 female mice at 24, 48 or 72 hours after dosing (Adams et al., 1992).

In a more recent study, the genotoxicity of Caramel Colour A (described by the authors as being in accordance with the definition, properties, identity test results and purity test results stipulated for Class I Plain Caramel) was investigated *in vitro* in a bacterial reverse mutation test and a chromosome aberration test, and a mouse micronucleus test *in vivo* (Harigae, 2000b). Negative results were reported at 5000 µg Caramel Colour A/plate without and with metabolic activation in the reverse mutation test, carried out with five bacterial strains, *S. typhimurium* TA100, TA98, TA1535 and TA1537, and *Escherichia coli* WP2uvrA, using the pre-incubation method. The chromosome aberration test was carried out using cultured mammalian fibroblasts derived from Chinese hamster lung tissue. In a cell growth inhibition test performed in the same culture system prior to the chromosomal aberration test the IC₅₀ of Caramel Colour A was 3309 µg/ml without S9, but the IC₅₀ was not reached up to 5000 µg/ml in the presence of S9. In the chromosomal aberration test, no effects were noted at concentrations up to and including 5000 µg/ml with metabolic activation and up to and including 3500 µg/ml without activation. However, in the absence of metabolic activation Caramel Colour A at 4000 µg/ml, a concentration inducing overt cytotoxicity, caused chromosomal aberrations, although the aberration potential was considered by the authors to be weak and without a marked dose-dependency.

The mouse micronucleus test was performed in CD-1 mice, dosed orally by gavage at levels of 500, 1000 or 2000 mg/kg bw and killed 24 or 48 hours after administration of the second of two doses,

given 24 hours apart. Negative results for micronuclei induction were obtained at 2000 mg/kg bw, the highest dose level tested (Harigae, 2000b). The Panel noted that there was no change in the NCE/PCE ratio in animals receiving Caramel Colour A compared with controls.

3.2.3.2. Class II Caustic Sulphite Caramel

As reported by JECFA, Class II Caustic Sulphite Caramel at 2.5-20 µl/plate was neither mutagenic nor bacteriotoxic in the Ames test using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, with or without metabolic activation by rat liver S-9 fraction (Richold and Jones, 1980c; Richold et al., 1984a, unpublished studies not available to the Panel). No mutagenic activity of Class II Caustic Sulphite Caramel in *S. typhimurium* at exposure levels of up to 20 mg/plate and no *in vitro* evidence for clastogenic effects in CHO cells at exposure levels of up to 5 mg/ml were reported in the study of Allen et al., which examined these two endpoints for all four classes of caramel colours (Allen et al., 1992). The Panel noted that it is not clear whether the result reported for Class II Caustic Sulphite Caramel in the publication of Allen et al. (1992) was also that reported in the earlier unpublished reports of Richold and Jones (1980c) and Richold et al. (1984a).

Class II Caustic Sulphite Caramel was tested for potential mutagenic activity based on induction of DNA repair (unscheduled DNA synthesis) in cultured human epithelial (HeLa 53) cells. Caustic Sulphite Caramel was incorporated in the culture medium at concentrations of 25-51 200 µg/ml and the test was performed on two occasions both in the presence and absence of rat liver S-9 mix. In both tests, in the absence of the S-9 mix a small (not further specified) but statistically significant increase in the number of silver grains over nuclei was observed at a concentration of 25 600 µg/ml; no significant increases were seen at higher concentrations in this test or at any concentration in the repeat test (Allen and Proudlock, 1984).

Class II Caustic Sulphite Caramel was not clastogenic to cultured CHO cells at concentrations of 500, 2500, or 5000 µg/ml either in the presence or absence of rat liver S-9 mix (Allen et al., 1984).

No additional information has been found on the genotoxicity of Class II Caustic Sulphite Caramel.

3.2.3.3. Class III Ammonia Caramel

As reported by JECFA, Class III Ammonia Caramel was evaluated in a bacterial mutagenicity (Ames) test using *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, and also in a yeast mutagenicity assay using *Saccharomyces cerevisiae* (*S. cerevisiae*) (strain D4), again with or without metabolic activation. The dose range employed was 1-50 mg/plate for TA100 and 1-20 mg/plate for all other testers. Class III Ammonia Caramel was non-mutagenic under these conditions (Jagannath and Brusick, 1978a, unpublished study not available to the Panel). Negative results were also obtained in a further series of bacterial mutagenicity studies using a range of caramel concentrations and *S. typhimurium* strains, with or without metabolic activation (e.g. Jagannath and Brusick, 1978b, Kawana et al., 1980; Richold and Jones, 1980d; Ashoor and Monte, 1983; Richold et al., 1984b; Allen et al., 1992).

In an Ames test using *S. typhimurium*, a series of samples taken at various stages in the manufacture of Class III Ammonia Caramel were found to be non-mutagenic in strains TA98 and TA1535 with and without metabolic activation. However, exposure of strain TA100 to samples taken late in the production process, increased the number of revertants in the absence of metabolic activation (Jensen et al., 1983).

As reported by JECFA, the same batch of Class III Ammonia Caramel as that tested by Richold et al. (1984b) in an Ames test study with negative results was investigated for its ability to induce unscheduled DNA synthesis in cultured human epithelial (HeLa 53) cells with and without metabolic activation. The test was performed on two occasions at Class III Ammonia Caramel concentrations of

0.025-51.2 mg/ml in the culture medium. In both tests, Class III Ammonia Caramel caused a statistically significant increase in nuclear grains at concentrations of 6.4 and 12.8 mg/ml only in the absence of metabolic activation (Allen and Proudlock, 1984, unpublished study not available to the Panel).

Five samples of caramel colour (one being Class III Ammonia Caramel) gave positive results in a chromosome aberration test in a cultured Chinese hamster lung fibroblast cell line, with or without metabolic activation (aberrations in 20% of metaphase cells). No further details were provided in the publication (Ishidate and Yoshikawa, 1980). In contrast, Class III Ammonia Caramel was not clastogenic to cultured CHO cells (no further details) at concentrations of 0.5, 2.5, or 5 mg/ml either in the presence or absence of metabolic activation (Allen et al., 1984, unpublished study not available to the Panel). In another *in vitro* cytogenetic assay using cultured CHO cells, Class III Ammonia Caramel was found to induce a significant increase in chromosome aberrations at concentrations of 3 mg/ml and above in the absence of metabolic activation. In the presence of metabolic activation of the colour no cytogenetic activity was observed at concentrations up to 5 mg/ml (Galloway and Brusick, 1981a, 1981b, unpublished study not available to the Panel).

In an *in vivo* mouse micronucleus test, Class III Ammonia Caramel was not clastogenic when administered to animals (5 per sex) by gavage at doses of 0, 1050, or 3500 mg/kg bw (2 doses 24 hours apart), with sampling of the bone marrow 6 hour after the last dose (Cimino and Brusick, 1981; Brusick et al., 1992). The Panel noted that the bone marrow sampling regime in this study was not in line with that recommended in OECD TG 474.

No additional information has been found on the genotoxicity of Class III Ammonia Caramel.

3.2.3.4. Class IV Sulphite Ammonia Caramel

A number of studies concerning the genotoxicity of Class IV Sulphite Ammonia Caramel are described by JECFA (JECFA, 1987).

Class IV Sulphite Ammonia Caramel was evaluated for mutagenicity using the Ames *S. typhimurium* bacterial mutagenicity test and the *S. cerevisiae* mutagenicity test. The samples tested were blends of 3 samples of Class IV Sulphite Ammonia Caramel (low colour intensity) and 3 samples of Class IV Sulphite Ammonia Caramel (high colour intensity). The *Salmonella* strains used were TA98, TA100, TA1535, TA1537, and TA1538. Tests were conducted with or without metabolic activation (metabolic activation system was a liver microsomal enzyme preparation from Aroclor-induced rats). Tests were conducted over a range of concentrations from 1-50 mg/plate. No signs of genotoxic activity were observed with any of the samples of Sulphite Ammonia Caramel tested using either the *S. typhimurium* or *S. cerevisiae* test organisms (Jagannath and Brusick, 1978a). Similar negative results had also been obtained for Class IV Sulphite Ammonia Caramel in an earlier study by one of the authors (Brusick, 1974), and in a subsequent study by Ashoor and Monte (Ashoor and Monte, 1983).

No mutagenic activity of Class IV Sulphite Ammonia Caramel (low and high colour intensity) in *S. typhimurium* and no *in vitro* evidence for clastogenic effects in CHO cells were reported in the study of Allen et al. (Allen et al., 1992), which examined all four classes of caramel colours. Similar results were reported by Brusick and co-workers (Galloway and Brusick, 1981a, 1981b; Brusick et al., 1992).

No additional information has been found on the genotoxicity of Class IV Sulphite Ammonia Caramel.

Overall the Panel concluded that there were no concerns regarding the genotoxic potential of caramel colours.

3.2.4. Chronic toxicity and carcinogenicity

3.2.4.1. Class I Plain Caramel and Class II Caustic Sulphite Caramel

No studies on the chronic toxicity and/or carcinogenicity of Class I Plain Caramel or Class II Caustic Sulphite Caramel were found in any of the previous evaluations, and no additional information has been found on the chronic toxicity and/or carcinogenicity of these classes of caramel colours.

3.2.4.2. Class III Ammonia Caramel

In the JECFA evaluation three chronic studies with Class III Ammonia Caramel are described in detail (JECFA, 1987).

Three groups of B6C3F1 mice (50 animals/sex/group) were given drinking water containing 0, 1.25, or 5% Class III Ammonia Caramel (equivalent to approximately 0, 2.1, or 8.4 g/kg bw/day) for 96 weeks followed by 8 weeks of drinking water without caramel colour (the Class III Ammonia Caramel contained less than 25 mg THI/kg; pyridoxine content of the diet not specified). No consistent differences compared with controls were noted with respect to growth or water intake. Although the cumulative mortality of males in the 5% group was higher than controls in weeks 100-104, no clear pathological differences in any organs and no treatment-related abnormalities in urinalyses were noted. In males of both treatment groups statistically significant elevations of the total leucocyte counts were observed, but were considered by the authors to be within the range encountered for B6C3F1 mice. No treatment-related gross pathology was noted during or at the end of the experiment and no significant differences in tumour incidence were found compared to controls (Hagiwara et al., 1983).

Four groups of Wistar rats (48 animals/sex/group) were fed diets containing 0, 1, 3, or 6% Class III Ammonia Caramel (equivalent to 0, 0.5, 1.5, and 3 g/kg bw/day) for 2 years (Evans et al., 1976). The material used in the study was described as a straight ammonia-catalysed "half open-half closed" caramel, and while general specifications for the material tested were provided, no information on the THI or 4-MEI content of the sample was provided, nor were dietary pyridoxine levels given. A significant decrease in body weight was observed in males receiving caramel in the diet compared with controls at all dose levels, of the order of 11% lower in the 1% group, 17% in the 3% group and 18% in the 6% group. Body weight gain was also reduced at all dose levels in males compared with controls (statistical analysis not presented in the paper). Food intake in males was reduced in males at a number of time intervals for which data were reported, although these reductions were not statistically significant. Body weights and hence body weight gains were also reduced in females at all dose levels compared with controls, particularly towards the end of the study, although decreases were not consistently dose-related and were never statistically significant (< 10% in any treated group). Water consumption was reported not to be affected (data not presented in the publication). Weights of major organs were reduced in both sexes in parallel with the reduced body weights, however these were not statistically significantly different from controls when adjusted for body weight. In particular, absolute spleen weights were reported to be reduced in a dose-related manner, being statistically significantly different from controls in all male treated animals and in females receiving 3% or 6% Class III Ammonia Caramel in the diet. Data for absolute spleen weights were not provided in the publication, which only provided data for organ weights relative to body weight. Reductions in spleen weight were not however statistically significant when adjusted for body weight. The only statistically significant changes in relative organ weights were reported for relative caecal (full) weights, which were increased in males receiving 3% or 6% caramel in the diet and in females receiving 1% or 6% caramel; for relative brain weight, increased in males receiving 3% or 6% caramel; and for relative thyroid and kidney weight, increased in females receiving 3% or 6% caramel.

In this study, a significant reduction in leucocyte counts compared with controls in both males and females receiving 6% Class III Ammonia Caramel in the diet was associated with a lymphocytopenia in the early part of the study. This effect was sustained until week 80 and 52 in males and females respectively. Lymphocytopenia was reported at week 13 in both males and females receiving 3%

caramel in the diet, and in males at this dose level at week 52. Neoplastic findings found in control and treated rats were typical of rats of this age and strain and none were considered by the authors to be treatment-related. There was no evidence of a carcinogenic effect. The authors concluded that a no-observed-effect level could not be identified for the caramel used in this particular study, on the basis of the reduction of absolute spleen weights observed in males at all dose levels of caramel and in females receiving 3% and 6% caramel, which they considered to be linked to the lymphocytopenia seen in the animals at earlier stages in the study (Evans et al., 1976). The authors attributed the other main finding in the study, the reduction in body weight gain in animals receiving caramel, to be due to a dilution effect of the test material when given in the diet (i.e. reduction in the amount of diet available to the animals due to the high levels of caramel present), with the effect in the treated males being exacerbated by a reduction in food intake.

The Panel concluded that the lymphocytopenia seen in the animals, together with the splenic effects, could be attributed to the presence of THI in the material tested, by analogy with other studies showing this effect (e.g. Mackenzie 1985b; Mackenzie et al. 1992b), and that since information was not provided on the level of THI in the material tested, these endpoints could not be used as the basis for identifying a NOAEL in the study. The Panel noted that other findings in the study were typical of those reported generally for the caramel colours (reduced body weight gain and increased caecal weight), considered by Mackenzie and others not to be toxicologically important (Mackenzie 1985b), and on this basis the Panel considered that a NOAEL of 3 g/kg bw/day (the highest dose tested) could be identified.

Three groups of F344 rats (50 animals/sex/group) received Class III Ammonia Caramel in drinking water at levels of 0, 1, or 4% (equivalent to 0, 0.5, or 2 g/kg bw/day) for 104 weeks. The Class III Ammonia Caramel used contained less than 25 mg THI/kg; the basal diet contained 11-12 mg pyridoxine/kg. After the exposure period animals received drinking water without caramel for 9 weeks. No treatment-related differences in growth or survival rates were noted. In the pituitary gland of males of the 4% group a significantly higher incidence of tumours was observed. This finding was however attributed by the authors to the variability of spontaneous tumour incidence in F344 rats. No dose-related effects were found regarding incidence and/or induction time of tumours in various other organs and tissues (Maekawa et al., 1983). The only parameters monitored in this study other than histopathological investigations of neoplastic change were clinical signs, mortality and body weight changes. The authors did not report a NOAEL for the study, however on the basis of the absence of any treatment-related effect in the study the Panel concluded that 2 g/kg bw/day (the highest dose tested) could be considered a NOAEL.

No additional information has been found on the chronic toxicity or carcinogenicity of Class III Ammonia Caramel.

3.2.4.3. Class IV Sulphite Ammonia Caramel

The 1987 JECFA evaluation describes a number of chronic toxicity/carcinogenicity studies with Class IV Sulphite Ammonia Caramel.

Six samples of Class IV Sulphite Ammonia Caramel (3 samples of low colour intensity and 3 samples of high colour intensity) were tested in a long-term toxicity study in rats (Sinkeldam et al., 1975; Sinkeldam et al., 1976). The Panel noted that the report of this study indicates that the material tested was "ammonia caramel". The Panel concluded that this was Class IV Sulphite Ammonia Caramel, as described by JECFA, based on the fact that information was provided on levels of 4-MEI only, not on 4-MEI and THI, as would have been the case for Class III Ammonia Caramel. Each test group consisted of 40 male or 40 female weanling Wistar rats, except the control group which had double this number of animals. Animals were selected from the first litter of parents fed diets containing the various caramel colours from weaning age (see section 3.2.5, reproduction studies, Til and Spanjers, 1973). The dose levels tested were 5, 10, or 15% (nominal) Class IV Sulphite Ammonia Caramel (low colour intensity) and 2, 4, or 6% (nominal) Class IV Sulphite Ammonia Caramel (high colour

intensity). The caramel samples contained, respectively, 202, 400 or 600 mg 4-MEI/kg (5, 10, or 15% low intensity caramel) or 350, 639 or 852 mg 4-MEI/kg (2, 4 or 6% high intensity caramel). The study design was such that the highest daily intake of 4-MEI was provided by a dose level of 10% (nominal) low colour intensity Class IV Sulphite Ammonia Caramel in the diet, containing 600 mg 4-MEI/kg, equivalent to approximately 3 mg 4-MEI/kg bw/day. Parameters investigated were body weight, food intake, mortality, blood biochemistry, haematology, liver and kidney function tests, urine composition, organ weights, macroscopic and microscopic pathology. Haematological examinations were carried out at weeks 13, 26, 52, 79 and 98, while blood biochemical parameters and urinalysis were investigated at 13, 26, 52 and 98 weeks. For interim investigations, 10 males and 10 females per treatment group were killed at 52 weeks.

No treatment-related effects on behaviour, growth, food intake, mortality, liver and kidney function tests, urine composition, or organ weights were observed. No clinical changes were observed, except for slightly decreased haemoglobin and haematocrit values at weeks 78 and 98 in males fed high colour intensity Class IV Sulphite Ammonia Caramel. Leucocyte counts were decreased in females fed 10 and 15% of one sample of low colour intensity Class IV Sulphite Ammonia Caramel at weeks 13 and 52, but these changes were not consistent and decreases in lymphocyte counts were not observed in the differential count. In males, no such effect was noted and in a subsequent experiment in which the same sample of Class IV Sulphite Ammonia Caramel was fed to female rats at levels as high as 15 and 30%, no indications of decreased leucocyte counts were observed after 4, 8, 12, or 16 weeks. At autopsy, an increased incidence of greenish discoloured mesenteric lymph nodes was observed in most groups fed high levels of Class IV Sulphite Ammonia Caramel. Microscopically, an increased pigment-phagocytosis in the mesenteric lymph nodes in all test groups (except the group fed 2% high colour intensity Class IV Sulphite Ammonia Caramel) was observed. Enlarged caecae were observed in a number of animals at 52 weeks, this change was not evident at the terminal kill. No evidence of any other adverse structural or cellular alteration was found. Gross and microscopic examination of the other organs did not reveal any pathological changes attributable to the ingestion of Class IV Sulphite Ammonia Caramel. After about 14 months, mortality attributed to intercurrent disease was observed in both control and treated groups. Approximately three-quarters of the animals died or were killed before the experiment was terminated at week 104. An increase in the incidence of neoplastic lesions in the different groups was not found (Sinkeldam et al., 1976). The Panel considered that the haematological findings in this study could not be attributed to treatment, and that the other findings in the study (pigmentation of lymph nodes and caecal enlargement) were non-adverse effects of administration of large amounts of caramel, reported in most long-term studies with the caramel colours. The Panel concluded therefore that the highest dose tested, of 15% low intensity caramel, estimated to be equivalent to 7.5 g/kg bw/day, was a NOAEL. This caramel contained a level of 202 mg 4-MEI/kg caramel, representing a daily intake of 1.5 mg 4-MEI/kg bw/day, which could be taken as a NOAEL for 4-MEI. However, since no treatment-related effects were identified in the dose group receiving 10% (nominal) low colour intensity Class IV Sulphite Ammonia Caramel in the diet, containing 600 mg 4-MEI/kg, the Panel considered that an intake (and hence a NOAEL) of 3 mg/kg bw/day could be identified for 4-MEI in this study.

Five groups of F344 rats (each group consisting of 50 male and 50 female weanling rats) were given drinking water containing Class IV Sulphite Ammonia Caramel at 2.5, 5.0, or 10 g/kg bw/day for 24 months (MacKenzie, 1985c; MacKenzie et al., 1992c). In addition, groups of F344 rats (25 male and 25 female weanling rats/group) were given drinking water containing Class IV Sulphite Ammonia Caramel at 2.5, 5.0, 7.5 or 10 g/kg bw/day for 1 year (chronic toxicity phase). Class IV Sulphite Ammonia Caramel did not affect survival in either the chronic toxicity or carcinogenicity phases of this study and, other than dark-stained and soft faeces, there were no treatment-related ante-mortem observations. Body weights were reduced for both males and females at the 5 and 10 g/kg bw/day dose levels, but the effects were correlated with reduced water and food consumption at these dose levels and were considered by the authors to reflect the reduced palatability of the drinking water, as already described in the 13-week dose range finding study (section 3.2.2.4). According to the authors, clinical chemistry and haematological studies at 6 and 12 months in the chronic toxicity study did not reveal changes of toxicological concern. At 6 months, serum concentrations of blood urea nitrogen and

creatinine were reduced in male groups treated with 5.0, 7.5, and 10 g/kg bw/day and in female groups treated with 7.5 and 10 g/kg bw/day caramel colour. Similar changes were noted at 12 months. Creatinine levels were within the normal range for the F344 rat, whereas blood urea nitrogen levels were slightly outside this range. Decreased levels of serum total protein, albumin, and globulin were also noted at the 6-month sampling, particularly in male rats. These changes, which were less marked at 12 months, were not accompanied by any pathology in the liver or kidneys and were not considered by the authors to be of toxicological importance. The urinalysis studies revealed generally reduced urine volume and increased specific gravity in both sexes.

At necropsy the changes noted were characteristic of the feeding of high levels of caramel colour, and consisted primarily of brown staining of the gastrointestinal tract and mesenteric lymph nodes and caecal enlargement. There was no evidence of reactive hyperplasia to the pigment in the mesenteric lymph nodes of the gastrointestinal tract. Increased kidney weights were noted in both sexes in animals fed Class IV Sulphite Ammonia Caramel; which the authors considered to be related to the water imbalance in these animals, since no histological alterations were present that could be associated with the increased renal weight. Random variations in both benign and malignant neoplasms typical of the F344 strain and this age of animal were observed; however, there were no treatment-related differences. The authors concluded that the feeding of Class IV Sulphite Ammonia Caramel at doses up to 10 g/kg bw/day for 24 months did not induce neoplastic changes or non-neoplastic changes of toxicological importance. Although statistically significant changes were noted in some parameters, they were not considered to be toxicologically important. The highest dose fed, 10 g/kg bw/day, was considered to be the NOAEL (MacKenzie, 1985c; MacKenzie et al., 1992c). The Panel agreed with this NOAEL.

Class IV Sulphite Ammonia Caramel was administered in drinking water at dose levels of 2.5, 5.0, or 10.0 g/kg bw/day to B6C3F1 mice (groups of 50 male and 50 female mice) for 104 weeks (MacKenzie, 1985d; MacKenzie et al., 1992c). A complete necropsy was performed on all animals dying on test or sacrificed in a moribund condition, and on all survivors at termination. Except for some sporadic significant differences in mean body weights of treated male and female groups compared to controls, no consistent effects on body weight were observed. Males of the 10 g/kg bw/day dose group had lower mean food consumption than the control groups for 67 of the 104 weeks and males receiving 2.5 g/kg bw/day had lower mean food consumption for 21 of the 104 weeks. There were no consistent differences in food intake of males at the 5 g/kg bw/day dose level nor in any of the females. Decreased fluid intake was noted, particularly in males at the higher dose levels, but not in females. There were no treatment-related differences in survival rates. At necropsy, there were dose- and/or treatment-related effects on the gastrointestinal tract and mesenteric lymph nodes, including dark gastrointestinal contents, staining of the mucosa, and diffusely red and congested mesenteric lymph nodes. These changes were not considered by the authors to be toxicologically important and there were no other changes of toxicological significance. There was no evidence of treatment-related neoplastic lesions in any organs (MacKenzie, 1985d; MacKenzie et al., 1992c). The Panel agreed with the authors' conclusions and considered that the dose level of 10.0 g/kg bw/day was a NOAEL for Class IV Caramel in B6C3F1 mice.

No additional information has been found on the chronic toxicity or carcinogenicity of Class IV Sulphite Ammonia Caramel.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Class I Plain Caramel and Class II Caustic Sulphite Caramel

No studies on the reproductive and/or developmental toxicity of Class I Plain Caramel and Class II Caustic Sulphite Caramel were described in any of the previous evaluations, and no additional information has been found on the reproductive and/or developmental toxicity of these classes of caramel colours.

3.2.5.2. Class III Ammonia Caramel

JECFA describes three developmental toxicity studies on Class III Ammonia Caramel in mice, rats and rabbits (Morgareidge, 1974a). The Panel noted that the reports of these studies indicate that the material tested was described as “Caramel; Bakers & Confectioners”, sample description FDA 71-82, and that bakers’ caramel and confectioners’ caramel are synonyms for Class III Ammonia Caramel.

Groups of pregnant CD1 mice (22-23 animals/group) received Class III Ammonia Caramel by gavage at doses of 0, 16, 74, 345, and 1600 mg/kg bw/day on days 6 to 15 of gestation (Morgareidge, 1974a). After Caesarean section on day 17 no treatment-related effects were observed regarding the number of implantation sites and resorption sites, or maternal and fetal survival. In addition dams were examined for urogenital anatomical abnormalities, fetal weight was recorded and fetuses were examined for gross external abnormalities, visceral abnormalities, and skeletal defects. The number of abnormalities did not differ from those occurring spontaneously in controls and no treatment-related effects were noted on fetal weight (Morgareidge, 1974a).

Pregnant Wistar rats (21-24 animals/group) were given Class III Ammonia Caramel by gavage at doses of 0, 16, 74, 345, and 1600 mg/kg bw/day on days 6 to 15 of gestation. After Caesarean section on day 20 no treatment-related effects were observed regarding the numbers of implantation sites and resorption sites, or maternal and fetal survival. In addition dams were examined for urogenital anatomical abnormalities, fetal weight was recorded and fetuses were examined for gross external abnormalities, visceral abnormalities, and skeletal defects. No treatment-related effects were seen in the urogenital tract of the dams, nor on fetal body weight, and fetuses displayed no gross external, visceral, or skeletal abnormalities other than those occurring spontaneously in controls (Morgareidge, 1974a).

Pregnant Dutch-belted female rabbits (11-12 animals/group) were administered caramel colour by gavage at doses of 0, 16, 74, 345, and 1600 mg/kg bw/day on days 6 to 18 of gestation. After Caesarean section on day 29 no treatment-related effects were observed on numbers of corpora lutea, implantation sites and resorption sites, or maternal and fetal survival. In addition dams were examined for urogenital anatomical abnormalities, fetal weight was recorded and fetuses were examined for gross external abnormalities, visceral abnormalities, and skeletal defects. No treatment-related effects were seen in the urogenital tract of the dams, nor on fetal body weight, and fetuses displayed no gross external, visceral, or skeletal abnormalities other than those occurring spontaneously in controls (Morgareidge, 1974a).

No additional information has been found on the reproductive and/or developmental toxicity of Class III Ammonia Caramel. The Panel noted that no multigeneration study was available on Class III Ammonia Caramel.

3.2.5.3. Class IV Sulphite Ammonia Caramel

JECFA described three developmental toxicity studies on Class IV Sulphite Ammonia Caramel in mice, rats and rabbits (JECFA, 1987).

Fifteen male and female Wistar rats were given 0 or 10% Class IV Sulphite Ammonia Caramel solution as their sole fluid source until day 100 and were then mated. Animals of the F1 generation (25 males and 25 females) were weaned and again given 0 or 10% caramel solution until day 100. There were no adverse effects with regard to the number of litters born and the number of pups/litter. No influence on haematology, growth, food consumption, gross pathology, or histopathology of the F1-generation at 100 days of age was observed (Haldi and Wynn, 1951). The full report of this unpublished study was unavailable for independent evaluation by the Panel.

Six different samples of Class IV Sulphite Ammonia Caramel, three double strength and three single strength, each containing a different level of 4-MEI (between 200 and 850 mg 4-MEI/kg caramel) were tested in a reproduction study in rats (Til and Spanjers, 1973). The Panel noted that the report of this study indicates that the material tested was “ammonia caramel”. The Panel concluded that this was Class IV Sulphite Ammonia Caramel, as described by JECFA, based on the fact that information was provided on levels of 4-MEI only, not on 4-MEI and THI, as would have been the case for Class III Ammonia Caramel. Twelve groups of 10 male and 20 female weanling Wistar rats were provided with diets containing 2-15% caramel. At week 12, the rats were mated in subgroups of 5 males and 10 females. After a 3-week mating period, the females were caged individually. Following parturition, the numbers of pups per litter and the weight of the litter were recorded on days 1, 10 and 20, and the young were inspected grossly for club-feet, cleft palate and hydrocephalus. After weaning, the dams were killed and numbers of implantation sites per dam were recorded. No consistent, dose-related effects on growth of the F0 animals were noted. No adverse effects were seen on female fertility, litter size, average weight and growth of the pups, or number of implantation sites or sex ratio of the young. In one group (10% SS (single strength) Class IV Sulphite Ammonia Caramel + 600 mg 4-MEI/kg) there was a slight increase in mortality at birth. No developmental effects were found (Til and Spanjers, 1973). The Panel estimated that the maximum intake of 4-MEI in this study would have been approximately 6 mg/kg bw/day, based on consumption of a diet containing 10% single strength Sulphite Ammonia Caramel with measured level of 600 mg 4-MEI/kg caramel, and assuming a body weight of 100 g for the rats at the start of the study.

Mature F344 rats (12 animals/sex/group) were given Class IV Sulphite Ammonia Caramel at concentrations of 0, 10, 15, 20, or 25% in drinking water (8-28 g/kg bw/day) for 21 days prior to mating until lactation (Tierney, 1980). In the 20- and 25% dose groups of both generations there was a higher incidence of soft stools than in the controls. In addition, dose-related depression of body-weight gain was noted in animals of the F0 and F1 generation. Mating, pregnancy, and fertility rates were considered by the authors to be comparable for all groups. Pups in the 25% dose group showed a higher incidence of alopecia compared with controls, and a generalised poor condition during the last 7 days of suckling. The authors attributed these findings to the fact that the bodies of most of the pups in this dose group were covered with faecal/test material and to a lack of grooming during this period. One pup at this dose level had an arched spine, which was again attributed to the general poor condition of the pups in this group. Alopecia was also seen in the pups in the 20% Class IV Sulphite Ammonia Caramel in the diet group, and in the F0 animals in these two dose groups, attributed by the authors to the increased grooming activity of the animals in an effort to remove test substance/faecal material. The Panel agreed with the conclusions of the authors regarding these findings. The number of implantation sites and of live pups at days 0, 4, and 21 of lactation in the 20%-dose groups were significantly lower than control values. The Panel noted however that there was no dose-related trend, since in the 25% dose group these parameters were not statistically different from controls. No significant haematological changes were observed at 45 or 90 days post-weaning. Blood urea nitrogen values were lower than controls at 45 and 90 days, as has been noted in several other studies in which animals received high levels of caramel in the diet, but other clinical chemistry values were normal. At necropsy, dose-related increases in absolute and relative weights of the liver, kidneys, and caecum (full and empty) were observed from animals in the 15% and higher dose groups. The only gross treatment-related morphological changes reported were brown/black or green colouration of the contents and mucous membrane of the lower gut and mesenteric lymph nodes (Tierney, 1980).

The study report did not identify a NOAEL. The Panel concluded that the effects observed were in line with the effects on body and organ weights observed in other subchronic and chronic studies and that for reasons already outlined above these were not considered adverse. The Panel also considered that the findings in the pups in the 25% dose group were attributable to the general condition of the dams and the body contamination with faecal/test material rather than a specific developmental or toxic response, while the decreases in implantation sites and numbers of live pups seen in the 20% group did not show a dose response relationship. Therefore the Panel concluded that the dose level of 25% Class IV Sulphite Ammonia Caramel in the diet, the highest dose level tested was a NOAEL. This dietary dose level was reported by the authors to be equivalent to an intake of 25-30 g/kg bw/day for the

female rats during the period of mating, gestation and parturition, although intakes by the males in this dose group were lower, at approximately 20-25 g/kg bw/day. The Panel considered that a NOAEL of 25-30 g/kg bw/day for female rats could be identified in this study, for the purposes of risk assessment.

In addition, JECFA described a study on developmental toxicity in three different species (Morgareidge, 1974b). The Panel noted that the material tested was described as caramel beverage, sample description FDA 71-83, which the Panel concluded was Class IV Sulphite Ammonia Caramel, as described by JECFA.

Pregnant albino CD1 mice (19-22 animals/group) were administered 0, 16, 74.3, 345, and 1600 mg/kg bw/day Class IV Sulphite Ammonia Caramel by gavage, from day 6-15 of gestation. After Caesarean section on day 17 no treatment-related effects were observed regarding the numbers implantation sites and resorption sites, or maternal and fetal survival. In addition dams were examined for urogenital anatomical abnormalities and fetuses were examined for gross external abnormalities, visceral abnormalities, and skeletal defects. The number of abnormalities did not differ from those occurring spontaneously in controls (Morgareidge, 1974b).

Pregnant Wistar rats (21-24 animals/group) were given Class IV Sulphite Ammonia Caramel by gavage at doses of 0, 16, 74, 345, and 1600 mg/kg bw/day on days 6 to 15 of gestation. After Caesarean section on day 20 no treatment-related effects were observed regarding the numbers of implantation sites and resorption sites, or maternal and fetal survival. Fetuses displayed no gross external, visceral, or skeletal abnormalities other than those occurring spontaneously in controls. Finally, the body weights of live pups were recorded but the effects were not described (Morgareidge, 1974b).

Pregnant Dutch-belted female rabbits (11-12 animals/group) were administered Class IV Sulphite Ammonia Caramel by gavage at doses of 0, 16, 74, 345, and 1600 mg/kg bw/day on days 6 to 18 of gestation. After Caesarean section on day 29 no effects were observed regarding numbers of corpora lutea, implantation sites and resorption sites, or maternal and fetal survival. Fetuses displayed no gross external, visceral, or skeletal abnormalities other than those occurring spontaneously in controls (Morgareidge, 1974b).

No additional information has been found on the reproductive and/or developmental toxicity of Class IV Sulphite Ammonia Caramel. The Panel noted that no multigeneration study was available on Class IV Sulphite Ammonia Caramel.

3.2.6. Hypersensitivity (intolerance/allergenicity)

No studies on intolerance to and/or allergenicity of any of the caramel colours were found in any of the previous evaluations, and no additional information has been found on these aspects.

3.2.7. Haematotoxicity and immunotoxicity

A large number of studies have been carried out to investigate the potential immunotoxicity of Class III Ammonia Caramel containing THI and also that of THI alone, reflecting the lymphocytopenia seen in studies with Class III Ammonia Caramel (see also section 3.2.2.3). A number of relevant studies on Class III Ammonia Caramel are described below while summaries of other relevant studies have been included in Annex B as background information, or in section 3.2.9, relating to the toxicity of 4-MEI and THI, or Annex D, relating to specific toxicity studies carried out with THI.

The effects of Class III Ammonia Caramel and THI and the influence of dietary pyridoxine on these effects were investigated in two studies in rats (Houben et al., 1992a). Weanling rats fed a diet containing 2-3 mg pyridoxine/kg and exposed to 4% Class III Ammonia Caramel or 5.72 mg THI/kg

bw in drinking water for 4 weeks showed reduced cell numbers in spleen and popliteal lymph nodes, as well as in the blood. Flow cytometric analyses demonstrated a comparable reduction in B and T lymphocytes. In blood, spleen, and popliteal lymph nodes, CD4⁺ lymphocytes were more reduced than CD8⁺ cells. The number of bone marrow cells was not affected. Although thymus weight and cell number were also not affected, a decreased cortex over medulla area ratio and an increase in medullary cell density was observed, largely due to an increase in CD4⁺ thymocytes. Decreased numbers of ED2⁺ macrophages were observed in the thymic cortex and in the spleen red pulp. All effects observed were either less pronounced or absent in a study with rats fed a diet containing 11-12 mg pyridoxine/kg in addition to Class III Ammonia Caramel. The effects of Ammonia Caramel and THI on the immune system were similar. Whereas the lymphocytopenia induced by Class III Ammonia Caramel exposure was associated with changes in vitamin B₆ status, THI did not induce obvious effects on vitamin B₆ parameters. The authors proposed that the effects of Class III Ammonia Caramel and THI on the immune system are not caused by a disturbance of vitamin B₆ metabolism, but may in fact result from a disturbance of a specific pyridoxal phosphate-dependent process (Houben et al., 1992a).

Rats were exposed to 0.4 or 4% Class III Ammonia Caramel or to 5.72 mg THI/kg bw in drinking water during and for 28 days prior to the start of immune function assays (Houben et al., 1993). Resistance to *T. spiralis* was examined in an oral infection model and clearance of *L. monocytogenes* upon an intravenous infection was studied. In addition, natural cell-mediated cytotoxicity of splenic and non-adherent peritoneal cells and the antibody response to sheep red blood cells were studied. From the results the authors concluded that exposure of rats to Class III Ammonia Caramel or THI influenced various immune function parameters. Thymus-dependent immunity was suppressed, while parameters of non-specific resistance were also affected, as shown by a decreased natural cell-mediated cytotoxicity in the spleen and an enhanced clearance of *L. monocytogenes* (Houben et al., 1993).

Female Balb/c mice (3 week-old) fed a diet with a relatively high vitamin B₆ content were exposed to 2 or 10% (equivalent to 3 or 15 g/kg bw/day) of a commercial Class III Ammonia Caramel preparation with a low THI content (less than 25 mg THI/kg) in the drinking water for 9 week. Although this treatment did not induce a lymphocytopenia in the exposed mice, flow cytometric analysis of lymphocyte subpopulations demonstrated reductions in the CD4⁺ and CD8⁺ cell populations. In addition, the proliferative response of spleen cells to B and T cell mitogens was significantly reduced in the mice exposed to 2% Class III Ammonia Caramel. No changes were observed in natural killer cell activity or in the humoral antibody response to a viral antigen. The authors considered that their results indicate that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B₆ status (Thuvander and Oskarssen, 1994). The Panel agreed with the conclusions of the authors that this study is indicative of effects of Class III Ammonia Caramel on the immune system in mice, with a Low-Observed-Adverse-Effect-Level (LOAEL) of 3 g/kg bw/day, but considered that the study should not be used as a pivotal study for the purposes of risk assessment without further substantiation, given a number of studies in rats showing no effect on haematological parameters over longer periods and at higher dose levels than those used in the study of Thuvander and Oskarssen.

The results of similar studies on THI alone are described in Section 3.2.9 and in Annex D.

3.2.8. Human studies

No studies with Class I Plain Caramel or Class II Caustic Sulphite Caramel in human volunteers were found in any of the previous evaluations and no additional information has been found on human studies with these caramel colours.

Houben et al. (1992b; 1992c) reported on a detailed study with Class III Ammonia Caramel in elderly men selected because of the presence of marginal vitamin B₆ deficiency. In this study, groups of 8 male human volunteers (above 65 years of age) were given a dessert twice a day for 7 days containing

100 mg Class III Ammonia Caramel/kg bw with either 23 mg THI/kg (commercial sample) or 143 mg THI/kg (research sample). The intake of THI from the latter Class III Ammonia Caramel sample was estimated to be 28.6 µg/kg bw/day. A control group was given a dessert twice a day without Class III Ammonia Caramel. Two weeks prior to and during the experiment Class III Ammonia Caramel and foods rich in vitamin B₆ were excluded from the diet. Intake of Class III Ammonia Caramel by these volunteers did not induce effects on blood lymphocyte numbers or on proliferative responses to mitogenic stimulation. In addition, no effect was seen on other haematological parameters including serum immunoglobulin levels and on immunogen production *in vitro*. Houben et al. (1992b) state that spontaneous vitamin B₆ deficiency in humans is not seen frequently, but it may occur under certain conditions. For example, chronic alcohol abusers, females on oral contraceptives, pregnant women, breast-fed infants, and elderly men and women may have increased risk of vitamin B₆ deficiency. Houben et al. (1992c), in comparing the results obtained in this study with data in rats maintained on normal and pyridoxine-reduced diets, suggested that, with regard to oral intake of THI, humans are less sensitive to Caramel Colour III-induced lymphocytopenia than are rats (Houben et al., 1992c).

A pilot study was carried out in which 9 volunteers ingested 1.5 g of Class III Ammonia Caramel daily (approximately 20-25 mg/kg bw/day; no details on THI content) for 21 days. No changes in total circulating leucocytes, lymphocytes, and erythrocytes, or in haemoglobin concentrations were found that could be attributed to treatment with Class III Ammonia Caramel. Three volunteers occasionally had soft stools; no control groups were used (BIBRA, 1976).

Tolerance studies of Class IV Sulphite Ammonia Caramel were conducted in human volunteers. The subjects, 10 men and 10 women, ingested Class IV Sulphite Ammonia Caramel once daily in simulated soft drinks over 3 test periods of 21 days each separated by 7-day rest intervals. The test doses were 6 g/day during the first test period, 12 g/day during the second period, and 18 g/day (equivalent to 100, 200 or 300 mg/kg bw/day for a 60 kg person in the respective test periods). Haematological, clinical chemical and routine urinary parameters were studied at the beginning of each ingestion period, after 10 days of ingestion, and at the end of each ingestion period. Most individual values for haemoglobin, haematocrit, RBC, corrected sedimentation rate, WBC, and differentials (neutrophils, basophils, eosinophils, monocytes, and lymphocytes) were found to be within normal limits. There were a few instances of values outside the normal range, indicating mild neutropenia and mild lymphocytosis, but these were not consistent and were unrelated to the ingestion of Class IV Sulphite Ammonia Caramel. On the other hand, caramel ingestion was associated with an increased frequency of bowel movements and softening or increased liquidity of faeces (Marier et al., 1977a; 1977b).

3.2.9. Studies on 4-methylimidazole (4-MEI) and 2-acetyl-4-tetrahydroxy-butylimidazole (THI)

The imidazole constituents 4-MEI and THI, the former occurring in both Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, and the latter occurring only in Class III Ammonia Caramel, have been investigated independently in a number of studies because of their role in the toxicological profile of these two classes of caramel colours.

4-MEI

4-MEI is formed by interaction of ammonia with reducing sugars, a chemical reaction occurring during the ammonia process used in the production of both Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. 4-MEI has been identified as a toxic by-product of fermentation in ammoniated hay forage for livestock animals (Ray et al., 1984) and has also been identified as the causal agent of convulsions in cattle and sheep fed ammonia-treated molasses (Wiggins, 1956a,b; Bartlett and Broster, 1957). Neurologic signs have been reported in calves of nursing cows fed ammoniated hay and sheep fed ammoniated hay (Weiss et al., 1986; Motoi et al., 1997) and were attributed to the presence of 4-MEI in the feed (Motoi et al., 1997).

4-MEI has been shown to cause convulsions at acutely toxic doses, in a range of species and by different routes. The LD₅₀ values for 4-MEI were determined to be 370 mg/kg bw in mice and 599 mg/kg bw in chicks, and the Convulsive Doses (CD₅₀) were only slightly lower than the LD₅₀ values. In a study testing the convulsant effects of a range of imidazoles, 4-MEI was found to be the most potent, producing convulsions in rabbits, mice, and chicks at single oral doses of 360 mg/kg bw (Nishie et al., 1969, 1970). The LD₅₀ values of several other imidazoles were also determined and found to be at least four times higher than those for 4-MEI (Nishie et al., 1969, 1970).

Further relevant publications on the toxicology of 4-MEI are presented in Annex D, for information.

JECFA at its twenty-first meeting concluded that the toxicity of 4-MEI as a constituent in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel was no longer a cause for concern since the introduction of chemical specifications limits the concentration of 4-MEI in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel produced by the ammonia process, to a level of 200 mg 4-MEI/kg caramel (JECFA, 1978). JECFA concluded that exposure to 4-MEI as a result of use of Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel as food colours at the levels foreseen would be substantially below the levels found to induce neurological effects in a range of animal species.

More recently, the National Toxicology Programme (NTP) of the US National Institutes of Health has carried out a series of toxicity studies on 4-MEI and its structural isomer 2-MEI (NTP, 2004), and has also completed chronic toxicity and carcinogenicity studies on 4-MEI in Fischer 344 rats and in B6C3F1 mice (NTP, 2007). The NTP summaries of these studies relevant to 4-MEI are provided in Annex D. Notably, the NTP has concluded that *“Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of 4-methylimidazole in male F344/N rats exposed to 625, 1,250, or 2,500 mg/kg. There was equivocal evidence of carcinogenic activity of 4-methylimidazole in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was clear evidence of carcinogenic activity of 4-methylimidazole in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Exposure to 4-methylimidazole resulted in non-neoplastic lesions in the liver of male and female rats and the lung of female mice and in clinical findings of neurotoxicity in female rats.”* (NTP, 2007).

The Panel noted that NTP concluded that 4-MEI is not genotoxic *in vitro* or *in vivo* (NTP, 2007). The Panel also noted that in rats the increased incidence of mononuclear cell leukemia was only seen at the highest dose of 2500 mg 4-MEI in the diet, equivalent to 170 mg 4-MEI/kg bw/day. In mice, the statistically significant effects noted by the NTP referred in the main to benign neoplastic lesions of the lung (adenomas), the incidences for malignant neoplastic lesions of the lung (carcinomas) only being statistically significantly increased in male mice at the top dose of 1250 mg 4-MEI/kg diet, equivalent to 170 mg 4-MEI/kg bw/day. The Panel concluded that the carcinogenic effect of 4-MEI seen in mice in this study was thresholded, and that the intermediate dose of 625 mg 4-MEI/kg diet, equivalent to 80 mg 4-MEI/kg bw/day could be considered to be a NOAEL for these effects.

The Panel noted that 4-MEI has recently been listed by the US State of California as a chemical known to cause cancer (OEHHA, 2011)

THI

The key toxicological effect of THI, as already indicated in previous sections of this opinion, is its immunosuppressive potential, mediated by its effect on components of the immune system, primarily the immunomodulatory molecule S1P (Hla, 2004, 2005; Schwab et al., 2005; Rivera et al., 2008; Yu et al., 2010). S1P is a lysophospholipid that plays an important role as a regulator of the immune and cardiovascular systems, acting through G-protein-coupled receptors on cell surfaces and regulating immune cell trafficking between lymphoid tissue and the blood and other tissues of the body (Hla, 2004, 2005; Rivera et al., 2008). Levels of S1P are normally low in most tissues, including lymphoid tissue, but are higher in blood and lymph, providing a driving force for migration of lymphocytes and

other immunocompetent cells into the peripheral circulation (Rivera et al., 2008). THI has been shown to mediate an increase in S1P in lymphoid tissue via inhibition of S1P lyase, the enzyme involved in degradation of S1P in the cell, and thereby inhibiting the egress of immunocompetent cells from the lymphoid organs (Schwab et al., 2005). This is considered to be the underlying mechanism for the lymphocytopenia seen in studies in rodents with THI alone and with Class III Ammonia Caramel containing levels of THI in levels in excess of 25 mg/kg caramel (the level established in the JECFA specifications (JECFA, 2006)).

As reported in more detail in Annex B, in studies in rats Sinkeldam and co-workers demonstrated significant reductions in peripheral blood lymphocytes and total leucocytes at a dose level of 1% Class III Ammonia Caramel in drinking water, equivalent to 1 g caramel/kg bw/day and estimated to be equivalent to 200 µg THI/ kg bw/day (Sinkeldam et al., 1988). Similar statistically significant reductions in lymphocytes were observed when the rats were given 200 µg, 500 µg or 2 mg THI/kg bw/day alone in drinking water. The authors reported decreases in lymphocyte counts in rats receiving as little as 0.1% Class III Ammonia Caramel in drinking water for 1 week and maintained on a low-pyridoxine diet (2-3 mg/kg). Class III Ammonia Caramel intake at this level in drinking water was 0.1 g kg bw/day, equivalent to 20 µg THI/ kg bw/day. Statistical analysis was not reported for this aspect of the study, but evaluation of the data provided would indicate that while lymphocytopenia was evident at this dose level, the reduction was unlikely to be statistically significant.

The 90-day oral toxicity study in rats on Class III Ammonia Caramel carried out by MacKenzie et al. also included an evaluation of the toxicity of THI after a 4-week dosing period (MacKenzie, 1985b; MacKenzie et al., 1992b). Groups of 10 rats per sex were given 0, 1, 2, 4, 16 or 32 mg THI/l in drinking water for 4 weeks (equivalent to approximately 0.1, 0.2, 0.5, 1.9 and 3.7 mg THI/kg bw/day in the treated groups), and groups of 20 rats per sex were given 0, 8 or 64 mg THI/l, equivalent to 0, 0.9, or 7.2 mg THI/kg bw/day. The rats were maintained on a normal rat chow, with adequate levels of pyridoxine. After 4 weeks 10 rats per sex of the 0, 0.9 and 7.2 mg/kg groups were given water without THI for 2 weeks (recovery phase). Decreased mean body weights in all test groups after 2 and 4 weeks were noted but ascribed to fasting for blood sampling and urinalysis. Gross and microscopic pathology in a large range of organs and tissues revealed no treatment-related effects. Treatment-related decreases in white blood cell and lymphocyte counts, and increases in neutrophil counts were observed. These changes returned to normal after 3 days recovery phase and remained normal. The NOAEL for the reduction in total lymphocytes was determined to be 4 mg THI/l in drinking water, equivalent to 380 µg/kg bw/day, in males and 1 mg THI/l in drinking water, equivalent to 120 µg/kg bw/day, in females (MacKenzie et al., 1992b).

Houben et al. (1992c) reported a range of estimated intakes of THI or Class III Ammonia Caramel that, in combination with manipulation of dietary pyridoxine levels, resulted in lymphocytopenia and could therefore be regarded as LOAELs for this effect. They ranged from an estimated intake of 1600 µg THI/ kg bw/day (provided by a level of 8% Class III Ammonia Caramel in drinking water) at a dietary pyridoxine level of 1.2 mg/kg, 800 µg THI/kg bw/day (provided by a level of 4% Class III Ammonia Caramel in drinking water) at a dietary pyridoxine levels of 8.2 mg/kg, 200 µg THI/kg bw/day in drinking water at dietary pyridoxine level of 1.8, 4.9 or 8.2 mg/kg and 20 µg THI/kg bw/day at a dietary pyridoxine level of 2-3 mg/kg. The majority of the LOAELs reported by Houben et al. (1992c) were derived from the paper of Sinkeldam et al. (1988) referred to above, although the authors also provided a LOAEL of 57.2 µg THI/ kg bw/day (provided by a level of 0.4% Class III Ammonia Caramel in drinking water) derived from their own work (Houben, 1992).

Houben et al. suggested that since rats fed a diet containing 2-3 mg pyridoxine/kg diet may have a vitamin B₆ status comparable to that of marginally vitamin B₆-deficient humans, the absence of effects indicative of immunotoxicity in humans exposed to approximately 28 µg THI/kg bw/day in Class III Ammonia Caramel may indicate that humans are less sensitive to THI- or Class III Ammonia Caramel-induced immunotoxic effects than are rats (Houben et al. 1992c; Houben and Penninks, 1994).

Comparison of the effects of Class III Ammonia Caramel in rats and mice indicate that mice are equally sensitive to the immunotoxic effects of THI as rats (Houben and Penninks, 1994; Thuvander and Oskarssen, 1994). A more recent study by Schwab et al. (2005) has shown that in mice (age not indicated) receiving 7.5 mg/kg bw/day THI (50 mg/l in drinking water) for 3 consecutive days, THI induced a marked decrease in the number of circulating lymphocytes and suppressed immunity. The mean THI plasma level was 135 ng/ml. S1P levels in lymphoid tissues of mice increased more than 100-fold after THI treatment, due to inhibition of the S1P degrading enzyme S1P lyase, an intracellular degrading enzyme which is expressed by hematopoietic cells (Schwab et al., 2005).

In a study exploring the relationship between systemic THI exposure, splenic S1P concentrations, and lymphocytopenia in rats, a single dose of THI was administered by gavage at levels of 10 and 100 mg/kg bw to male rats (n=8), and blood samples taken at intervals of up to 48 hours after dosing for determination of THI absorption and blood lymphocyte counts (Yu et al., 2010). Another 30 male rats gavaged at levels of 10 and 100 mg/kg bw were sacrificed at intervals of up to 48 hours after dosing for assessment of splenic levels of S1P. The study showed that THI was rapidly absorbed and reached a plasma peak level at 1 hour after dosing. Splenic S1P increased and reached a peak level at 24 hours after dosing. THI plasma concentration was linked to splenic S1P concentration and as the splenic S1P level increased, blood lymphocyte counts decreased. According to the authors, the results of this pharmacokinetic–biomarker–pharmacodynamic model explain the discrepancies between plasma THI concentration and the pharmacological response and the relationship of THI exposure, S1P, and lymphocytopenic response (Yu et al., 2010).

Annexes B and D provide further summaries of a range of studies carried out on the immunomodulatory effects of THI, in particular its effects on lymphocytes, leading to the lymphocytopenia seen in studies on Class III Ammonia Caramel containing THI as a constituent. Based on a review of these studies, including the more recent study of Schwab et al. (2005) showing effects on S1P, an important immunoregulatory substance, THI can be considered to be a potent immunosuppressive agent. The Panel noted that additional studies on THI to determine a consistent NOAEL for the immunomodulatory effects, based on Tier I and Tier II immunotoxicity parameters, would provide useful information to further characterise the immunotoxicity of THI.

3.2.10. Overview of the toxicological profiles of furan and 5-HMF, LMW constituents of the caramel colours

Furan

In 2004, the EFSA Panel on Contaminants in the Food Chain (CONTAM) considered available data on exposure to furan as a result of its presence in a number of foods that undergo heat treatment, such as canned and jarred foods (EFSA, 2004b). The CONTAM Panel additionally reviewed the available data on the toxicity of furan, and noted that *“The toxicity database of furan is incomplete as no data are available on reproductive and developmental toxicity. There are also no human studies. Furan is cytotoxic and the liver is the primary target organ of furan toxicity after oral application. Furan is clearly carcinogenic to rats and mice, showing a dose-dependent increase in hepatocellular adenomas and carcinomas in both sexes. In rats, also a dose-dependent increase in mononuclear leukaemia was seen in both sexes. A very high incidence of cholangiocarcinomas of the liver was present in both sexes, even at the lowest dose. Taking into account all the presently available data on the mode of action of furan, the Panel concluded that the weight of evidence indicates that furan-induced carcinogenicity is probably attributable to a genotoxic mechanism. However, chronic toxicity with secondary cell proliferation may indirectly amplify the tumour response. From the presently available data it appears that there is a relative small difference between possible human exposures and the doses in experimental animals that produce carcinogenic effects, probably by a genotoxic mechanism. However, a reliable risk assessment would need further data on both toxicity and exposure.”* (EFSA, 2004b). The ANS Panel agreed with the conclusions of the CONTAM Panel.

5-hydroxymethyl-2-furfural (5-HMF)

5-HMF has been identified in a wide variety of heat-processed foods including coffee, milk, fruit juices, spirits, honey, etc, in addition to the caramel colours. Along with many other flavour- and colour-related substances, 5-HMF is formed in the Maillard reaction as well as during caramelisation. 5-HMF is thus a ubiquitous component of many foodstuffs. It is also a flavouring substance [FL-no: 13.139], and is included in the Community Register of flavouring substances used in or on foodstuffs. 5-HMF as a flavouring substance has been evaluated by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (EFSA, 2010) and is currently under re-evaluation in the light of new data on genotoxicity, subchronic toxicity and carcinogenicity.

For 5-HMF, the Panel noted that substantial toxicological data are available, including a 3-week and 13-week subchronic studies, and chronic and carcinogenicity studies in B6C3F1 mice and F344/N rats (NTP, 2008). The carcinogenicity study in mice demonstrated that 5-HMF may induce liver tumours, but these were considered not to be relevant for human risk assessment. In contrast, no carcinogenic responses were reported in the study with rats. The critical non-neoplastic effect seen in these NTP studies was cytoplasmic alterations in renal proximal tubule epithelium in mice, observed in the 13-weeks study with mice at 188 mg/kg bw/day and above with an intermittent dose regimen of 5 days per week.

In relation to the genotoxicity of 5-HMF, taking into account additional data on metabolism, the Panel noted that the substance is negative in the conventional Ames test. Mutagenicity is observed only upon inclusion of 3'-phosphoadenosine-5'-phosphosulphate (PAPS), a sulpho-group donor and liver cytosol into the metabolic system, suggesting the formation of a sulphate-ester; 5-sulphoxymethyl-2-furfural (SMF). SMF itself was mutagenic in the absence of any metabolic activation system. In an *in vitro* assay, 5-HMF induced dose-dependent increase in DNA damage (Comet assay), but the Panel noted that this study has major drawbacks and inconsistencies and has to be considered of limited validity. In another Comet assay in HepG2 cells, able to express both cytochrome P450 (CYP) and sulphotransferase (SULT) enzymes, some indication of DNA damage were observed, but the substance did not induce clastogenic or aneugenic effects (micronucleus assay) in the same cell system. *In vivo*, a non-standard micronucleus assay in peripheral blood erythrocytes, conducted as part of a sub-chronic study in mice, provided no indication of a genotoxic potential (NTP, 2008). The Panel noted however that this study has limited validity since no bone marrow cell toxicity was observed.

4. DISCUSSION

The Panel was not provided with newly submitted dossiers on the caramel colours and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation.

Caramel colours are colouring substances authorised as food additives in the EU, and are classified according to the reactants used in their manufacture as follows: Class I Plain Caramel or Caustic Caramel (E 150a); Class II Caustic Sulphite Caramel (E 150b); Class III Ammonia Caramel (E 150c) and Class IV Sulphite Ammonia Caramel (E 150d).

The four classes of caramel colours have been previously evaluated on a number of occasions by the SCF, JECFA and TemaNord. Both JECFA and the SCF concluded that a numerical ADI was not necessary for Class I Plain Caramel, considering that it contains no added ammonia or sulphite and that it is likely to be produced in normal cooking processes (SCF, 1989). For Class II Caustic Sulphite Caramel, JECFA established an ADI of 0-160 mg/kg bw/day based on a NOAEL of 16 g/kg bw/day identified in the 90-day study in rats of MacKenzie et al. (1992a) and using an uncertainty factor of 100 (JECFA, 2001). The SCF included Class II Caustic Sulphite Caramel within the ADI of 200

mg/kg bw/day that it had already established for Class IV Sulphite Ammonia Caramel based on information indicating that its chemical composition was similar to and intermediate between that of Class I Plain Caramel and Class IV Sulphite Ammonia Caramel (SCF, 1997).

For Class III Ammonia Caramel, the SCF allocated an ADI of 200 mg/kg bw/day, apparently based on the 90-day rat study carried out on this caramel by MacKenzie (1985b), with the proviso that the content of THI should not exceed 10 mg/kg colour on a colour intensity basis (SCF, 1997). In 1987 JECFA identified a NOAEL of 20 g/kg bw/day from the same 90-day rat study, and consequently allocated an ADI of 200 mg/kg bw/day to Class III Ammonia Caramel using an uncertainty factor of 100 (JECFA, 1987). For Class IV Sulphite Ammonia Caramel, the SCF established an ADI of 200 mg/kg bw/day (SCF, 1989), based on a NOAEL for Class IV Sulphite Ammonia Caramel of 10% in the diet in an oral feeding study in rats, probably the long-term/carcinogenicity study (MacKenzie, 1985c). JECFA applied an uncertainty factor of 50 to the NOAEL of 10 g/kg bw/day in this long-term/carcinogenicity study in the rat (MacKenzie, 1985c) to derive an ADI of 200 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The Panel noted that the design of various studies in the previously evaluated dataset on the caramel colours would not be in full compliance with current regulatory protocols. However, from the study descriptions available, the Panel considered that these studies were of sufficient quality for evaluation.

Specifications for the four classes of caramel colours have been defined in Commission Directive 2008/128/EC and by JECFA (2006). The different classes of caramel colours are variously defined (in addition to their method of production) by the degree of binding to DEAE cellulose and to phosphoryl cellulose (for Classes I Plain Caramel and Class III Ammonia Caramel), by the absorbance ratio (Classes II Caustic Sulphite Caramel and Class IV Sulphite Ammonia Caramel) and by their colour intensity. The solids content for the different classes range from 62-77% (Class I Plain Caramel), 65-72% (Class II Caustic Sulphite Caramel), 53-83% (Class III Ammonia Caramel) or 40-75% (Class IV Sulphite Ammonia Caramel). The Panel noted minor differences between the EU specifications and those of JECFA but did not consider that these were generally of significance for the safety evaluation of the caramel colours.

The Panel noted that maximum levels of the imidazole 4-MEI are restricted to ≤ 250 mg/kg in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel under Commission Directive 2008/128/EC. The Panel is aware, however that a revised limit for 4-MEI in Class III and Class IV caramels of 200 mg/kg, to bring the limit in line with that in the JECFA specifications, is proposed in the draft Commission Regulation laying down specifications of food additives listed in Annex II and III of (EC) Regulation 1333/2008, currently under discussion. The Panel also noted that maximum levels of the imidazole THI are restricted to ≤ 10 mg/kg in Class III Ammonia Caramel under Commission Directive 2008/128/EC.

The Panel noted that the caramel colours are poorly characterised, and it is not clear whether the controls on manufacturing processes are sufficient to minimise batch-to-batch variability, particularly with respect to levels of individual LMW constituents. The wide range of starting materials and reactants that may be used for the production of caramel colours may result in a variation of end products, with different physical, chemical and toxicological properties. The Panel noted that concerns about e.g. chemical composition, purity and similarity of various caramel colours have also been raised in the past by the SCF. The Panel also noted that a number of the identified or theoretical LMW constituents of caramel colours, e.g. furan, acrylamide and 5-HMF, are genotoxic under certain experimental conditions and in some cases have carcinogenic potential, which may be relevant to the toxicological profile of the caramel colours. The Panel considered that the toxicological studies carried out on specific caramel colours would have involved exposure to these compounds, and therefore the anticipated toxicological effect should have been detected in these studies, as exemplified by the toxicological profiles of Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel due to the presence of the imidazoles THI and 4-MEI.

According to information from industry, slight changes in time and temperatures of the reaction, or in starting materials, can produce changes in the chemical composition and physical properties of the final product. Furthermore industry indicated that variation of manufacturing process parameters allows the production of a large range of different qualities of products within each caramel class. Concerning Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel there is also a wide range of products made by caramel manufacturers, with differences in chemical composition including some variation in THI and/or 4-MEI concentrations, respectively (EUTECA, 2011a).

The Panel noted that there was limited information about the relationship between processing parameters for the caramel colours and the formation and nature of heat-derived constituents of these colours.

The Panel noted that data on the toxicokinetics of the caramel colours are very limited. Data available concerning the toxicokinetics of Class IV Sulphite Ammonia Caramel indicate that the majority of the high molecular weight fraction of orally administered Class IV Sulphite Ammonia Caramel is directly excreted in the faeces. The small fraction that is absorbed is distributed to lymphoreticular tissue, and eventually excreted in the urine. The Panel considered that individual constituents of the LMW fraction of caramel colours (e.g. MW less than 500 g/mol) are likely to be absorbed, although little information is available to confirm this assumption.

The caramel colours are of low toxicity both in short-term tests and in chronic toxicity/carcinogenicity studies. A large number of short-term oral toxicity studies are available, particularly on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, including 90-day feeding studies in the rat. The results of these studies do not indicate any significant differences in the toxicological profile between the four classes. The only significant toxic effect, namely the lymphocytopenia produced by Class III Ammonia Caramel, particularly in animals with low pyridoxine levels, has been specifically linked with the presence of THI in this caramel. Animal studies on Class III Ammonia Caramel have shown evidence of lymphocyte depression and other evidence of immunotoxicity, as described in section 3.2.2.3 and Annex B and discussed further below.

The available short-term studies, employing generally high dose levels in drinking water, show some dose-related effects, including mild diarrhoea, reduced body weight gain associated with reduced food and fluid consumption, pigmentation of mesenteric lymph nodes, enlargement of the caecum, reduced urinary output associated with increases in specific gravity of the urine, and increases in caecal and kidney weights, unaccompanied by any histopathological change. The authors of the studies concluded that the effects on body weight gain were in part due to the reduced water intake caused by poor palatability of the drinking water containing high levels of caramel rather than toxic effects of the caramel colours *per se*, and that the other effects seen (increases in caecal and kidney weight and changes in urinary parameters), were secondary both to the reduced fluid intake and the intake of large quantities of osmotically-active caramel material. The authors also suggested that a reduction in food intake is an invariable response to either voluntary or forced restriction of water intake by the rat (MacKenzie et al., 1992b). The Panel agrees that these are likely explanations for the treatment-related effects on body weight and other parameters seen in studies with caramel colours, and considers that these effects are not of toxicological significance in establishing the safety of the caramel colours.

Caramel colours have been extensively tested for genotoxic potential in a variety of assays *in vitro*. Overall the results in a range of *in vitro* systems were generally negative, with a few marginally positive findings (e.g. Kawachi et al., 1980; Ishidate and Yoshikawa, 1980; Jensen et al., 1983; Ishidate et al., 1984; Yu et al., 1984; Aeschbacher et al., 1986). The caramel colours investigated in these publications were generally commercial caramel colours whose nature and classification was not further specified by the authors (JECFA, 1987). On the basis of these marginally inconclusive results, the ITCA in the period 1980–1990 carried out a comprehensive testing programme with samples of well-defined caramel colours to determine the genotoxic potential of the different caramel classes in a battery of genotoxicity tests *in vitro* and *in vivo*. The results of these studies largely indicated a lack of

genotoxic potential *in vitro*, with occasional positive responses being seen at very high exposure levels showing cytotoxicity. *In vivo* mouse micronucleus studies on Class I Plain Caramel and Class III Ammonia Caramel have not shown evidence of cytogenetic damage. Overall the Panel concluded that there were no concerns regarding the genotoxic potential of caramel colours.

No studies on the chronic toxicity and/or carcinogenicity of Class I Plain Caramel or Class II Caustic Sulphite Caramel are available. The findings in the long-term toxicity studies carried out with Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel did not reveal any pattern of toxicity not already seen in the 90-day oral toxicity studies carried out with these caramel colours. The only effects of Class III Ammonia Caramel observed in the chronic toxicity studies in rats or mice were decreased spleen weight, increased caecum weight, and (transient) lymphocytopenia. The Panel noted that decreases in splenic weight in the chronic study with Class III Ammonia Caramel in rats carried out by Evans *et al.* (1976) were significant in male rats at all dose levels, and were associated with reductions in lymphocyte numbers seen at earlier stages in the study. Evans *et al.* concluded that a NOAEL could not be identified in this study. The Panel noted however that the lymphocytopenia seen in the animals, together with the splenic effects, could be attributed to the presence of THI in the material tested, by analogy with other studies showing this effect (e.g. Mackenzie 1985b; Mackenzie *et al.*, 1992b), and that since specific information was not provided on the level of THI in the material tested, this endpoint could not be used to identify a NOAEL for Class III Ammonia Caramel in this study.

Similarly, in the 2-year carcinogenicity study carried out with Class III Ammonia Caramel by Maekawa *et al.*, 1983) the only parameters monitored, other than histopathological investigations of neoplastic change, were clinical signs, mortality and body weight changes. The authors did not report a NOAEL for the study, however on the basis of the absence of any treatment-related effect in the study the Panel concluded that 2 g/kg bw/day (the highest dose tested) could be considered a NOAEL.

In the 2-year oral toxicity study on Class IV Sulphite Ammonia Caramel in rats carried out at dose levels of up to 10 g/kg bw/day by MacKenzie (Mackenzie, 1985c; MacKenzie *et al.*, 1992c) dark-stained and soft faeces were observed, as was caecal enlargement and increased kidney weight. In addition, body weight, food intake and water intake in male and female rats were decreased in the groups receiving 5 or 10 g Class IV Sulphite Ammonia Caramel/kg bw/day, probably reflecting a reduced palatability of the caramel containing drinking water. No pathological changes attributable to the treatment were found after examination of the organs. The highest dose tested, 10 g/kg bw/day, was considered by the authors to be the NOAEL in rats, on the basis that the effects seen were primarily related to reduced fluid intake, as discussed for the short-term toxicity studies on Class III and Class IV caramels above, and are of no toxicological significance. The Panel agreed with this NOAEL.

No evidence of carcinogenicity was seen in 2-year studies in rats on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. In a parallel study in mice, there was similarly no evidence for a carcinogenic potential of Class IV Sulphite Ammonia Caramel. The Panel noted that no carcinogenicity data were available for Class I Plain Caramel and Class II Caustic Sulphite Caramel. The Panel considered however that given the fact that Class I Plain Caramel is likely to be produced in normal cooking processes, also considering the long-term toxicity and carcinogenicity data available on Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel and the relatively similar toxicological profile of all the caramel colours, there are no concerns regarding the carcinogenicity of Class I Plain Caramel and Class II Caustic Sulphite Caramel, nor regarding the carcinogenicity of Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel.

In relation to the reproductive and developmental toxicity of the caramel colours, the Panel noted that no data were available for Class I Plain Caramel and Class II Caustic Sulphite Caramel. There were no indications of reproductive and or developmental toxicity in mice, rats, or rabbits given Class III Ammonia Caramel by gavage at levels of up to 1600 mg/kg bw/day (Morgareidge, 1974a). In a reproduction study with Class IV Sulphite Ammonia Caramel in rats (Tierney *et al.*, 1980), at the top dose of 25% in the diet, equivalent to approximately 28 g/kg bw/day, pups showed a higher incidence

of alopecia compared with pups in the control group and a generalized poor condition during the last 7 days of suckling. One pup at this dose level also had an arched spine, an incidence which was not statistically significant compared with controls. The number of implantation sites, litter size and of live pups at days 0, 4, and 21 of lactation in the 20%-dose group were significantly lower than control values, the Panel noted however that there was no dose-related trend, since in the 25% dose group these parameters were not statistically different from controls. The Panel considered that a NOAEL of 25-30 g/kg bw/day for female rats could be identified in this study.

The Panel noted that no multigeneration study is available on any of the four classes of caramel colours.

Due to a lack of data, no conclusion can be drawn with respect to the intolerance and allergenicity of the four caramel colours under evaluation. The Panel noted, however that no cases of intolerance and allergenicity linked to caramel exposure have been reported in published literature.

In relation to the potential haematotoxicity/immunotoxicity of caramel colours, effects have been identified in animal studies with Class III Ammonia Caramel, but not with the other classes of caramel colours. Lymphocytopenic and immunomodulatory effects have been seen in a number of studies with Class III Ammonia Caramel, and the overall conclusion of the authors of these studies was that THI, a constituent of Class III Ammonia Caramel, together with deficiency of pyridoxine, as a dietary influence, were primarily responsible for these effects. The THI-induced lymphocyte depression was largely ameliorated by pyridoxine. The Panel agreed with this interpretation and noted that where these changes were reported, the effects were (apparently) transient in nature, disappearing in the later stages of longer-term studies with Class III Ammonia Caramel. A recent study (Schwab et al., 2005) has shown that in mice receiving 7.5 mg/kg bw/day THI (50 mg/l drinking water) for 3 consecutive days, the level of S1P in plasma increased more than 100-fold. Treatment with THI also induced a marked decrease in the number of circulating lymphocytes and suppressed immunity. The migration of lymphocytes from the thymus and peripheral lymphoid organs is regulated by levels of S1P in these tissues relative to that in the peripheral circulation and inhibition of migration of immunocompetent cells occurs in response to elevated concentrations of circulatory S1P. S1P is thus an important immunoregulatory substance (Hla, 2004, 2005; Schwab et al., 2005; Rivera et al., 2008; Yu et al., 2010). THI has been shown to inhibit the S1P degrading enzyme S1P lyase, an intracellular degrading enzyme which is expressed by hematopoietic cells (Schwab et al., 2005), and these findings may provide a mechanistic explanation for the immunosuppressive effects of THI.

The Panel noted that in the pivotal 90-day study on Class III Ammonia Caramel (MacKenzie et al., 1985b, 1992b), in which rats were dosed with up to 20 g/kg bw/day caramel colour, containing either 15 mg THI/kg caramel or 295 mg THI/kg caramel, on a solids basis, no dose-related lymphocytopenia occurred in the animals fed caramel containing approximately 15 mg THI/kg. Class III Ammonia Caramel containing 295 mg THI/kg, at a dose level of 20 g/kg bw/day, induced a statistically significant decrease in lymphocyte counts in both sexes at 2 weeks and only in male rats at 6 weeks. All lymphocyte values in these groups were normal at the termination of the study.

Comparison of the effects of Class III Ammonia Caramel in rats and mice indicates that both species are equally sensitive to the immunotoxic effects of THI, although in contrast humans may be less sensitive than rodents (Houben and Penninks, 1994), as discussed below. The Panel noted that the results of a study carried out by Thuvander and Oskarssen indicate that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B₆ status (Thuvander and Oskarssen, 1994). Sinkeldam and co-workers demonstrated significant reductions in lymphocytes and total leucocytes at a dose level of 1% Class III Ammonia Caramel in drinking water, estimated to be equivalent to 200 µg THI/ kg bw/day (Sinkeldam et al., 1988). Similar treatment-related, statistically significant reductions in lymphocytes were observed when the rats were given 200 µg, 500 µg or 2 mg THI/ kg bw/day alone in drinking water. The 90-day oral toxicity study in rats carried out by Mackenzie et al. included an evaluation of the toxicity of THI after a 4-week dosing

period (MacKenzie, 1985b; MacKenzie et al., 1992b). In rats maintained on a pyridoxine-replete diet, the short-term NOAEL of THI for the reduction of total lymphocytes was determined to be 120 µg/kg bw/day in female rats, and 380 µg/kg bw/day in male rats (MacKenzie et al., 1992b). Houben et al. (1992; 1992c) reported intakes of THI alone or Class III Ammonia Caramel containing THI that, in combination with manipulation of dietary pyridoxine levels, resulted in lymphocytopenia and could therefore be regarded as LOAELs for this effect. These ranged from 57.2 µg THI/kg bw/day (provided by a level of 0.4% Class III Ammonia Caramel in drinking water, in rats maintained on a low-pyridoxine diet, 2-3 mg/kg diet) to levels of 200 µg THI/kg bw/day or higher. Sinkeldam and co-workers reported decreases in lymphocyte counts in rats receiving 0.1% Class III Ammonia Caramel in drinking water, equivalent to 20 µg THI/ kg bw/day, for 1 week and maintained on a low-pyridoxine diet (2-3 mg/kg diet) (Sinkeldam et al., 1988). The Panel noted that statistical analysis was not reported for this aspect of the study, but evaluation of the data indicated that the decrease in lymphocytes was unlikely to be statistically significant at this dose level. The Panel considered that these findings in pyridoxine-deficient rats may be of limited relevance for human health risk assessment.

Overall the Panel concluded that a NOAEL for the lymphocytopenic effects of THI in pyridoxine-replete rats may lie in the range of 120-400 µg/kg bw/day as indicated by the studies of MacKenzie et al., Sinkeldam et al. and Houben et al.

The Panel noted that, in contrast to the findings in rats and mice, in the available short-term human studies consumption of Class III Ammonia Caramel had no effects on total or specified white blood cell counts at dose levels of up to 200 mg/kg bw/day (with THI levels almost 20 times above those permitted according to current specifications) albeit during short term exposure periods (BIBRA, 1976; Marier et al., 1977a; 1977b; Houben et al., 1992b; 1992c). The study of Houben et al. included subjects with (mild) pyridoxine deficiency, and the authors, in comparing the results obtained in this study with data in rats maintained on normal and pyridoxine-reduced diets, suggested that, *“with regard to oral intake of THI, humans are less sensitive to Caramel Colour III-induced lymphocytopenia than are rats”* (Houben et al., 1992c; Houben and Penninks, 1994). The Panel agreed with this interpretation, based on the available data.

The Panel considered that while theoretically THI could also be a constituent of Class IV Sulphite Ammonia Caramel, since formation of this imidazole is linked to the use of the ammonia process in the manufacture of Class III and Class IV caramels, THI has only been identified in Class III Ammonia Caramel Colour using current analytical methodologies (EUTECA, 2010a). No maximum limit for THI has therefore been established in the specifications for Class IV Sulphite Ammonia Caramel. It is suggested by the industry that the presence of sulphite/sulphur dioxide in Class IV Sulphite Ammonia Caramel may interfere with or inhibits the formation of THI (EUTECA, 2010a). Its absence in Caramel Classes I and II is due to the fact that there is no nitrogen source present that would allow its formation (EUTECA, 2010a).

The Panel noted that, in addition to the influence of the imidazole THI on the toxicological profile of Class III Ammonia Caramel, another imidazole contaminant of this class of caramel colours, 4-MEI, also found in Class IV Sulphite Ammonia Caramel, is considered to be responsible for the convulsions observed after administration of high doses of Class III Ammonia Caramel to a range of species and by different routes. The Panel considered, in line with the JECFA opinion (1978), that the acute toxicity of 4-MEI as a constituent in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel is not of toxicological concern since maximum levels of 4-MEI are legally restricted to ≤ 250 mg/kg in these caramel colours, at which level these acute toxic effects of 4-MEI will not be manifest. The Panel also noted however that in recent carcinogenicity studies, NTP has concluded that 4-MEI shows *“clear evidence of carcinogenic activity in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms, and also “equivocal evidence of carcinogenic activity of 4-methylimidazole in female F344/N rats based on increased incidences of mononuclear cell leukaemia.”* The Panel considered, however, that the carcinogenic effect of 4-MEI seen in mice in this study was thresholded, based on the lack of genotoxicity of 4-MEI (NTP, 2007), also noting that

alveolar/bronchiolar neoplasms occur spontaneously at high incidence in B6C3F1 mice (Fox et al., 2006). The Panel concluded therefore that the intermediate dose of 625 mg 4-MEI/kg diet, equivalent to 80 mg 4-MEI/kg bw/day could be considered to be a NOAEL in this study. The Panel noted that 4-MEI has recently been listed by the US State of California as a chemical known to cause cancer (OEHHA, 2011).

The Panel, in evaluating the overall toxicological database on the four classes of caramel colours, considered that while the constituents 4-MEI (present in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel) and THI (found in Class III Ammonia Caramel only) must be taken into account in the safety evaluation of these caramel colours as food additives, as discussed above, all four caramel colours are otherwise similar in their toxicological effects. These effects include, variously, mild diarrhoea, reduced body weight gain associated with reduced food and fluid consumption, pigmentation of mesenteric lymph nodes, enlargement of the caecum, reduced urinary output associated with increases in specific gravity of the urine, and increases in caecal and kidney weights, unaccompanied by any histopathological change, although not all these changes were reported in every study with the individual caramel colours. Such effects can be expected following administration of a complex mixture of high molecular weight, insoluble materials, such as the caramel colours, and the commonality of effect can be compared to e.g. the polyol sweeteners, producing local physiological effects including osmotic diarrhoea in the gastrointestinal tract. The Panel noted that the available human volunteer studies with the caramel colours (e.g. BIBRA, 1976; Marier et al., 1977a; 1977b) indicate that repeated caramel ingestion at levels of up to 300 mg/kg bw/day can result in an increased frequency of bowel movements and softening or increased liquidity of faeces. The Panel also noted that the effects produced by the caramel colours can be anticipated to be additive in nature.

The Panel considered that, in spite of the absence of full chemical characterisation of the four classes of caramel colours, given the consistency in the toxicological database, the caramel colours can be considered as a single group in terms of assessing their safety. The Panel considered therefore that a group ADI can be established for the caramel colours. Given, however, concerns regarding the immunotoxicity of THI, present in Class III Ammonia Caramel, the Panel decided to define an individual ADI for this caramel within the overall group ADI, based on the currently available database. While the Panel noted the toxicological data gaps for caramel colours, including the generally sparse database on reproductive toxicity for caramel colours as a whole and the absence of long-term toxicity and carcinogenicity studies on Class I Plain Caramel and Class II Sulphite Caramel, the Panel considered that missing data for these classes of caramel colours can be accounted for by data from another class. The Panel noted that specific constituents of concern resulting from differences in the production processes for the four classes of caramel colours, including especially THI in Class III Ammonia Caramel and 4-MEI in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel should be dealt with separately as discussed in detail later in this Discussion section.

The Panel considered that several studies carried out in rats with the different caramel colours are relevant for establishment of a group ADI for the caramel colours. These include the three studies used previously by JECFA and SCF to define the respective ADIs for the individual classes:

- a 90-day study with Class II Caustic Sulphite Caramel (MacKenzie, 1985a; MacKenzie et al., 1992a), providing a NOAEL of 16 g/kg bw/day, the highest dose tested,
- a 90-day study with Class III Ammonia Caramel (MacKenzie, 1985b, MacKenzie et al., 1992b) providing a NOAEL of 20 g/kg bw/day, the highest dose tested,
- a 2-year oral toxicity study in rats carried out by MacKenzie (MacKenzie, 1985b, MacKenzie et al., 1992c) with Class IV Sulphite Ammonia Caramel, providing a NOAEL of 10 g/kg bw/day, the highest dose tested,

The Panel considered that several additional toxicological studies should be taken into account when establishing the group ADI, and these include:

- a 13-week toxicity study in rats with Class IV Sulphite Ammonia Caramel in drinking water (Heidt and Rao 1980), providing a NOAEL of 30 g/kg bw/day, the highest dose level tested,
- another 13-week drinking water study in rats with Class IV Sulphite Ammonia Caramel (MacKenzie, 1985b; MacKenzie et al., 1992c) providing a NOAEL of 30 g/kg bw/day, the highest dose level tested,
- a 90-day study in Beagle dogs with Class IV Sulphite Ammonia Caramel (Kay and Calandra, 1962c) providing a NOAEL of 6.25 g/kg bw/day, the highest dose tested,
- a 96-week drinking water study in mice with Class III Ammonia Caramel (Hagiwara et al., 1983), providing a NOAEL of 8.4 g/kg bw/day, the highest dose tested,
- a 2-year dietary study in rats with Class III Ammonia Caramel (Evans et al., 1976) providing a NOAEL of 3 g/kg bw/day, the highest dose tested,
- a 104-week study in rats with Class III Ammonia Caramel (Maekawa et al., 1983), providing a NOAEL of 2 g/kg bw/day, the highest dose tested,
- a reproductive toxicity study in rats with Class IV Sulphite Ammonia Caramel (Tierney, 1973) providing in the dams a NOAEL of 25-30 g/kg bw/day.

Given that:

- the NOAELs in all these studies were the highest dose levels tested,
- the effects of the caramel colours in 90-day studies were generally similar to those reported in the long-term studies,
- available reproduction and developmental toxicity studies, although limited, do not reveal any effects of concern,
- the studies reveal no effects on the reproductive organs,
- the effect of most concern, i.e. lymphocytopenia, can, as also stated by JECFA, best be evaluated from short term studies, and
- the long-term studies support the conclusion that the caramel colours are not carcinogenic,

the Panel decided to use the highest NOAEL of 30 g/kg bw/day reported in several of these studies, still the highest dose level tested, as the basis to derive a group ADI for the caramel colours. The Panel noted that whilst there were arguments for increasing the default uncertainty factor of 100, to compensate for limitations in the toxicological databases on reproductive toxicity, equally compelling arguments could be advanced for deriving a chemical-specific adjustment factor below the default uncertainty factor. The Panel therefore applied an uncertainty factor of 100 to the NOAEL of 30 g/kg bw/day to derive a group ADI of 300 mg/kg bw/day for the caramel colours. Overall, based on the available database, the Panel considered that this would provide a sufficient margin of safety.

In relation to Class III Ammonia Caramel, the Panel considered the available data on the immunotoxicity of THI, a constituent of this caramel class only. The Panel noted that no dose-related effects on haematological parameters were reported in the 90-day study of MacKenzie (1985b, 1992b),

using a Class III Ammonia Caramel containing a THI level of 15 mg/kg, while with a Class III Ammonia Caramel containing a much higher level of THI of 295 mg/kg, only transient effects on lymphocytes were seen. The MacKenzie study provided a NOAEL of 20 g/kg bw/day for Class III Ammonia Caramel, the highest dose tested. The Panel also noted, however, the results of the study of Thuvander and Oskarssen, indicating that Class III Ammonia Caramel that meets the limit of less than 25 mg THI/kg established in the JECFA specifications may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B₆ status (Thuvander and Oskarssen, 1994). While the Panel considered that this study should not be used as a pivotal study for the purposes of risk assessment without further substantiation, given a number of studies in rats showing no effect on haematological parameters over longer periods and at higher dose levels than those used in the study of Thuvander and Oskarssen, the Panel considered that it should be taken into account in establishing an ADI for Class III Ammonia Caramel. The Panel applied an additional uncertainty factor of 2 together with the default uncertainty factor of 100 to the NOAEL of 20 g/kg bw/day identified from the MacKenzie study. The Panel therefore establishes, within the group ADI for all caramel colours and based on the currently available database, an ADI of 100 mg/kg bw/day for Class III Ammonia Caramel.

The Panel noted that this means that within the group ADI of 300 mg/kg bw/day established for the four caramel colours, only 100 mg/kg bw/day of this 300 mg/kg bw/day can be made up by Class III Ammonia Caramel.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers. As caramel colours Class I, II, III and IV are authorised *quantum satis* in almost all categories, the refined exposure estimates have been performed only for Tier 3 using the maximum reported use levels or when no usages were reported to EFSA, values defined by decision rules for *quantum satis* usages were used (Table 6).

Exposure estimates for children (1-14 years old) have been done by the Panel for 11 European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland, Germany, Denmark, Cyprus, Greece) based on detailed individual food consumption data provided by the EXPOCHI consortium (Huybrechts et al., 2010). As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5- 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2008). For the adult population, the Panel has selected the UK population as representative of the EU consumers for estimates of exposure.

The mean dietary exposure of European children including UK pre-school children ranged from 76.9 to 427.2 mg/kg bw/day for Class I Plain Caramel, from 8.7 to 34.6 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 21.7 to 302.4 mg/kg bw/day for Class III Ammonia Caramel, and from 23.2 to 506.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel. At the 95th or 97.5th percentile, estimates ranged from 179.6 to 882.2 mg/kg bw/day for Class I Plain Caramel, from 18.5 to 117.3 mg/kg bw/day for Class II Caustic Sulphite Caramel, from 107.9 to 757.3 mg/kg bw/day for Class III Ammonia Caramel, and from 129.7 to 1480.2 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors (>10% in all or several countries) to the total anticipated exposure of children were for Class I Plain Caramel: non alcoholic flavoured drinks (12% to 55%), fine bakery wares (15% to 32%), desserts including flavoured milk products (11% to 48%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 56%), soups (11% to 32%) and malt bread (16% to 49%). For Class II Caustic Sulphite Caramel the main contributors were fine bakery wares (12% to 53%), desserts including flavoured milk products (11% to 41%), edibles ices (11% to 22%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 45%), soups (18% to 54%) and malt bread (19% to 55%). For Class III Ammonia Caramel the main contributors were fine bakery wares (13% to 45%), desserts including flavoured milk products (12% to 44%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (12% to 79%), and vinegar (12% to 45%), while in one country non alcoholic flavoured drink, malt bread confectionery, and sausages, pates and terrines contributed

29%, 15%, 13% and 10%, respectively. For Class IV Sulphite Ammonia Caramel the main contributors were non alcoholic flavoured drinks (13% to 51%), confectionery (20% to 81%), fine bakery wares (10% to 29%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (10% to 24%), and malt bread (10% to 34%).

The anticipated dietary exposure reported for the UK adult population gives a mean of 136.6 mg/kg bw/day and a 97.5th percentile of 429.3 mg/kg bw/day for Class I Plain Caramel; a mean of 21.7 mg/kg bw/day and a 97.5th percentile of 109.5 mg/kg bw/day for Class II Caustic Sulphite Caramel; a mean of 60.8 mg/kg bw/day and a 97.5th percentile of 295.0 mg/kg bw/day for Class III Ammonia Caramel; and a mean of 89.4 mg/kg bw/day and 97.5th percentile of 368.9 mg/kg bw/day for Class IV Sulphite Ammonia Caramel.

The main contributors to the total anticipated exposure of adults were for Class I Plain Caramel non alcoholic flavoured drinks (30%), beer and cidre bouché (27%), soups (16%), and sauces, seasonings (e.g. curry powder, tandoori) and pickles (10%). For Class II Caustic Sulphite Caramel the main contributors were beer and cidre bouché (50%) and soups (20%). For Class III Ammonia Caramel the were beer and cidre bouché (48%) and sauces, seasonings (e.g. curry powder, tandoori) and pickles (22%). For Class IV Sulphite Ammonia Caramel the main contributors were confectionery (65%) and non alcoholic flavoured drinks (23%).

The Panel also evaluated combined anticipated dietary exposure to all four classes of caramel colours, taking into account the highest maximum reported level for all caramel classes, described in Table 8, from each food category. When considering this scenario, as presented in Table 10, anticipated mean combined dietary exposure reported for European children, including UK pre-school children, ranged from 83.5 to 698.3 mg/kg bw/day. At the 95th/97.5th percentile, estimates ranged from 224.8 to 1672.3 mg/kg bw/day. For the UK adult population this scenario gave a range of exposures of 194.8 and 474.3 mg/kg bw/day for the mean and the 97.5th percentile, respectively.

The main contributors to the total combined anticipated exposure to caramel colours for children were non alcoholic flavoured drinks (11% to 28%), confectionery (19% to 58%), fine bakery wares (15% to 29%), desserts including flavoured milk products (10% to 31%), sauces, seasonings (e.g. curry powder, tandoori) and pickles (14% to 44%), and malt bread (16% to 46%). Soups were estimated to contribute from 25% to 28% in two countries and vinegar was estimated to contribute 20% in one country. For the adult population the main contributors were confectionery (30%), non alcoholic flavoured drinks (21%), beer, cidre bouché (19%) and soups (11%).

The Panel noted that the anticipated dietary exposure of the adult population at the 97.5th percentile to Class I Plain Caramel exceeds the group ADI of 300 mg/kg bw/day proposed for the caramel colours. Similarly, the anticipated dietary exposure of the adult population at the 97.5th percentile to Class IV Ammonia Caramel exceeds this group ADI. For children, the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for Class I Plain Caramel exceed the group ADI of 300mg/kg bw/day. Similarly, for children the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for Class IV Sulphite Ammonia Caramel exceed the group ADI of 300 mg/kg bw/day.

The anticipated dietary exposure to Class II Sulphite Caramel for both adults and children was below the group ADI of 300 mg/kg bw/day.

For Class III Ammonia Caramel the upper end of the mean intake range for children exceeds the individual ADI of 100 mg/kg bw/day established for this colour within the group ADI, while the 97.5th percentile anticipated dietary exposures of both the child and adult populations are above this ADI of 100 mg/kg bw/day.

Anticipated combined dietary exposures of both adults and children to all caramel colours exceed the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile, while the ADI is also exceeded by the

combined mean intake for children. In the case of children, this exceedance applies to the upper end of the exposure range only.

Reflecting the concerns regarding the immunotoxicity seen in a number of studies with either Class III Ammonia Caramel or with THI alone, found as a constituent in Class III Ammonia Caramel, and the carcinogenicity of 4-MEI, found as a constituent in Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel, the Panel has estimated exposure to THI and 4-MEI as a result of consumption of these caramel colours in the diet (Table 11). Additionally the Panel has also estimated exposure to sulphur dioxide, present in Class II and Class IV caramels as a result of the production method (Table 11). The Panel considered overall that the outcome of these exposure estimates did not give rise to concern and that the maximum levels established for THI, 4-MEI and sulphur dioxide in the specifications in the relevant caramel classes are sufficiently protective. The Panel noted in particular that for THI the reduction of the ADI for Class III Ammonia Caramel from 200 mg/kg bw/day (as established by the SCF in 1987) to 100 mg/kg bw/day, together with the outcome of the exposure estimates indicating that this ADI is likely to be exceeded by the upper end of the mean intake range for children and by the 97.5th percentile intakes of both the child and adult populations may result in lower intakes of THI in the future than those indicated in Table 11.

The Panel additionally considered the possible risks of exposure to furan and 5-hydroxymethylfurfural (5-HMF), present as constituents of the caramel colours as a result of the heat-treatment processes involved in the manufacture of the caramel colours. The Panel noted that furan has carcinogenic effects in experimental animals, probably produced by a genotoxic mechanism. The Panel noted that while 5-HMF is genotoxic in *in vitro* studies, the conclusion from the toxicological database on 5-HMF is that overall no *in vivo* genotoxic or relevant carcinogenic response is seen in studies in rodents and that no genotoxicity or carcinogenicity is expected in humans.

The Panel noted that variations in the manufacturing processes of the caramel colours may result in a wide variability in the nature and levels of the various constituents, including constituents of toxicological concern such as 5-HMF and furan. Given this likely variability, the Panel considered that in order to further guarantee the safety of caramel colours with respect to their minor constituents, such as THI, 4-MEI, 5-HMF and furan, it would be prudent to reduce their levels as much as technologically feasible. The Panel considers therefore that the specifications for the caramel colours should be updated and extended to also include maximum levels for constituents of possible concern not yet included in the specifications, such as for example 5-HMF and furan.

CONCLUSIONS

Caramel colours are colouring substances authorised as food additives in the EU, and are complex mixtures of compounds produced by heating carbohydrates under controlled heat and chemical processing conditions. The caramel colours are classified according to the reactants used in their manufacture as follows: Class I Plain Caramel or Caustic Caramel (E 150a); Class II Caustic Sulphite Caramel (E 150b); Class III Ammonia Caramel (E 150c) and Class IV Sulphite Ammonia Caramel (E 150d).

Both JECFA and SCF have concluded that a numerical ADI was not necessary for Class I Plain Caramel, while both bodies have established an ADI of 200 mg/kg bw/day for Class III Ammonia Caramel and Class IV Sulphite Ammonia Caramel. This is also the ADI established by the SCF for Class II Caustic Sulphite Caramel, while JECFA have established an ADI of 160 mg/kg bw/day for this class.

The Panel concludes that, given the toxicological similarity of all four classes of caramel colours and the consistency in the toxicological database, the caramel colours can be considered as a single group in terms of assessing their safety, and that a group ADI can be derived. The Panel establishes a group ADI of 300 mg/kg bw/day for the caramel colours. Given, however, concerns regarding the immunotoxicity of THI, present in Class III Ammonia Caramel, the Panel decided to define an

individual ADI of 100 mg/kg bw/day for this caramel within the group ADI, based on the currently available database. This means that within the group ADI of 300 mg/kg bw/day, only 100 mg/kg bw/day of this 300 mg/kg bw/day can be made up by Class III Ammonia Caramel.

The Panel notes that the anticipated dietary exposure of the adult population at the 97.5th percentile to Class I Plain Caramel exceeds the group ADI of 300 mg/kg bw/day proposed for the caramel colours. Similarly, the anticipated dietary exposure of the adult population at the 97.5th percentile to Class IV Ammonia Caramel exceeds this group ADI. For children, the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for Class I Plain Caramel exceed the group ADI of 300mg/kg bw/day. Similarly, for children the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for Class IV Sulphite Ammonia Caramel exceed the group ADI of 300 mg/kg bw/day.

The anticipated dietary exposure to Class II Sulphite Caramel for both adults and children was below the group ADI of 300 mg/kg bw/day.

For Class III Ammonia Caramel the upper end of the mean intake range for children exceeds the individual ADI of 100 mg/kg bw/day established for this colour within the group ADI, while the 97.5th percentile anticipated dietary exposures of both the child and adult populations are above this ADI of 100 mg/kg bw/day.

Anticipated combined dietary exposures of both adults and children to all caramel colours exceed the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile, while the ADI is also exceeded by the combined mean intake for children. In the case of children, this exceedance applies to the upper end of the exposure range only.

The Panel concludes overall that the exposure estimates for THI, 4-MEI or sulphur dioxide are not of concern, but notes remaining uncertainties regarding the effects of THI on the immune system. The Panel would welcome additional studies to clarify these effects .

The Panel notes that variations in the manufacturing processes of the caramel colours may result in a wide variability in the nature and levels of the various constituents, including constituents of toxicological concern such as 5-HMF and furan. Given this likely variability, the Panel considers that in order to further guarantee the safety of caramel colours with respect to their minor constituents, such as THI, 4-MEI, 5-HMF and furan, it would be prudent to reduce their levels as much as technologically feasible. The Panel considers therefore that the specifications for the caramel colours should be updated and extended to also include maximum levels for constituents of possible concern not yet included in the specifications, such as for example 5-HMF and furan.

The Panel additionally concludes that there is limited information about the relationship between processing parameters for the caramel colours and the formation and nature of heat-derived constituents which is also relevant for the control of manufacturing processes. Future research work is recommended in this respect.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
2. Caramel colours (E150a-d): preliminary EUTECA input containing info on use levels, production methods, safety evaluation studies. EUTECA, Secretariat, Brussels, March, 2007.
3. A report "Screening potential intake of Caramel Colours" (D. Tennant). EUTECA, Secretariat, Brussels, July, 2007.

4. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009).
5. MacKenzie, K.M. (1985). 90-day toxicity study of caramel color (caustic sulfite process) in rats. Vols. I & II. Unpublished report No. 6154-105 from Hazleton Laboratories America Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association. Unpublished report from report provided by EUTECA (2010).
6. MacKenzie, K. M. (1985). 90-day toxicity study of caramel color (ammonia process) in rats. Vols. I and II. Unpublished report No. 6154-107 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association. (MacKenzie, K.M. (1985c). Carcinogenicity and chronic toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats (Chronic toxicity portion, Vols. I-V; Carcinogenicity portion, Vols. I-VIII). Unpublished study No. 81184 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association. Unpublished report from report provided by EUTECA (2010).
7. MacKenzie, K.M. (1985). Carcinogenicity and chronic toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats (Chronic toxicity portion, Vols. I-V; Carcinogenicity portion, Vols. I-VIII). Unpublished study No. 81184 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association Unpublished report from report provided by EUTECA (2010).
8. MacKenzie, K.M. (1985). Carcinogenicity study of caramel color (sulfite ammonia process) type SAC2 in mice. Vols. I-VI. Unpublished study No. 81185 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association. Unpublished report from report provided by EUTECA (2010).
9. Evans, J. G., Butterworth, K. R., Gaunt, I. F. and Grasso, P. (1976). Long-term toxicity study in the rat of a caramel produced by the "half-open-half closed pan" ammonia process. Unpublished report No. 6/1976 from the British Industrial Biological Research Association, Carshalton, Surrey, England. Unpublished report from report provided by EUTECA (2010).
10. Re-evaluation of caramel colours. Additional data follow-up to Email sent by EFSA on 24 May 2010. EUTECA, Secretariat, Brussels, June, 2010.
11. Til HP and Spanjers M, 1973. Reproduction study in rats with six different Ammonia Caramels. Unpublished report No. R 4068 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Unpublished report provided by EUTECA (2010).
12. Tierney WJ, 1980. An in-utero range-finding study of caramel colour no. 3, sample 3-1, in rats. Unpublished project No. 79-2405 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by International Technical Caramel Association. Unpublished report provided by EUTECA (2010).
13. Heidt M and Rao GN, 1980. 90-day subacute toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats. Unpublished report No. 79028 from Raltech Scientific Services, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association. Unpublished report provided by EUTECA (2010).
14. Sinkeldam EJ, van der Heyden CA and Beems RB, 1976. Chronic (two year) feeding study in rats with six different Ammonia Caramels. Unpublished report No. R 4961 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Unpublished report provided by EUTECA (2010).

15. Level of use of caramel colours provided by EUTECA to EFSA on 05.11.2010 a follow up of Email sent on 21 September 2010
16. Re-evaluation of caramel colours_Additional data follow-up to email sent on 14 December 2010. Data provided on 21 January 2011.
17. Re-evaluation of caramel colours_Additional data follow-up to email sent on 24 January 2011. Data provided on 28 January 2011

REFERENCES

- Aeschbacher HU, 1986. Genetic toxicology of browning and caramelizing products. In Progress in Clinical and Biological Research, Vol. 206, 133-144. Genetic Toxicology of the Diet. Edited by I. Knudsen. Alan R. Liss, New York (as referred to by SCF, 1997).
- Adams K, Allen JA, Brooker PC, Jones E and Proudlock RJ, 1992. Assessment of the genotoxic potential of Caramel Colour I in four short-term tests. Food and Chemical Toxicology 30, 397-403 (as referred to by SCF, 1997).
- Adams ML, Meyer ER and Cicero TJ, 1998. Imidazoles suppress rat testosterone secretion and testicular interstitial fluid formation In vivo. Biology of Reproduction 59(2), 248-54.
- Allen JA, Brooker I C, Jones E, Adams K and Richold M, 1992. Absence of mutagenic activity in Salmonella and of clastogenic activity in CHO cells of caramel colours I, II, III and IV. Food and Chemical Toxicology, 30, 389-395 (as referred to by SCF, 1997).
- Allen JA, Brooker PC, Birt DM and McCaffrey KJ, 1984. Analysis of metaphase chromosomes obtained from CHO cells cultured in vitro and treated with caramel colour II. Unpublished report No. ITC 3B/84966 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Allen JA and Proudlock RJ, 1984. Autoradiographic assessment of DNA repair in mammalian cells after exposure to caramel colour II. Unpublished report from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Ashoor SH and Monte WC, 1983. Mutagenicity of commercial caramels. Cancer Letters 18, 187-190 (as referred to by JECFA, 1987).
- Bartlett S and Broster WH, 1957. Feeding trials with ammoniated molasses in the diet of young dairy cattle. Journal of Agricultural Science 50, 60-63.
- Battelle Memorial Institute, 1971. Unpublished report dated 4 May 1971 (as referred to by JECFA, 1977).
- BIBRA (British Industrial Biological Research Association), 1976. A study of the haematological effects of caramel in human volunteers. Unpublished report No. 1/172/76 from the British Industrial Biological Research Association, Carshalton, Surrey, England (as referred to by JECFA, 1987).
- BIBRA (British Industrial Biological Research Association), 1977. The short-term (10 week) feeding study with three caramels in rats. Unpublished report No. 177/2/77 from the British Industrial Biological Research Association, Carshalton, Surrey, England.
- BIBRA (British Industrial Biological Research Association), 1978. The evaluation of the effect of sulfite Ammonia Caramels on the lymphocytes of the rat. Unpublished report No. 238/2/78 from the British Industrial Biological Research Association, Carshalton, Surrey, England.

- Bononi M and Tateo F, 2007. Determination of furan by headspace solid-phase microextraction-gas chromatography-mass spectrometry in balsamic vinegars of Modena. *Journal of Food Composition and Analysis* 22, 79-82.
- Bradbury MG, Doherty KV, Parish CR and Lyons AB, 1996. The immunosuppressive compound 2-acetyl-4-tetrahydroxybutyl imidazole inhibits the allogeneic mixed lymphocyte reaction by sequestration of a recirculating subpopulation of T cells. *Immunology*, 87(1), 80-5.
- Bradbury MG, Qiu MR and Parish CR, 1997. The immunomodulatory compound 2-acetyl-4-tetrahydroxybutyl imidazole causes sequestration of lymphocytes in non-lymphoid tissues. *Immunology and Cell Biology* 75(5), 497-502.
- Brusick D, 1974. Mutagenic Evaluation of Compound FDA 71-83, Caramel. Unpublished report submitted to the Food and Drug Administration.
- Brusick DJ, Jagannath DR and Galloway SM, 1992. Genotoxicity hazard assessment of caramel colours III and IV. *Food and Chemical Toxicology* 30, 403-410 (as referred to by SCF, 1997).
- CFR (Code of Federal Regulation), 2006. Title 21: Food and Drug. Chapter I: Food and Drug Administration, Department of health and human services. Part 182: Substances generally recognized as safe. Sec 182.1235: Caramel. Available at: <http://www.grokfood.com/regulations/182.1235.htm>
- Chacharonis P, 1960. Acute and chronic toxicity studies on caramel colours A and B. Unpublished report No. S.A. 54219 from Scientific Associates Inc., St. Louis, MO, USA (as referred to by JECFA, 1987).
- Chacharonis P, 1963. Acute oral toxicity study in rats on caramel colorings 25A-1, 30B-0 and 30F-1. Unpublished report No. S.A. 79105 from Scientific Associates Inc., St. Louis, MO, USA (as referred to by JECFA, 1987).
- CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009)
- Cimino MC and Brusick DJ, 1981. Mutagenicity evaluation of ETA 48-IH caramel color in the mouse micronucleus test. Unpublished report No. 22129 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Ciolino LA, 1998. Determination and classification of added caramel color in adulterated acerola juice formulations. *Journal of Agricultural and Food Chemistry* 46, 1746-1753.
- Code of Federal Regulations (CFR), 2006. Caramel. Title 21, Volume 3, Sec. 182.1235. e-CFR Data as of May 20, 2010.
- De Heer C, Schuurman HJ, Houben GF, Pieters RH, Penninks AH and van Loveren H, 1995. The SCID-hu mouse as a tool in immunotoxicological risk assessment: effects of 2-acetyl-4(5)-tetrahydroxybutyl-imidazole (THI) and di-n-butyltin dichloride (DBTC) on the human thymus in SCID-hu mice. *Toxicology*. 1995 Jun 26;100(1-3):203-11.
- Dross A and Baltes W, 1989. Über die Fraktionierung von Zuckercouleur-Inhaltsstoffen nach ihrer Molmasse. *Z Lebensm Unters Forsch* 188, 540-544.
- EC (European Commission), 1998. Report on Methodologies for the Monitoring of Food Additive Intake Across the European Union. Final Report Submitted by the Task Coordinator, 16 January 1998. Reports of a Working Group on Scientific Cooperation on Questions Relating to Food, Task 4.2. SCOOP/INT/REPORT/2 (Brussels: European Commission Directorate General I11 Industry).
- EC (European Commission), 2001. Commission of the European Communities (COM). 542 final. Report from the commission on dietary food additive intake in the European Union. Brussels, 01.10.2001. Available at:

http://eur-lex.europa.eu/LexUriServ/site/en/com/2001/com2001_0542en01.pdf

EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. The EFSA Journal (2004) 32, 1-197.

Available at: http://www.efsa.europa.eu/en/efsajournal/doc/opinion_nda_04_en1.1.pdf

EFSA (European Food Safety Authority), 2004b. Report of the Scientific Panel on Contaminants in Food Chain on provisional findings on furan in food. The EFSA Journal (2004) 137, 1-20.

Available at: <http://www.efsa.europa.eu/it/efsajournal/doc/137.pdf>

EFSA (European Food Safety Authority), 2010. Scientific opinion of the EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on Flavouring Group Evaluation 13, Revision 1 (FGE.13Rev1): Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14. EFSA Journal 2010;8(4):1403, 1-112.

Available at: <http://www.efsa.europa.eu/en/scdocs/doc/1403.pdf>

Emerton V (Editor), 2008. Ingredients Handbook: Food Colours. Leatherhead Ingredients Handbook series. Wiley, John and Sons, Incorporated. ISBN: 1905224443.

EUTECA, 2007a. European Technical Committee on Caramels, Caramel colour – production method (general description). Personal communication to EFSA, March 2007.

EUTECA, 2007b. Screening Potential Intakes of Caramel Colours. Report provided for the European Technical Committee on Caramels, July 2007.

EUTECA, 2010a. Revaluation of caramel colours – Additional data. Letter from EUTECA to EFSA on 17.06.2010 a follow up of Email sent on 24 May 2010.

EUTECA, 2010b. Revaluation of caramel colours –EUTECA/ITCA joint response to email sent by EFSA on 1st October. 27 October 2010.

EUTECA, 2010c. Revaluation of caramel colours –Level of use of caramel colours provided by EUTECA to EFSA on 05.11.2010 a follow up of Email sent on 21 September 2010.

EUTECA, 2011a. Revaluation of caramel colours –Additional data provided by EUTECA to EFSA on 21.01.2011 a follow up of Email sent on 14 December 2010.

EUTECA, 2011b. Revaluation of caramel colours –Additional data provided by EUTECA to EFSA on 28.01.2011 a follow up of Email sent on 24 January 2011.

Evans JG, Butterworth KR, Gaunt IF and Grasso P, 1976. Long-term toxicity study in the rat of a caramel produced by the "half-open-half closed pan" ammonia process. Unpublished report No. 6/1976 from the British Industrial Biological Research Association, Carshalton, Surrey, England.

Foote WL, Robinson RF and Davidson RS, 1958. Toxicity of caramel color products. Unpublished report of Battelle Memorial Institute, Columbus, OH, USA.

Galloway SM and Brusick DJ, 1981a. Mutagenicity evaluation of ETA-48-IH in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).

Galloway SM and Brusick DJ, 1981b. Mutagenicity evaluation of ETA-48-IH in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Part II. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).

Gaunt IF, Lloyd AG, Grasso P, Gangolli SP and Butterworth KR, 1977. A short-term study in the rat on two caramels produced by variations of the "ammonia process". Food and. Cosmetics Toxicology 15, 509-521.

- Gobin SJ, Legg RF, Paine AJ and Phillips JA, 1989. The effect of 2-acetyl-4-tetrahydroxybutylimidazole on lymphocyte subsets in peripheral blood of the rat. *International Journal of Immunopharmacology* 11(8), 937-46.
- Gobin SJ and Phillips JA, 1991. Immunosuppressive effects of 2-acetyl-4-tetrahydroxybutyl imidazole (THI) in the rat. *Clinical and Experimental Immunology* 1991 Aug;85(2), 335-40.
- Gobin and Paine AJ, 1992. Effects of 2-acetyl-4-tetrahydroxybutyl imidazole (THI) on the thymus of rats. *Thymus* 20(1), 17-30.
- Gobin SJ, Legg RF and Paine AJ, 1992. The role of lymphocyte production and migration in the lymphocytopenia caused by 2-acetyl-4-tetrahydroxybutyl imidazole. *International Journal of Immunopharmacology* 14(4), 687-97.
- Gugasyan R, Losinno C and Mandel T, 1995. The effect of 2-acetyl-4-tetrahydroxybutylimidazole on lymphocyte subsets during a contact hypersensitivity response in the NOD mouse. *Immunology Letters* 46(3), 221-7.
- Gugasyan R, Clouston D, Mandel T and Wicks I, 1997. Prevention of splenic granuloma formation in adjuvant arthritis by 2-acetyl-4-tetrahydroxybutylimidazole (THI). *Immunology Letters* 58(3), 133-8.
- Gugasyan R, Coward A, O'Connor L, Shortman K and Scollay R, 1998. Emigration of mature T cells from the thymus is inhibited by the imidazole-based compound 2-acetyl-4-tetrahydroxybutylimidazole. *Immunology* 93(3), 398-404.
- Haldi J and Wynn W, 1951. A study to determine whether or not caramel has any harmful physiological effect. I. Unpublished report from Emory University, Atlanta, GA, USA (as referred to by JECFA, 1987).
- Haldi J and Wynn W, 1958. A study to determine whether or not caramel has any harmful physiological effect. II. Unpublished report from Emory University, Atlanta, GA, USA.
- Hagiwara A, Shibata M, Kurata Y, Seki K, Furushima S and Ito N, 1983. Long-term toxicity and carcinogenicity test of ammonia-process caramel colouring given to B₆C₃F₁ mice in the drinking water. *Food and Chemical Toxicology* 21, 701-706.
- Harigae Y, Takimoto H, Tohi S, Hata S, Kondo J, Ohta T, Naito K, Nagase T, Kimura H and Okada M, 2000a. Repeated dose toxicity study of caramel I by 28-day oral administration in rats. *Nippon Shokuhin Kagaku Gakkaishi* 7, 35-40.
- Harigae Y, Takimoto H, Tohia S, Hata S, Kondo J, Miwa Y and Nagasawa Y, 2000b. Mutagenicity studies of caramel I. *Nippon Shokuhin Kagaku Gakkaishi* 7, 28-34.
- Hagiwara A, Shibata M, Kurata Y, Seki K, Furushima S and Ito N, 1983. Long-term toxicity and carcinogenicity test of ammonia-process caramel colouring given to B6C3F1 mice in the drinking water. *Food and Chemical Toxicology* 21, 701-706 (as referred to by JECFA, 1987).
- Heidt M and Rao GN, 1980. 90-day subacute toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats. Unpublished report No. 79028 from Raltech Scientific Services, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.
- Heidt M and Rao GN, 1981. Subchronic toxicity study of Ammonia Caramel color type AC2 in rats. Unpublished report No. 80036 from Raltech Scientific Services, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Heyns K, 1971. Unpublished report of Technical Caramel Committee (as referred to by JECFA, 1977).
- Hla T, 2004. Physiological and pathological actions of sphingosine-1-phosphate. *Semin. Cell Dev. Biol.* 15(5), 513-520.
- Hla T, 2005. Dietary factors and immunological consequences. *Science* 309, 1682-1683.

- Houben GF, 1992. Vitamin B₆ status dependent immunomodulation by caramel III. ISBN 90-393-0181-6. University of Utrecht, Utrecht.
- Houben GF, van den Berg H, Kuijpers MH, Lam BW, van Loveren H, Seinen W and Penninks AH, 1992a. Effects of the color additive caramel color III and 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI) on the immune system of rats. *Toxicology and Applied Pharmacology* 113(1), 43-54.
- Houben GF, van Dokkum W, van Loveren H, Penninks AH, Seinen W, Spanhaak S and Ockhuizen T, 1992b. Effects of caramel colour III on the number of blood lymphocytes: A human study on caramel colour III immunotoxicity and a comparison of the results with data from rat studies. *Food and Chemical Toxicology* 30, 427-430.
- Houben GF, Abma PMH, van den Berg H, van Dokkum W, van Loveren H, Penninks AH, Seinen W, Spanhaak S, Voss JG and Ockhuizen T, 1992c. Effects of the colour additive caramel colour III on the human system: A study with human volunteers. *Food and Chemical Toxicology* 30, 749-758 (as referred to by SCF, 1997).
- Houben GF, Penninks AH, Seinen W, Vos JG and van Loveren H, 1993. Immunotoxic effects of the color additive Caramel Color III and 2-acetyl-4(5)-tetrahydroxybutyl-imidazole (THI); immune function studies in rats. *Fundamental and Applied Toxicology* 20, 30.
- Houben GF and Penninks AH, 1994. Immunotoxicity of the colour additive Caramel Colour III: a review of complicated issues in the safety evaluation of a food additive. *Toxicology* 91, 289-302.
- Huybrechts I, Sioen I, Boon PE, De Neve M, Amiano P, Arganini C, Bower E, Busk L, Christensen T, Hilbig A, Hirvonen T, Kafatos A, Koulouridaki S, Lafay H, Liukkonen K-H, Papoutsou S, Lourdes R-B, Ruprich J, Rehurkova I, Mathilde K, Serra-Majem L, Turrini A, Verger E, Westerlund A, Tornaritis M, van Klaveren JD and De Henauw S, 2010. Long-term dietary exposure to different food colours in young children living in different European countries. Scientific report submitted to EFSA. EFSA-Q-2010-00787. Available from: <http://www.efsa.europa.eu/en/scdocs/doc/53e.pdf>
- Iscaro A, Mackay IR and O'Brien C, 1988. Lymphopenic effects on mice of a component of Ammonia Caramel, 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI). *Immunology and Cell Biology* 6 (Pt 5-6), 395-402.
- Ishidate M Jr and Yoshikawa K, 1980. Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation - a comparative study on mutagens and carcinogens. *Archives of Toxicology Supplements* 4, 41-44 (as referred to by JECFA, 1987).
- Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology* 22, 623-636.
- ITCA, 1990. Caramel Colour II. Toxicological and chemical properties. Monograph prepared by the International caramel Association (ITCA, Washington DC, August 1990) and submitted to the European Commission by the European Technical Caramel Association, 3 September 1990) (document not available to the Panel).
- Jagannath DR and Brusick D, 1978a. Mutagenicity evaluation of ETA 4-10, ETA 4-11, ETA 4-15 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Jagannath DR and Brusick D, 1978b. Mutagenicity evaluation of ETA 4-8, ETA 4-9, ETA 4-12, ETA 4-13, ETA 4-14 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1970. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers,

- stabilizers, anticaking agents, and certain other substances. 13th Report of JECFA. WHO Technical Report Series, No. 445, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972a. Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological evaluations and specifications and a review of the technological efficacy of some antioxidants. 14th Report of JECFA. WHO Technical Report Series, No. 488, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972b. Toxicological evaluation of some enzymes, modified starches, and certain other substances. 15th Report of JECFA. WHO Food Additives Series, No 1.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1977. Summary of toxicological data of certain food additives. 21st Meeting of JECFA. WHO Food Additives Series, No 12. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1978. Evaluation of certain food additives. 21st report of JECFA. WHO Technical Report Series 617. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1980. Evaluation of certain food additives. 24th report of JECFA. WHO Technical Report Series, No. 653, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1986. Evaluation of certain food additives and contaminants. 29th report of JECFA. WHO Technical Report Series, No. 733, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1987. Toxicological evaluation of certain food additives and contaminants. 29th Meeting of JECFA. WHO Food Additives Series, No 20.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Evaluation of certain food additives and contaminants. 55th report of JECFA. WHO Technical Report Series, No 901, 8-10.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Combined compendium of food additive specifications. Monographs 1. Available at: <http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-102.pdf>
- Jensen NJ, Willumsen D and Knudsen I, 1983. Mutagenic activity at different stages of an industrial Ammonia Caramel process detected in Salmonella typhimurium TA 100 following pre-incubation. Food and Chemical Toxicology 21, 527-530 (as referred to by JECFA, 1987; SCF, 1997).
- Kamuf W, Nixon A, Parker O and Barnum GC, 2003. Overview of Caramel Colors. Cereal foods world, Vol. 42, No. 2, 64-69. Available at: <http://www.aaccnet.org/cerealfoodsworld/pdfs/W03-0205-01F.pdf>
- Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M and Sugiyama T, 1980. Cooperative programme on short-term assays for carcinogenicity in Japan. In Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Scientific Publication No. 27, 323-330. International Agency for Research on Cancer, Lyon, France (as referred to by JECFA, 1987).
- Kawana K, Akema R, Nakaoka T, Ikeda H, Takimoto T and Kawauchi S, 1980. Studies on mutagenicities of natural food additives. II. Mutagenicities and antibacterial activities of caramels. Eisei Kagaku 26, 259-263 (as referred to by JECFA, 1987).
- Kay JH and Calandra JC, 1962a. Subacute oral toxicity of caramel colorings (coded Sample A) - albino rats. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.
- Kay JH and Calandra JC, 1962b. Ninety-day subacute oral toxicity of caramel coloring (coded Sample B) - albino rats. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.

- Kay JH and Calandra JC, 1962c. Subacute oral toxicity of caramel coloring (coded Sample A) - dogs. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.
- Kitts DD, Wu CH, Kopec A and Nagasawa T, 2006. Chemistry and genotoxicity of caramelized sucrose. *Mol Nutr. Food Res* 50, 1180-1190.
- Komoto M, 1962. *Journal of Agricultural and Food Chemistry* **36**, 305 (as referred to by JECFA, 1977).
- Kroplien U, 1984. Letter dated 17 December 1984 to International Technical Caramel Association, submitted to WHO (as referred to by JECFA, 1987).
- Kroplien U, Rosdorfer J, van der Greef J, Long RC Jr and Goldstein JH, 1985. 2-Acetyl-4(5)-(tetrahydroxybutyl)-imidazole: Detection in commercial caramel colour III and preparation by a model browning reaction. *The Journal of Organic Chemistry* 50, 1131-1133 (as referred to by JECFA, 1987).
- Licht BH, Shaw K, Smith C, Mendoza M, Orr J and Myers DV, 1992a. Development of specifications for caramel colours. *Food and Chemical Toxicology* 30(5), 383-387.
- Licht BH, Shaw K, Smith C, Mendoza M, Orr J and Myers DV, 1992b. Characterization of caramel colours I, II and III. *Food and Chemical Toxicology* 30(5), 375-382.
- Licht BH, Shaw K, Smith C, Mendoza M, Orr J and Myers DV, 1992c. Characterization of caramel colour IV. *Food and Chemical Toxicology* 30(5), 365-373.
- MacKenzie KM, 1985a. 90-day toxicity study of caramel color (caustic sulfite process) in rats. Study No. 6154-105. Volumes I and II. Hazleton Laboratories America Inc., Madison, WI, USA. 11 January 1985. Unpublished report. Submitted to WHO by International Technical Caramel Association.
- MacKenzie KM, 1985b. 90-day toxicity study of caramel color (ammonia process) in rats. Study No. 6154-107. Volumes I and II. Hazleton Laboratories America, Inc., Madison, WI, USA. 11 January 1985. Unpublished report. Submitted to WHO by International Technical Caramel Association.
- MacKenzie KM, 1985c. Carcinogenicity and chronic toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats (Chronic toxicity portion, Volumes I-V; Carcinogenicity portion, Volumes I-VIII). Study No. 81184. Hazleton Laboratories America, Inc., Madison, WI, USA. 11 January 1985. Unpublished report. Submitted to WHO by International Technical Caramel Association.
- MacKenzie KM, 1985d. Carcinogenicity study of caramel color (sulfite ammonia process) type SAC2 in mice. Volumes I-VI. Study No. 81185. Hazleton Laboratories America, Inc., Madison, WI, USA. 14 January 1985. Unpublished report. Submitted to WHO by International Technical Caramel Association.
- MacKenzie KM, Carter JL, Petsel SR, Chappel CI, Emerson JL and Stanley J, 1992a. Subchronic toxicity study of Caramel Colour II in F344 rats. *Food and Chemical Toxicology* 30(5), 411-6.
- MacKenzie KM, Boysen BG, Field WE, Petsel SRW, Chappel CI, Emerson JL and Stanley J, 1992b. Toxicity studies of caramel colour III and 2-acetyl-4(5)-tetrahydroxybutyl imidazole in F344 rats. *Food and Chemical Toxicology* 30, 417-425.
- MacKenzie KM, Boysen BG, Field WE, Petsel SR, Chappel CI, Emerson JL and Stanley J, 1992c. Toxicity and carcinogenicity studies of Caramel Colour IV in F344 rats and B6C3F1 mice. *Food and Chemical Toxicology* 30(5), 431-443.
- Maekawa A, Ogiu T, Matsuoka C, Onodera H, Furuta K, Tanigawa H, Hayashi Y and Odashima S, 1983. Carcinogenicity study of ammonia-process caramel in F344 rats. *Food and Chemical Toxicology* 21, 237-244 (as referred to by JECFA, 1987).

- Mandel TE, Koulmanda M and Mackay IR, 1992. Prevention of spontaneous and cyclophosphamide-induced diabetes in non-obese diabetic (NOD) mice with oral 2-acetyl-4-tetrahydroxybutylimidazole (THI), a component of caramel colouring III. *Clinical and Experimental Immunology* 88(3), 414-9.
- Marier G, Berry G, and Orr JM, 1977a. Tolerance study of single strength ammonia sulfite caramel in human volunteers. Unpublished project No. 5612, report No. 2, from Bio- Research Laboratories Ltd., Pointe Claire, Quebec, Canada.
- Marier G, Berry G, and Orr JM, 1977b. Tolerance study of double strength ammonia sulfite caramel in human volunteers. Unpublished project No. 5711, report No. 2, from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.
- Morgan SE and Edwards WC, 1986a. Bovine bonkers: new terminology for an old problem. A review of toxicity associated with ammoniated feeds. *Vet Hum Toxicol* 28, 240-242.
- Morgareidge K, 1974a. Teratologic evaluation of FDA 71-82 (caramel, bakers and confectioners) in mice, rats and rabbits. Unpublished report No. PB 234-870 from Food and Drug Research Laboratories Inc., Waverly, NY; USA (as referred to by JECFA, 1987).
- Morgareidge K, 1974b. Teratologic evaluation of FDA 71-83 (caramel beverage) in mice, rats and rabbits. Unpublished report No. PB 234-867 from Food and Drug Research Laboratories Inc., Waverly, NY; USA.
- Motoi Y, Yoshioka M, Hirose H, Ishino S, Nakajima Y, Miyazaki S, Miyamoto S, Sudou M, Monda T and Murai M, 1997. Central nervous disorder of calves consuming colostrums containing 4 methylimidazole or colostrums from cows fed excess ammoniated hay. *Japan Agricultural Research Quarterly* 31, 225-231.
- Murkovic M and Pichler N, 2006. Analysis of 5-hydroxymethylfurfural in coffee, dried fruits and urine. *Mol Nutr. Food Res* 50, 842-846.
- Myers DV and Howell JC, 1992. Characterization and specifications of caramel colours: an overview. *Food Chemistry Toxicology* 30, 359-363.
- Nees PO, 1964. Toxicological feeding study of caramel E. Unpublished report from Wisconsin Alumni Research Foundation, Madison, WI, USA.
- Nishie K, Waiss AC and Keyl AC, 1969. Toxicity of methylimidazoles. *Toxicology and Applied Pharmacology* 14, 301-307 (as referred to by JECFA, 1987).
- Nishie K, Waiss AC and Keyl AC, 1970. Pharmacology of alkyl and hydroxyalkylpyrazines. *Toxicology and Applied Pharmacology* 17, 1 (as referred to by JECFA, 1987).
- NTP, 2004. National Toxicology Program Toxicity Report Series Number 67 NTP Technical Report on the Toxicity Studies of 2- and 4-Methylimidazole (CAS No. 693-98-1 and 822-36-6) Administered in Feed to F344/N Rats and B6C3F1 Mice April 2004 NIH Publication No. 04-4409 U.S. Department of Health and Human Services Public Health Service National Institutes of Health
- NTP (National Toxicology Program), 2007. NTP Technical Report 535 on the Toxicology and Carcinogenesis Studies of 4-Methylimidazole (CAS No. 822-36-6) in F344/N Rats and B6C3F1 Mice. NIH Publication No. 07-4471. U.S. Department of Health and Human Services.
- NTP, 2008. National Toxicology Program. Toxicology and carcinogenesis studies of 5-(hydroxymethyl)-2-furfural (CAS No. 67-47-0) in F344/N rats and B6C3F1 mice (gavage studies) (Draft). National Toxicology Program, Research Triangle, NC, USA. TR-554. NIH Publication No. 08-5895. [Online] <http://ntp.niehs.nih.gov/>.
- O'Brien J, Nursten HE, James M and Crabbe MJC, 1998. The Maillard reaction in foods and medicine. Published by the Royal Society of Chemistry, Cambridge, UK. ISBN 0-85404-733-6.
- OEHHA, 2011. OEHHA Proposition 65 - Request for Relevant information - Dibromoacetic acid, 4-Methylimidazole. http://www.oehha.ca.gov/prop65/prop65_list/010711list.html

- Oser BL, 1963. Toxicological feeding study of "acid-proof" caramel. Unpublished report No. 83911 from Food and Drug Research Laboratories, Inc., Waverly, NY, USA
- Phillips JA and Paine AJ, 1990. Effect of pyridoxine on the disposition and lymphopenic effects of 2-acetyl-4(5)-tetrahydroxybutyl imidazole in the rat. *Xenobiotica* 20(6), 555-62.
- Patey AL, Shearer G, Knowles ME and Denner WHB, 1985. Ammonia caramels: specifications and analysis. *Food Additives and Contaminants* 2 (2), 107-112.
- Patey AL, Startin JR, Rowbottom PM and Shearer G, 1987. Identification of substituted hydroxypyridines and hydroxypyrazines in caramel food colourings. *Food Additives and Contaminants* 4, 9-15.
- Prier RF, 1960. The toxicity of double strength acid proof caramel in rats - 12 week feeding test. Unpublished report No. 9070599 from Wisconsin Alumni Research Foundation, Madison, WI, USA.
- Procter BG, 1976. A preliminary evaluation of the potential toxicological effects of Ammonia Caramel in mice. Unpublished report No. 5705 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada (as referred to by JECFA, 1987).
- Procter BG, Berry G and Chappel CI, 1976. A toxicological evaluation of various caramels fed to albino rats. Unpublished report No. 4244 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada (as referred to by JECFA, 1987).
- Procter BG, 1977. A preliminary evaluation of the potential toxicological effects of Ammonia Caramel (U.S. origin) in rats. Unpublished report No. 5617 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Ray AC, Raisor MJ, Herd DB, Murphy MJ and Reagor JC, 1984. Methylimidazole content of ammoniated forages associated with toxicity in cattle. In *American Association of Veterinary Laboratory Diagnosticians 27th Annual Proceedings*, pp. 337-348.
- Richold M and Jones E, 1980a. Ames metabolic activation test to assess the potential mutagenic effect of ETA-38-1W. Unpublished report No. FDC 8/80361 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.
- Richold M and Jones E, 1980b. Ames metabolic activation test to assess the potential mutagenic effect of ETA-38-2H. Unpublished report No. FDC 8/80359 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.
- Richold M and Jones E, 1980c. Ames metabolic activation test to assess the potential mutagenic effect of ETA-38-3N. Unpublished report No. FDC 8/80356 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.
- Richold M and Jones E, 1980d. Ames metabolic activation test to assess the potential mutagenic effect of ETA-48-1H. Unpublished report No. FDC 8/80358 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Richold M, Jones E and Fenner LA, 1984a. Ames metabolic activation test to assess the potential mutagenic effect of caramel colour II. Unpublished report No. ITC 1B/84709.
- Richold M, Jones E and Fenner LA, 1984b. Ames metabolic activation test to assess the potential mutagenic effect of caramel colour III. Unpublished report No. ITC 1B/84709.
- Rivera J, Prola RL, Olivera A, 2008. The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat. Rev. Immunol.* 8(10), 753-763.
- Royle L and Radcliffe CM, 1999. Analysis of caramels by capillary electrophoresis and ultrafiltration. *Journal of the Science of Food and Agriculture* 79, 1709-1714.

- Royle L, Ames JM, Castle L and Nursten HE, 2002. Analysis of malts by capillary electrophoresis. *Journal of the Science of Food and Agriculture* 82, 443-451.
- Sádecká J and Tóthová J, 2010. Determination of caramel in non-aged mixed wine spirits by synchronous fluorescence spectroscopy. *European Food and Research Technology*, 230;797-802.
- SCF (Scientific Committee on Food), 1975. Reports of the Scientific Committee for Food, 1st series. Opinion expressed 31 December 1975, 17-20.
- SCF (Scientific Committee on Food), 1984. Reports of the Scientific Committee for Food, 14th series. EUR 8752. Luxembourg: Office for Official Publications of the European Communities, 1983, ISBN 92-825-3893-1. Opinion expressed 1983, 56-57.
- SCF (Scientific Committee on Food), 1989. Reports of the Scientific Committee for Food, 21st series. EUR 11617. Luxembourg: Office for Official Publications of the European Communities, 1998. ISBN 92-826-0823-9. Opinion expressed 1987, 3-8.
- SCF (Scientific Committee on Food), 1997. Reports of the Scientific Committee for Food, 36st series. Opinion of the SCF on Causitic sulphite caramel and Ammonia caramel. Luxembourg: Office for Official Publications of the European Communities, 1997, ISBN 92-827-9580-2. 1-8.
- Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y and Cyster JG, 2005. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. *Science* 309(5741), 1682-3.
- Selim S, Chappel CI and Schoenig GP, 1992. Absorption, distribution and excretion of the colour fraction of Caramel Colour IV in the rat. *Food and Chemical Toxicology* 1992 May, 30(5), 445-51.
- Sharratt M, 1971. Unpublished report (as referred to by JECFA, 1987).
- Sinkeldam EJ and van der Heyden CA, 1975. Short-term feeding study with caramel SS 202 in albino rats. Unpublished report No. R 4777 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam EJ, Willems MI and van der Heyden CA, 1975. One year feeding study in rats with six different Ammonia Caramels. Unpublished report No. R 4767 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam EJ and van der Heyden CA, 1976a. Short-term feeding test with three types of caramels in albino rats. Unpublished report No. R4789 from CIVO/TNO, Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.
- Sinkeldam EJ and van der Heyden CA, 1976b. Short-term (10 week) feeding study in rats with three different Ammonia Caramels. Unpublished report No. R 5120 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands (as referred to by JECFA, 1987).
- Sinkeldam EJ, van der Heyden CA and Beems RB, 1976. Chronic (two year) feeding study in rats with six different ammonia caramels. Unpublished report No. R 4961 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam EJ, 1979. Paired feeding test in rats with caramel (AC3) in drinking water. Unpublished report No. R 6157 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, Bruyntjes JP and Kuper CF, 1980a. Sub-chronic (13-week) oral toxicity study with a modified Ammonia Caramel (ETA-26-1) in rats. Unpublished report No. R 6483 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, Roverts WG and Kuper CF, 1980b. Sub-chronic (13-week) oral toxicity study with Ammonia Caramel (AC2) in rats. Unpublished report No. R 6121 from Centraal Instituut voor

- Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, 1981. Quantitative relationship between dietary pyridoxine content and lymphocyte counts in rats fed Ammonia Caramel. Unpublished report No. V81.390/211704 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, 1982a. Quantitative relationship between dietary pyridoxine content and lymphocyte counts in mature rats fed Ammonia Caramel. Unpublished report No. V82.102/220102 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, 1982b. Short-term (7 day) bioassay in rats with three dose levels of 2-acetyl-4(5)-tetrahydroxybutylimidazole (13-A-82). Unpublished report No. V82.291/221153 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, de Groot AP, van den Berg H and Chappel CI, 1984. The effect of vitamin B6 on the number of lymphocytes in blood of rats fed caramel colour (ammonia process). Unpublished report submitted to WHO by International Technical Caramel Association (as referred to by JECFA, 1987).
- Sinkeldam EJ, de Groot AP, van den Berg H and Chappel CI, 1988. The effect of pyridoxine on the number of lymphocytes in blood of rats fed Caramel Colour III. *Food and Chemical Toxicology* 26(3), 195-203.
- TemaNord, 2002. Food additives in Europe 2000; Status of safety assessments of food additives presently permitted in the EU. *TemaNord* 2002, 560, 145-148.
- Tennant, D, 2007. Screening potential intakes of caramel colours. Report provided for the European Technical Committee on Caramels (EUTECA).
- Tennant D, 2008. Screening potential intakes of colour additives used in non-alcoholic beverages. *Food and Chemical Toxicology* 46 (2008) 1985–1993.
- Thuvander A and Oskarsson A, 1994. Effects of subchronic exposure to caramel colour III on the immune system in mice. *Food and Chemical Toxicology* 32, 7-13 (as referred to by TemaNord, 2002).
- Tierney WJ, 1979. A four-week dose range-finding study of caramel colour no. 3, sample 3-1, in mice. Unpublished project No. 79-2380 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by International Technical Caramel Association.
- Tierney WJ, 1980. An in-utero range-finding study of caramel colour no. 3, sample 3-1, in rats. Unpublished project No. 79-2405 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by International Technical Caramel Association.
- Til HP and Spanjers M, 1973. Reproduction study in rats with six different Ammonia Caramels. Unpublished report No. R 4068 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- UNEP (United Nations Environment Programme), 2005. SIDS Initial Assessment Report for imidazole. OECD SIAM meeting 17, Italy, 2003. <http://www.chem.unep.ch/irptc/sids/OECDsids/288324.pdf>
- Vinggaard AM, Hass U, Dalgaard M, Andersen HR, Bonefeld-Jørgensen E, Christiansen S, Laier P and Poulsen M E, 2006. Prochloraz: an imidazole fungicide with multiple mechanisms of action. *International Journal of Andrology*, 29: 186–192.
- Weiss WP, Conrad HR, Martin CM, Cross RF and Shockey WL, 1986. Etiology of ammoniated hay toxicosis. *Journal of Animal Sciences* 63, 525-532. *Sugar Journal* 18, 18-20.

- Wiggins LF, 1956a. The ammoniation of molasses. Proceedings of the International Society of Sugar Chemistry Technology 2, 525-529.
- Wiggins, L.F., 1956b. Some recent studies on ammoniated molasses. Sugar Journal 18, 18-20.
- Yu YN, Chen XR, Ding C, Cai ZN and Li QG, 1984. Genotoxic activity of caramel on Salmonella and cultured mammalian cells. Mutation Research 139, 161-165 (as referred to by SCF, 1997).
- Yu XQ, Kramer J, Moran L, O'Neill E, Nouraldeen A, Oravec T, Wilson AGE (2010). Pharmacokinetic/pharmacodynamic modelling of 2-acetyl-4(5)-tetrahydroxybutyl imidazole-induced peripheral lymphocyte sequestration through increasing lymphoid sphingosine 1-phosphate. Xenobiotica 40(5), 350-356.
- Yuan JH and Burka LT, 1995. Toxicokinetics of 4-methylimidazole in the male F344 rat. Xenobiotica 25(8), 885-94.

ANNEX A.

Rules defined by the Panel to deal with quantum satis (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

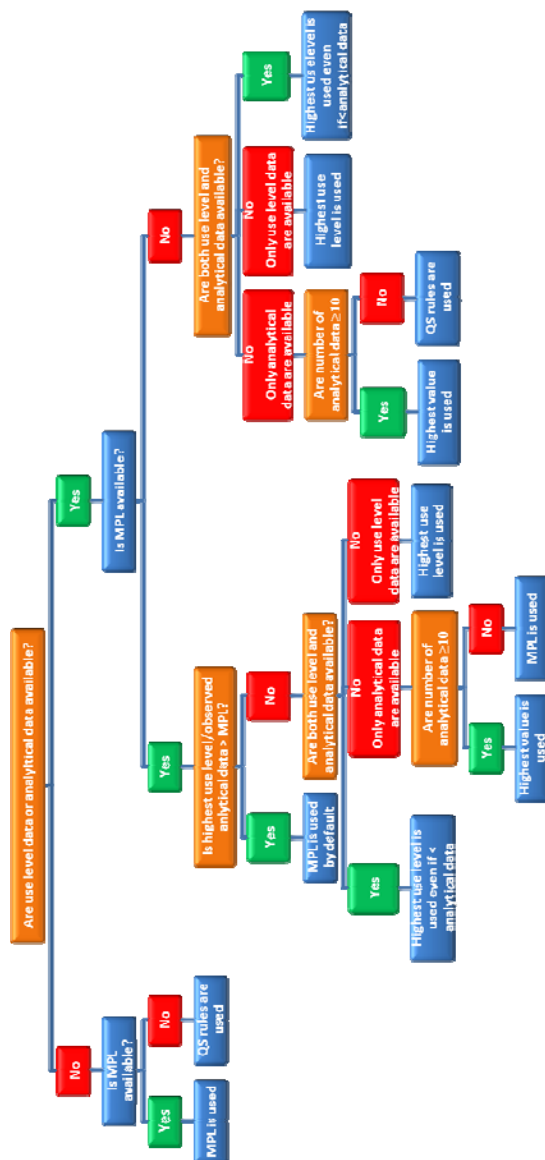
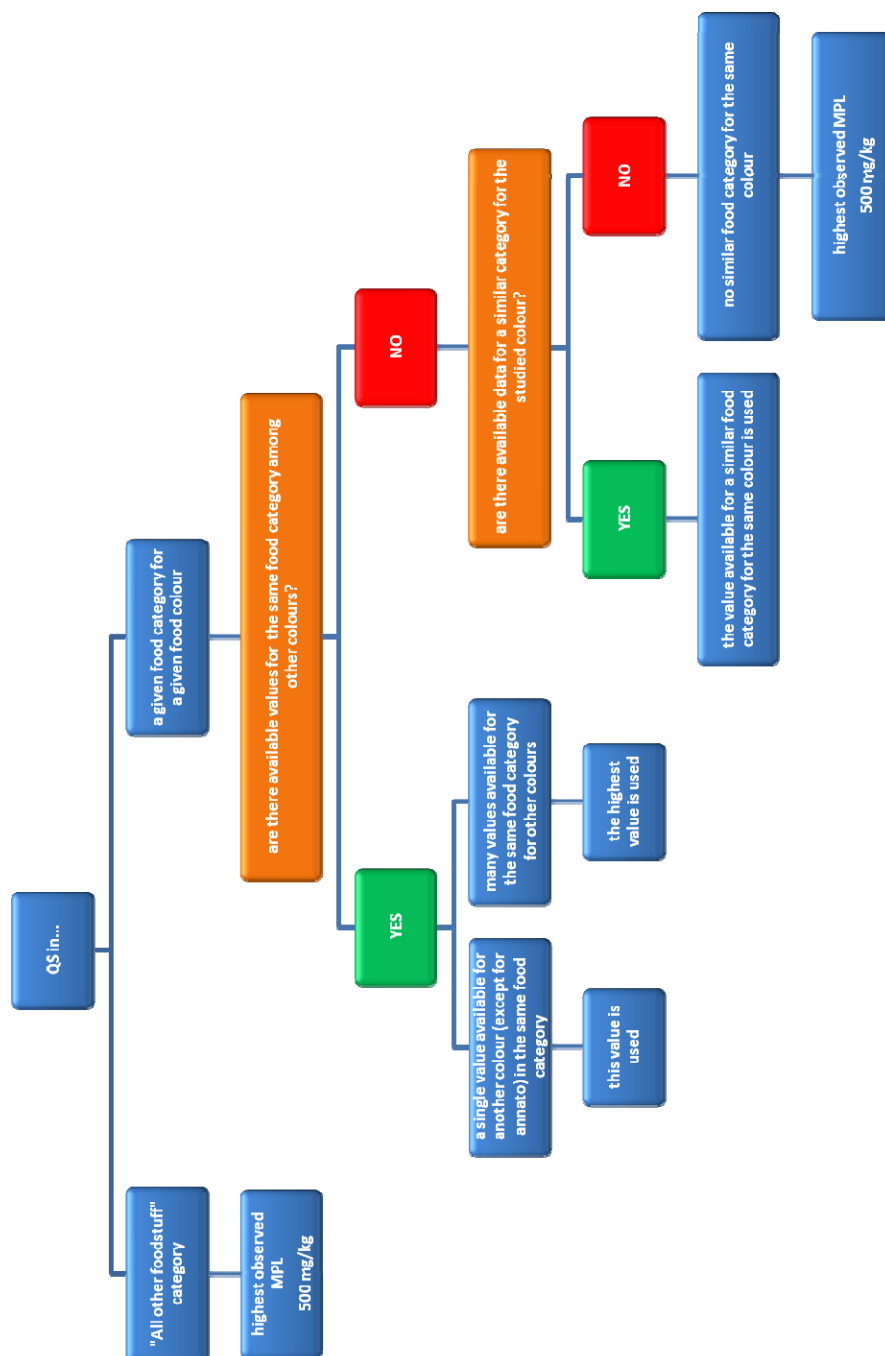


Figure 2: Rules defined by the Panel to deal with *quantum satis* (QS) authorisation.



ANNEX B

Summaries of short-term toxicity studies focussing on toxicity of Class III Ammonia Caramel including studies investigating the influence of 2-acetyl-4-tetrahydroxy-butylimidazole (THI) and pyridoxine on the toxic effects seen.

Four groups of rats (10 animals/sex) received either 0 or 10% of 2 different samples of Class III Ammonia Caramel in their diet (equivalent to 0 or 5 g/kg bw/day) for 90 days. (No information on the THI content of the sample or dietary pyridoxine (vitamin B₆) levels was provided). Weight gains were slightly reduced whereas food consumption was normal in all groups. No abnormalities were noted regarding haematology, urinalysis, gross pathology, or histopathology (Chacharonis, 1963).

Four groups of rats (no detail on group size or sex ratio) received 0, 4, 8, or 16% Class III Ammonia Caramel in their diet (equivalent to 0, 2, 4, or 8 g/kg bw/day) for 3 months. No convulsions or other behavioural abnormality or signs of neurological damage were seen. No macroscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971).

Groups of Sprague-Dawley (CFE strain) rats (15 animals/sex; 25 animals/sex in the control group) were fed either “open” or “closed” pan process Class III Ammonia Caramel at dietary levels of 0, 4, 8, or 16% (equivalent to 0, 2, 4, or 8 g/kg bw/day) for 13 weeks (no information on the THI content of the sample or dietary pyridoxine levels is given). Both caramel colours decreased body-weight gain at all dietary levels. At necropsy, thymus and spleen weights were decreased at the 8 and 16% dose levels. At the same dose levels the caecal weights were increased. Relative liver and kidney weights were also increased suggesting an effect on these organs. Histopathological examination did not reveal treatment-related changes. Numbers of leucocytes and lymphocytes were significantly decreased at all dose levels after 6 and 10 weeks; after 13 weeks these changes were observed only in males. Haemoglobin concentrations were reduced at the highest dietary levels after 6 weeks, while at the lower levels this effect was less clear. At week 13, significantly decreased haemoglobin concentrations were noted at all dose levels in males but only at the 8 and 16% levels in females. In some groups there was a (less consistent) decrease in the total number of red blood cells after 6 and 13 weeks (Gaunt et al., 1977).

Female Wistar rats (20/group) were fed diets containing 0, 15, or 30% of Class III Ammonia Caramel (equivalent to 0, 7.5 or 15 g/kg bw/day) for 8 weeks followed by a 4-week recovery period (no information on the THI content of the sample or dietary pyridoxine levels was given). The colour caused dose-related decreases in body weights and food efficiency. The relative weights of the caecae of animals receiving Class III Ammonia Caramel, both filled and empty, were increased at 4 and 8 weeks but reverted to normal after the 4-week recovery period. Diarrhoea was observed at the 30% dietary level. Gross examination at autopsy after 8 weeks revealed a slight, dose-related brown discolouration of the mesenteric lymph nodes in a few animals of each test group. After recovery periods of 2 or 4 weeks the discolouration was less intensive, but still visible. Microscopically, the lymph nodes of the test rats showed accumulation of pigment-laden macrophages, which was not noticeably diminished after a 2 or 4 weeks recovery period. White blood cell counts were significantly increased at the highest dose level after 4 weeks but not after 8, 10, and 12 weeks (Sinkeldam and van der Heyden, 1976a).

Groups of Wistar rats (10 animals/sex/group) received diets containing Class III Ammonia Caramel at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 15.0% (equivalent to approximately 0, 0.6, 1.3, 2.5, 5, or 7.5 g/kg bw/day) for 10 weeks (no information on the THI content of the sample or dietary pyridoxine levels was provided). Class III Ammonia Caramel caused slight decreases in body weights at the 5% dietary level. Loose stools were observed, particularly at the 5% dietary level. The relative weight of the caecum (both full and empty) was increased by feeding Class III Ammonia Caramel. Minimal amounts of pigment were observed in mesenteric lymph nodes of several rats at dose levels of 1.25% and higher. White blood cell counts and lymphocyte counts were decreased in both sexes at a dose

level of 15%; at lower dose levels this effect occurred only in females (Sinkeldam and van der Heyden, 1976b).

Groups of Sprague-Dawley rats (15 animals/sex/group; 20 animals/sex in the control group) were fed diets containing 0, 10 or 15% Class III Ammonia Caramel (equivalent to 0, 5 or 7.5 g/kg bw/day) for 4 weeks (no information on the THI content of the sample or dietary pyridoxine levels was provided). There were no consistent differences in body-weight gain or food consumption and no mortality occurred. During the course of the study rats receiving the food colour had soft dark-coloured faeces, particularly at the highest dietary level. At necropsy, caecal weights were increased in both sexes. In addition, males showed a statistically significant increase in relative thymus weights. Histopathological studies were not conducted. Haematological studies were conducted prior to feeding the colour and after 2 and 4 weeks. At both dose levels a significant depression of differential lymphocytes (expressed as %), and a concomitant increase in segmented neutrophils were observed at both intervals in males and after 4 weeks in females. After 4 weeks female rats also displayed a significant depression of total white cell and lymphocyte counts at both dose levels (Procter et al., 1976).

Groups of Sprague-Dawley rats (15 animals/sex/group) were fed diets containing Class III Ammonia Caramel at concentrations of 0, 1.25, 2.5, 5, 10, or 15% (equivalent to approximately 0, 0.6, 1.3, 2.5, 5, or 7.5 g/kg bw/day) for 10 weeks (no information on the THI content of the sample or dietary pyridoxine levels was provided). Animals fed Class III Ammonia Caramel generally displayed reduced body-weight gains, particularly during the last 2-4 weeks of the study. Faeces became soft and contained increased water content at levels of 5% or higher. An increase in empty caecal weight was consistently evident at dose levels of 5, 10, and 15%. Histopathological examination did not reveal changes in the structure of the ileal or caecal mucosa nor in the reticuloendothelial components of the central or peripheral systems. No macroscopic or histopathological evidence of abnormal pigmentation of the mesenteric lymph nodes was found. Lymphocyte counts were significantly reduced whereas numbers of segmented neutrophils were increased at all dose levels. No changes were observed in haemoglobin levels or erythrocyte counts (Procter et al., 1977).

Six groups of weanling Wistar rats (15 animals/sex in the 0-4%-groups; 10 animals/sex in the 16%-group; 60 animals/sex in the control group) were fed Class III Ammonia Caramel in the diet at levels of 0, 0.5, 1, 2, 4, or 16% (equivalent to 0, 0.25, 0.5, 1, 2, or 8 g/kg bw/day) for 10 weeks. Another 2 groups of 10 rats received a diet containing 0 or 16% of the colour for 10 weeks followed by a 28-day recovery period (no information on the THI content of the sample or dietary pyridoxine levels was provided). Class III Ammonia Caramel depressed body-weight gain at dietary levels greater than 1%. Spleen weights were decreased and caecal weights were increased and at the highest dose level; the relative weights of the liver and kidney were increased at dietary levels of 2% and higher. All organ weight deviations returned to normal during the recovery phase, except for the kidney weights which only partially recovered. Microscopically, pigment was observed in the mesenteric lymph nodes of male and female rats at this dose level. Total white blood cell counts were decreased in the 2, 4, and 16% groups in males and the 4 and 16% groups in females. However, the lymphocyte/neutrophil ratio was significantly decreased in both sexes at all dose levels. In both sexes white cell counts, cell ratios, and total numbers of lymphocytes had returned to normal within 7 days of recovery (BIBRA, 1977).

Weanling Wistar rats (10 animals/sex/group) received drinking water containing concentrations of 0, 4, 6, 8, or 10% Class III Ammonia Caramel (equivalent to 0, 2, 3, 4, or 5 g/kg bw/day) for 13 weeks (the caramel sample used contained 78 mg THI/kg on an 'as is' basis, or 105 mg THI/kg on a solids basis; the diet fed to the rats contained approximately 13 mg pyridoxine/kg). There was a dose-related decrease in fluid consumption, decreased urinary output of more concentrated urine, decreased food consumption, and decreased body-weight gain in both sexes. These changes were related to the palatability of the drinking fluid. Necropsy revealed an increase in the relative weight of the caecum and a dose-dependent increase in the relative weight of the kidneys in several groups receiving the colour (not specified). No treatment-related histopathological changes were seen in the caecum, kidney, or a range of additional organs examined. The enlargement of the kidneys was attributed to the

decreased fluid consumption. No effects on the relative weights or histology of spleen or thymus were observed. Statistically significant decreases in lymphocyte counts were observed in males in all treatment groups after 29/30 days, but only in the 4 and 8% groups after 57/58 days. In females decreases were statistically significant only in the 8% group after 29/30 days. For both sexes the decreases in lymphocyte counts were not clearly dose-dependent and after 13 weeks there were no significant differences among any of the treatment groups compared to controls. No outstanding differences were observed in red blood cell analyses between test groups and controls (Sinkeldam et al., 1980b).

Class III Ammonia Caramel was given to weanling Wistar rats (10 animals/sex/group) in the drinking water at concentrations of 0, 5, 10, 15, or 20% (equivalent to 2.5, 5, 7.5, or 10 g/kg bw/day) for 13 weeks. (The caramel sample contained 0-3 mg THI/kg on an 'as is' basis; the diet fed to the rats contained 13.5 mg pyridoxine/kg.). Food intake was generally lower in all treated groups. Body-weights were decreased in a dose-related manner only in males. Treated animals of both sexes excreted less and more concentrated urine which was attributed to a dose-related decrease in water intake. The urine was darker-coloured at the 15 and 20% dose levels although urine composition was essentially normal. Although the relative weights of the caecum, liver, brain, kidneys, and testes were increased in several test groups (not specified), there were no pathological changes in any of these organs. Microscopic examination revealed a treatment-related increase in numbers of macrophages containing a yellow-brown pigment in the mesenteric lymph nodes. Lymphocyte counts of both sexes were in general relatively low in all test groups but only statistically significant in males of the 20%-group after 30 days and males of all treatment groups after 57 days. No significant differences in lymphocyte counts were observed in males of any group after 80 days, nor in females at any dose level at any time interval. Mean neutrophil counts were significantly increased in females receiving 20% Class III Ammonia Caramel for 81 days (Sinkeldam et al., 1980a).

Groups of weanling F344 rats (10 animals/sex/group) were given Class III Ammonia Caramel in the drinking water at concentrations of 0, 0.5, 1, 2, 4, or 8% (equivalent to 0, 0.25, 0.5, 1, 2, or 4 g/kg bw/day) for 4 weeks (the sample of Class III Ammonia Caramel used contained 70 mg THI/kg on an 'as is' basis; the diet used in this study contained 17 mg pyridoxine/kg.) No differences in body-weight gain were noted for any of the test groups, although food consumption of the males was significantly lower than controls throughout the study. At necropsy no differences in organ weights were noted and there were no gross or microscopic pathological changes related to treatment. As decreases in lymphocyte counts were noted among the treatment groups at the midpoint of the study but not at 1 month, these findings were considered transient. Other haematological and clinical chemistry parameters were normal for all groups (Heidt and Rao, 1981).

In order to determine appropriate dose levels for a chronic toxicity study, groups of F344 rats (10 animals/sex/group) were given 20 ml per day of solutions containing Class III Ammonia Caramel at concentrations of 0, 1.25, 2.5, 5, 10, or 20% (equivalent to 0, 0.5, 1, 2, 4, or 8 g/kg bw/day) for 13 weeks (the Class III Ammonia Caramel used contained 78 mg THI/kg on an 'as is' basis; the diet (*ad libitum*) contained 11-12 mg pyridoxine/kg.) Weight gains were reduced in all exposed groups from week 1. At week 13 weight gains compared to controls were 89*, 94, 84*, 76*, and 76%* for males, and 96, 98, 80*, 84*, and 92% for females, according to rising doses (asterisks denotate significance). At necropsy, no pronounced macroscopic changes were observed in any animals, although a few rats in the experimental groups were very emaciated. No histological changes related to administration of the colour were found in any experimental groups. No haematological changes were observed during or at the end of the experimental period (Maekawa et al., 1983).

Groups of mice (10 animals/sex) were fed Class III Ammonia Caramel in the diet at levels of 0, 1, 2, 4, 8, or 16% (equivalent to approximately 0, 1.4, 2.9, 5.7, 11.4, or 22.9 g/kg bw/day) for 4- to 6-weeks (the caramel sample contained 830 mg 4-MEI/kg; no information on the THI content of the sample or dietary pyridoxine levels are given.) In the highest dose group growth was decreased (especially in the third and fourth weeks) although no influence on food intake was observed. The faeces of the animals fed the higher dose levels were soft, tarry in appearance, poorly-formed, and sticky or pasty in

consistency. The mean relative weights of the caecae (full and empty) were increased at the 4, 8, and 16% dietary levels (no sex distinction). In the males fed 16% Class III Ammonia Caramel, an increase of neutrophilic leucocytes and a decrease in lymphocytes were observed. No other remarkable findings were observed on appearance, behaviour, or gross examination; histopathological examinations were not conducted (Procter, 1976).

In a study in female Balb/c mice, the animals received a diet containing 0, 2 or 10% (equivalent to approximately 0, 3.3, or 16.7 g/kg bw/day) of a commercial Class III Ammonia Caramel preparation in the drinking water for 9 weeks (the sample of Class III Ammonia Caramel used contained < 25 mg/kg THI; the diet contained relatively high vitamin B₆ content (not specified)). Although a treatment related lymphocytopenia was not evident, flow cytometric analysis demonstrated reductions in the CD4⁺ and CD8⁺ lymphocyte subpopulations in exposed mice. In addition, the proliferative response of spleen cells to B and T cell mitogens was significantly reduced in the mice exposed to 2% Class III Ammonia Caramel. No changes were observed in natural killer cell activity or in the humoral antibody response to a viral antigen (Thuvander and Oskarsson, 1994).

To investigate the effect of pyridoxine on lymphocyte counts, two groups of male weanling rats (10 per group) received a Spratt's diet containing 0 (basal diet; containing 2.3 mg pyridoxine/kg) or 8% Class III Ammonia Caramel (equivalent to 4 g/kg bw/day) for 12 days. A third group received a diet containing 8% Class III Ammonia Caramel supplemented with 10 mg pyridoxine/kg hydrochloride. After 12 days, rats receiving Spratt's diet with 8% Class III Ammonia Caramel displayed a marked reduction in total white blood cells and lymphocytes and an increase in neutrophil counts. Rats receiving the caramel diet supplemented with pyridoxine had white blood cells and lymphocytes in numbers similar to those fed the basal diet (Sinkeldam, 1981).

In a further study rats received a CIVO (Zeiss) stock diet with 0 (basal diet, containing 3 mg pyridoxine/kg) or 8% Class III Ammonia Caramel (no detail on duration). The colour induced a reduction in total white blood cells and lymphocyte numbers which were ameliorated by the addition of 10 mg pyridoxine/kg in the diet. In this study, neutrophils were not affected by the Class III Ammonia Caramel treatment, but the plasma pyridoxal phosphate levels were reduced by treatment (Sinkeldam et al., 1984).

In another study by Sinkeldam (1982a), groups of weanling and mature Wistar rats (10 males per group) were fed diets containing approximately 0, 2.5, 6, 12, or 24 mg/kg of pyridoxine (equivalent to 0, 0.13, 0.3, 0.6, or 1.2 mg/kg bw/day) for 14-days. At each dietary level, groups were given Class III Ammonia Caramel in the drinking water at levels of 0, 1, 4, or 8%. equivalent to 0, 0.5, 2, or 4 g/kg bw/day (the caramel sample contained 107 mg THI/kg on a colour equivalent basis, 204 mg THI/kg on an 'as is' basis, and 295 mg THI/kg on a solids basis). In Table 12 the effects on lymphocytes in weanling and mature rats are summarized:

Table 12. Lymphocyte reduction in weanling and mature rats after combined administration of pyridoxine and Class III Ammonia Caramel.

	Weanling rats								Mature rats							
	Day 6				Day 13				Day 6				Day 13			
	2.5	6	12	24	2.5	6	12	24	2.5	6	12	24	2.5	6	12	24
pyr.	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
THI	+	-	-	-	+	+	-	-	+	+	+	-	+	-	-	-
1	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-
4	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-
8	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-

pyr. = pyridoxine

+ = statistically significant lymphocyte reduction

- = no (statistically significant) lymphocyte reduction

Based on these findings, Sinkeldam concluded that a clear inverse dose relationship exists between the severity of lymphocyte depression and the pyridoxine level of the diet.

Sinkeldam and co-workers also examined the effect of vitamin E, folic acid, pyridoxine and choline on the reduction in circulating lymphocytes in the blood of rats administered Class III Ammonia Caramel in drinking water and maintained on normal or pyridoxine-deficient diets (Sinkeldam et al., 1988). The authors found that the reduction in the number of circulating lymphocytes in rats fed Class III Ammonia Caramel could be prevented by the addition of pyridoxine to the diet. The activity of Class III Ammonia Caramel in reducing the number of circulating lymphocytes also closely resembled that of the pyridoxine antagonist 4'-deoxypyridoxine. After the isolation and identification of THI, comparative studies indicated that THI was the constituent of Class III Ammonia Caramel responsible for reducing the number of circulating lymphocytes. Although the effect of Class III Ammonia Caramel was reduced or prevented by increasing dietary pyridoxine, the lymphocyte reduction associated with the administration of THI was not materially affected by the dietary level of pyridoxine. The authors demonstrated significant reductions in lymphocytes and total leucocytes at a dose level of 1% Class III Ammonia Caramel in drinking water, equivalent to 1 g caramel/kg bw/day and estimated to be equivalent to 200 µg THI/ kg bw/day. Similar statistically significant reductions in lymphocytes were observed when the rats were given 200 µg, 500 µg or 2 mg THI/kg bw/day alone in drinking water. The authors reported decreases in lymphocyte counts in rats receiving as little as 0.1% Class III Ammonia Caramel in drinking water for 1 week and maintained on a low-pyridoxine diet (2-3 mg/kg). Class III Ammonia Caramel intake at this level in drinking water was 0.1 g/kg bw/day, equivalent to 20 µg THI/ kg bw/day. Statistical analysis was not reported for this aspect of the study, but evaluation of the data provided would indicate that while lymphocytopenia was evident at this dose level, the reduction was unlikely to be statistically significant.

Houben et al.(1992b) found that effects induced by Class III Ammonia Caramel and/or THI were less pronounced or absent in rats fed a diet high in pyridoxine. Furthermore, THI did not induce obvious effects on vitamin B₆ status. Similar effects of THI and Class III Ammonia Caramel as those observed in rats were observed in mice on a basic diet with 2-3 mg/kg vitamin B₆ (pyridoxine) (equivalent to 0.3 to 0.45 mg/kg bw/day) (no further details) (Houben et al., 1992b).

ANNEX C.

Summaries of toxicity studies focussing on toxicity of Class IV Caramel (Sulphite Ammonia Caramel) not included in the main body of the opinion

Rats (5/group) received either 10 or 20% Class IV Sulphite Ammonia Caramel solution (equivalent to about 10 or 20 g/kg bw/day) as their sole source of fluid for up to 127 days. Only dark faeces and very mild diarrhoea were noted. No adverse effects were noted regarding general health, body weight, food and fluid consumption, haematology, gross pathology, or histopathology (Haldi and Wynn, 1951).

Weanling rats (5 animals/sex/group) received 0 or 10% Class IV Sulphite Ammonia Caramel solution as their sole fluid source for 100, 200, or 300 days. No adverse effects were noted regarding growth, food and fluid intake, haematology, gross pathology, or histopathology (Haldi and Wynn, 1951).

Rats (20 animals/sex/group) received either 0 or 11-14 g/kg bw of Class IV Sulphite Ammonia Caramel solutions for 100 days. Growth and food intake did not differ significantly between test and control animals. Gross pathology and histopathology showed no abnormal findings related to administration of the test compound (Haldi and Wynn, 1958).

Rats (5 animals/sex/group) were given 1 ml/kg bw/day of concentrated caramel colour (unspecified) for 21 days. Some diarrhoea was induced in all animals, but no other abnormalities were noted. Gross pathology and histopathology revealed no significant changes due to administration of the test compound (Foote et al., 1958).

Sprague-Dawley rats (10 animals/sex/group) received 0, 0.1, 1.0 or 10% Class IV Sulphite Ammonia Caramel in their diet for 12 weeks. No adverse effects were noted on growth, food consumption, urinalysis, haematology, gross pathology or histopathology that were related to administration of the caramel colour (Prior, 1960).

Rats (10 animals/sex/group) received 0, 5, or 10 g/kg (not specified whether per kg food or bw) Class IV Sulphite Ammonia Caramel in their diet for 3 months. Weight gain was normal in all groups. Food consumption, haematology, and urinalysis were comparable in all groups. Gross pathology and histopathology showed no test-related adverse findings (Chacharonis, 1960).

Sprague-Dawley rats (10 animals/sex/group) received 0, 5, 10, or 20% low or high colour intensity Class IV Sulphite Ammonia Caramel in their diet for 90 days. In addition, a paired-feeding study involving 5 male rats in 2 groups was run for 23 days (one sample was at the 20% level), and there were no differences in the rate of growth. The only effects attributable to treatment were a mild depression in growth of male rats at the 10 and 20% levels due to unpalatability of the test diet. No other adverse findings were noted in growth, behaviour, mortality, haematology, urinalysis, gross pathology, organ weights, or histopathology (Kay and Calandra, 1962a; 1962b).

Sprague-Dawley rats (10 animals/sex/group) received either 0 or 10% of 3 different caramel colours (one of which was a sample of Class IV Sulphite Ammonia Caramel) in their diet for 90 days. Weight gains showed a slight reduction compared with controls but food consumption was normal for all groups. No abnormalities were noted with regard to haematology, urinalysis, gross pathology, or histopathology (Chacharonis, 1963).

Rats (15 animals/sex/group) received 0, 5, 10, or 20% Class IV Sulphite Ammonia Caramel in their diet for 90 days. No adverse effects were noted on appearance, behaviour, survival, body weights, food intake, haematology, blood chemistry, urinalysis, organ weights, gross pathology, or histopathology (Oser, 1963).

Rats (10 animals/sex/group) received 0, 0.015, 0.3, or 3.0% Class IV Sulphite Ammonia Caramel in their diet for 90 days. No differences between test and control animals were noted regarding body weight, food consumption, haematology, urinalysis, gross pathology, or histopathology (Nees, 1964).

Four groups of rats received 0, 4, 8, or 16% Class IV Sulphite Ammonia Caramel in their diet for 3 months. No convulsions or other behavioural abnormalities or signs of neurological damage were seen. No macroscopic or microscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971).

B6C3F1 mice (10 animals/sex/group) were given concentrations of 0, 10, 15, 20, or 30% Class IV Sulphite Ammonia Caramel in drinking water for 4 weeks. At 28 days the body weights of male animals at the 30%-dose level were significantly decreased compared to the controls, and transient depressions of body weights were noted at the highest dose level in females. Fluid consumption was depressed throughout the study among all treatment groups. However, these changes were not consistent with time or dosage. There were no statistically significant differences in food consumption between treated animals and controls. At necropsy, the only treatment-related effect reported was enlargement of the caecum (Tierney, 1979).

In addition, one special study on haematology is described.

A study was conducted in rats to determine whether haematological changes were associated with the feeding of high dietary concentrations of high (16 or 22%) or low intensity (34 or 47%) Class IV Sulphite Ammonia Caramel. Male rats (10/group), mean initial body weights approximately 173 g, were fed Class IV Sulphite Ammonia Caramel in the diet for 28 days. Sixteen rats were assigned to a control group. Rats were bled from the retro-orbital sinus on days 16, 9, and 2 before treatment and again on days 7, 14, and 28 during treatment. Feeding of these high dietary levels of Class IV Sulphite Ammonia Caramel led to reduced rates of body-weight gain. No effect on relative lymphocyte counts was observed. Caecal weights were increased in all treatment groups (BIBRA, 1978).

ANNEX D.

Summaries of toxicity studies on 2-acetyl-4-tetrahydroxy-butylimidazole (THI) and 4-methylimidazole (4-MEI)

D.1 Studies on 2-acetyl-4-tetrahydroxy-butylimidazole (THI)

THI was administered to male weanling Wistar rats (10 per group) at levels of 0, 2, 5, or 20 mg/l in drinking water (equivalent to 0, 0.1, 0.25, or 1 mg/kg bw/day) for 7 days. A marked depression of lymphocyte counts at all dose levels was observed. The lymphocyte-depressing potency of 0.1 mg THI/kg bw/day was comparable to a positive control group that received 1% Class III Ammonia Caramel in the drinking water (equivalent to 500 mg/kg bw/day), indicating a level of approximately 200 mg THI/kg in the caramel sample (which compares with a value of 204 mg THI/kg on an 'as is' basis). In addition to lymphocyte depression, THI induced a dose-dependent increase in neutrophils (Sinkeldam, 1982b; Kroplien, 1984).

In a study in which the lymphocyte-depressing fraction of Class III Ammonia Caramel was isolated, THI was identified as the sole responsible constituent (no further details) (Kroplien et al., 1985).

The haematological effects were studied in mice of oral administration in drinking water of THI, the constituent of Class III Ammonia Caramel responsible for lymphocytopenia (Iscaro et al., 1988). Initially five groups of BALB/c mice (five mice per group) were given doses of THI ranging from 0 to 200 parts/10(6) and bled weekly. Doses of THI from 5 to 100 parts/10(6) had no effect on circulating leucocytes over 6 weeks, but lymphocytopenia occurred with 200 parts/10(6). An increase in the concentration of THI to 400 parts/10(6) in the group on the lowest dose resulted in lymphocytopenia. An increase in dosage in two groups of mice, to 1000 and 2000 parts/10(6), resulted in marked lymphocytopenia. The number of neutrophils, eosinophils and monocytes remained unchanged throughout the experiment. Measurement of the proportions of CD4(L3T4)+ and CD8(Ly2)+ lymphocytes in lymph nodes from mice on high doses of THI did not show a selective depression of either subset, although both were increased relative to non-T cells. The authors concluded that THI causes a selective lymphocytopenia in mice, as in rats, but at relatively higher doses, and merits investigation in mice as an experimental treatment for states of lymphocyte excess or overactivity (Iscaro et al., 1988).

Gobin and co-workers examined the effect of giving THI at a level of 1 mg/kg bw/day by gavage for 7 days on the numbers of lymphocytes in subsets in peripheral blood (Gobin et al., 1989). Both immunoglobulin light chain-bearing B-cells (MARK-1+) and CD5 marker-bearing T-cells (OX-19+) were reduced in number within 1 day of treatment. Within the pan-T-cell population, Class II MHC reactive helper T-lymphocytes (W3/25-) were more reduced than the Class I MHC reactive cytotoxic/suppressor T cells (OX-8+). The number of null cells (MARK-1-, OX-19-) was not affected; the majority of these cells appeared to be large granular lymphocytes.

In a further study by the same group, THI was given in the drinking water at doses of 1, 10 and 50 mg/l (equivalent to 0.1, 1 and 5 mg/kg per day) to animals on a vitamin B₆-deficient diet (Gobin and Phillips, 1991). After 1 week, the immune competence of the animals was assessed under continued THI treatment. No marked changes in thymus or spleen weight were observed after THI treatment, although there was an increased number of pyknotic cells in the thymic cortex, mainly engulfed by macrophages and there appeared to be a slight thinning of the cortex area. THI produced a significant loss in T and B lymphocytes in peripheral blood but not in the spleen. No change in Natural Killer (NK) cell activity against YAC-1 target cells was observed in the spleen. The observed increase in NK cell activity in peripheral blood was due to an increase in circulating Large Granular Lymphocytes (LGL). Although the serum antibody titre against Keyhole Limpet Haemocyanin (KLH) was not affected by THI treatment, B cells showed less proliferation when cultured with lipopolysaccharide. T cell function was impeded as measured in mitogen-induced proliferation assay, delayed-type hypersensitivity assay and host versus graft (popliteal lymph node) assay. The authors concluded that

their results indicate that THI is an immunosuppressor in the rat, in whom it can produce profound lymphocytopenia and suppression of cell-mediated immunity (Gobin and Phillips, 1991).

In further investigations, THI (1 mg/kg bw/day) was given for up to 7 days in the drinking water to Fischer 344 rats on a vitamin B₆ deficient diet (Gobin and Paine, 1992; Gobin et al., 1992). THI rapidly produced profound lymphocytopenia resulting in depletion of T- and B-lymphocytes in peripheral blood but not in bone marrow and spleen. No marked change in thymus weight was found but the cellularity was marginally decreased and flow cytometric analysis of the lymphocyte subsets revealed an increase in the number of CD4+CD8- and CD4-CD8+ single positive (SP) cells and a reduction in the number of CD4+CD8+ double positive (DP) thymocytes. This reduction was in agreement with histological findings of increased numbers of pyknotic cells in the cortex, mainly engulfed by macrophages. Mitogen-induced proliferation of thymocytes prepared from THI-treated animals was increased, concordant with the gradual increase in the percentage of mature SP cells. No change in normal proliferation of thymocytes cultured *in vitro*, or, in proliferation *in vivo*, detected as 5-bromo-2'-deoxyuridine (BrdU) incorporation, was found. It is concluded that THI produced an increase in death of immature DP cells. However, THI did not affect thymocyte proliferation or their differentiation into mature SP cells in the thymus, but rather impairs their migration into the circulation. The authors concluded that the mechanism of action of THI appears to be indirect, but that THI does not act through increasing the release of adrenal corticosteroids to supra-physiological levels as the same histopathological changes in the thymus were found in adrenalectomised rats (Gobin and Paine, 1992). The authors also concluded that collectively these results indicate that the lymphocytopenia in the peripheral blood compartment after THI treatment, is caused by a rapid sequestration of lymphocytes into the spleen and bone marrow rather than by a reduced lymphocyte production and release into the periphery. The fact that THI also caused lymphocytopenia in splenectomised rats indicates that the spleen does not play an active part in the change in migrational behaviour of lymphocytes after THI treatment. Finally, as there was no increase in the absolute number of lymphocytes found in the spleen or bone marrow it seems they are rapidly degraded (Gobin et al., 1992).

THI administered orally to rats markedly decreased the peripheral blood lymphocyte count within 16-24 hours (Phillips and Paine, 1990). The lymphocytopenic effect of THI was prevented by co-administration of pyridoxine. ¹⁴C-THI, administered orally, was absorbed in a dose-dependent manner and excreted unmetabolised. These processes were not affected by co-administration of pyridoxine. In contrast, the rate of excretion of ¹⁴C-THI administered i.v. was increased by both dietary and parenterally-administered pyridoxine, and pyridoxine decreased the amount of radiolabel associated with lymphoid tissues. The authors propose that their results show that the lymphocytopenic effect of THI is not sustained once it is excreted, and indicate that pyridoxine and THI may compete for the same binding site in lymphoid tissues (Phillips and Paine, 1990).

The effect of oral administration of THI was studied in spontaneous and induced murine diabetes mellitus (Mandel et al., 1992). Continuous administration of THI at 400 mg/kg in drinking water reduced the prevalence of spontaneous diabetes in female NOD/Lt mice from 63% in untreated controls to 8% in treated animals. Since cyclophosphamide (CP) accelerates and intensifies diabetes in NOD mice, the authors also studied the effect of THI in this model. Diabetes incidence was reduced from 100% in mice given only CP to 13-14% in mice given THI either concurrently or from 14 days previously. Histologically, THI greatly reduced the severity of insulitis. As measured by flow cytometry, all THI-treated mice had a 60-80% reduction in splenic CD4+ and CD8+ T cells. THI-treated mice showed no untoward effects and specifically no weight loss, or pathological changes in their livers, kidneys or lungs. However, there was moderate atrophy of the thymus cortex. The authors concluded that THI could be a useful immunosuppressive agent (Mandel et al., 1992).

Groups of 10 rats per sex were given 0, 1, 2, 4, 16 or 32 mg THI/l in drinking water for 4 weeks (equivalent to approximately 0.1, 0.2, 0.5, 1.9 and 3.7 mg THI/kg bw/day in the treated groups), and groups of 20 rats per sex were given 0, 8 or 64 mg THI/l, equivalent to 0, 0.9, or 7.2 mg THI/kg bw/day. The rats were maintained on a normal rat chow, with adequate levels of pyridoxine. After 4

weeks 10 rats per sex of the 0, 0.9 and 7.2 mg/kg groups were given water without THI for 2 weeks (recovery phase). Decreased mean body weights in all test groups after 2 and 4 weeks were noted but ascribed to fasting for blood sampling and urinalysis. Gross and microscopic pathology in a large range of organs and tissues revealed no treatment-related effects. Treatment-related decreases in white blood cell and lymphocyte counts, and increases in neutrophil counts were observed. These changes returned to normal after 3 days recovery phase and remained normal. The NOAEL for the reduction in total lymphocytes was determined to be 4 mg THI/l in drinking water, equivalent to 380 µg/kg bw/day, in males and 1 mg THI/l in drinking water, equivalent to 120 µg/kg bw/day, in females (MacKenzie et al., 1992b).

In another study in rats, Class III Ammonia Caramel and THI (no details on doses) both induced a reduction in cell numbers in blood, spleen and lymph nodes, which were ascribed to decreases in T and B lymphocyte numbers. Increases in the number of mature medullary thymocytes (T cell precursors) in the thymus, and decreases in the number of ER4⁺ (recent thymus emigrants) in the spleen, were considered to indicate a diminished migration of mature cells from the thymus to the periphery. The increase in the number of mature cells was also indicated by an increase in mitogen-induced thymidine incorporation by thymocytes from THI exposed rats (Houben, 1992b). Further evidence for a change in the function of accessory cells was demonstrated in *ex vivo* studies, as splenic cells from THI exposed rats did not show adequate response upon mitogenic stimulation (no further detail) (Houben, 1992b).

SCID-hu mice were exposed to THI, Class III Ammonia Caramel, or the organotin compound, di-n-butyltin dichloride (DBTC). Histopathological examination of the human thymus grafts of SCID-hu mice either exposed to THI or to DBTC showed a reduction in the relative size of the thymus cortex, an effect also described in rodents. The authors conclude that the human thymus is a target for the immunotoxic action of both THI and DBTC (de Heer et al., 1995).

In a study investigating the effects of THI on the murine immune system, Bradbury et al. (1996) showed that splenic T lymphocytes from mice treated with 50 mg/l THI in their drinking water were unable to launch a Mixed Lymphocyte Reaction (MLR) against allogeneic stimulator cells, and had decreased and delayed interleukin-2 (IL-2) production. However, these T cells exhibited a normal proliferative response to concanavalin A (Con A), immobilized anti-CD3 monoclonal antibody (mAb) and anti-CD3 plus anti-CD28 mAb. Furthermore, the MLR response could be restored by the addition of IL-2 to the MLR culture. Homing studies using intravenous injection of fluorescence-labelled splenocytes showed that THI treatment decreased absolute numbers of labelled T and B lymphocytes in the blood and the spleen. Furthermore, these labelled cells reappeared in the blood and the spleen when mice were taken off THI, indicating that lymphocyte recirculation and splenic homing were modified reversibly by THI treatment. Cessation of THI treatment also resulted in a rapid reappearance of MLR responsiveness in the spleen, indicating that THI treatment does not functionally impair recirculating T cells. Collectively the authors suggested that the data were collectively compatible with the concept that a rapidly recirculating population of T cells, which produce IL-2 in an allogeneic MLR, are lost from the blood and spleen following THI treatment, and are sequestered in other, yet to be identified, tissues (Bradbury et al., 1996).

The fate of cells lost from the blood and the spleen during THI treatment was followed via injection of fluorescently labelled splenocytes into THI-pretreated mice (Bradbury et al. 1997). The results showed that THI increased labelled cells in the liver, lungs and kidneys of THI-treated mice. Furthermore, the sequestration in the liver occurred just 1.5 hours after injection of labelled cells with the increase still being present at 24 hours after injection. Microscopic examination of liver sections indicated that fluorescent lymphocytes were clustered within the liver sinusoids in THI-treated mice, possibly associated with endothelial cells. The liver retention of lymphocytes was confirmed by immunohistochemical studies which showed a significant increase of T cells in the liver of THI-treated mice. To determine the subset of lymphocytes which are lost from the spleen and sequestered in non-lymphoid organs, lymphocytes remaining in the spleen after THI treatment were characterized. The

results of this study confirmed that THI reduced B cells, CD4⁺ and CD8⁺ T cells and cells expressing CD62L, CD44 and IL-2R in the spleen.

The ability of THI to inhibit T-cell export from the thymus was examined in a study in BALB/c mice maintained on drinking water containing THI for 5 days (Gugasyan et al., 1998). The mice showed a two-fold increase in the total number of mature medullary thymocytes (CD4⁺CD8⁻ and CD4⁺CD8⁺) as well as a slight decrease in the total number of immature double-positive cells (CD4⁺CD8⁺). The mature single-positive thymocytes were found to express high levels of the homing molecule L-selectin, suggesting that these potential emigrants were prevented from leaving the thymus. To confirm this, THI-treated mice were injected intrathymically with fluorescein isothiocyanate and the number of labelled T cells appearing in the lymph nodes and spleen was determined 16 hours later. A 10-fold decrease in the number of CD4⁺ and CD8⁺ recent thymic emigrants in the lymph nodes and spleen of THI-treated mice was observed. Previous studies have shown that THI does not affect other aspects of thymocyte development, such as proliferation and differentiation. Taken together, the authors proposed that their results suggest that the immunosuppressive effects of THI may be due, in part, to preventing of the final step of T-cell export out of the thymus (Gugasyan et al., 1998). These authors also reported that continuous oral treatment of non-obese diabetic (NOD) mice with THI reduced the number of CD4⁺ and CD8⁺ T cells in the peripheral blood (Gugasyan et al., 1995). In the draining lymph nodes THI treatment had no effect on lymphocyte subsets prior to contact sensitization, but subsequent sensitization and elicitation with OX could not stimulate a significant increase in the number of CD4⁺ T cells in the treated mice, whereas untreated control mice showed elevated numbers of these lymphocytes. The authors suggested that THI can inhibit a contact hypersensitivity response by preventing the recruitment of CD4⁺ T cells in the draining lymph nodes through an unknown mechanism (Gugasyan et al., 1995). In a study by the same group, THI marginally delayed the development of adjuvant-induced arthritis (AA) in Lewis rats, a widely used model of chronic inflammatory arthritis. However, THI had no effect on the incidence or severity of disease, although it totally prevented granuloma formation in the spleen and associated splenomegaly. The authors concluded that THI may be a useful adjunctive agent for some inflammatory diseases (Gugasyan et al., 1997).

Schwab et al. (2005) have shown that in mice (age not indicated) receiving 1 mg/kg bw/day THI (50 mg/l drinking water) for 3 consecutive days, THI induced a marked decrease in the number of circulating lymphocytes and suppressed immunity. The mean THI plasma level was 135 ng/mL. The migration of lymphocytes from the thymus and peripheral lymphoid organs depends on their membrane sphingosine 1-phosphate (S1P) receptor-1 and is thought to occur in response to elevated concentrations of circulatory S1P. S1P is a lysophospholipid that acts through G-protein-coupled receptors in the cell, and THI mediates the increase in S1P via inhibition of S1P lyase, the enzyme involved in degradation of S1P in the cell (Schwab et al., 2005). The authors have found that S1P levels in lymphoid tissues of mice are normally low but increase more than 100-fold after THI treatment. This treatment inhibited the S1P degrading enzyme S1P lyase, an intracellular degrading enzyme which is expressed by hematopoietic cells. Disruption of the S1P gradient caused mature T cells to remain stored in immune tissues such as the thymus and peripheral lymphoid organs and thus blocked lymphocyte trafficking.

D.2 Studies on 4-methylimidazole (4-MEI)

The toxicokinetics and metabolism of 4-MEI have been studied in the male F344 rat using ¹⁴C radiolabelled compound (Yuan and Burka, 1995). Radioactivity in plasma and urine was profiled by HPLC. After gavage administration of 50 mg/kg, about 85% of the administered radioactivity was recovered in urine within 48 hour. The majority of the radioactivity in urine or plasma was associated with the parent compound and only one minor hydrophilic metabolite was present in urine and in plasma. Elimination of radioactivity via faecal, biliary or respiration was negligible. Elimination of 4-MEI after an i.v. dose of 5 mg/kg can be described by a two-compartment process with an estimated half-life of 1.8 hours and an estimated apparent volume of distribution of 2.3 litre/kg. After gavage at doses of 5, 50 and 150 mg/kg, 4-MEI was readily absorbed with a estimated bioavailability of 60-70%.

Urinary excretion data indicated that renal clearance of 4-MEI accounted for about 80% of total body plasma clearance. Based on the estimated AUC of metabolite and the estimated renal clearance of 4-MEI, the formation of metabolite and the renal clearance of 4-MEI appeared to be a saturable process (Yuan and Burka, 1995).

Imidazole, 1-methylimidazole, 4-MEI, and ketoconazole, an oral imidazole antifungal agent, caused dose-dependent decreases in testosterone secretion and Testicular Interstitial Fluid (TIF) formation (Adams et al., 1998). Imidazole, 2-methylimidazole (2-MEI), and 4-MEI decreased Luteinizing Hormone (LH) secretion. 4-MEI decreased testosterone secretion 1-6 hours after injection, increased testosterone at 8-16 hours, decreased LH secretion at 4 hours, decreased TIF volumes at 1-8 hours, and slightly increased TIF volumes at 24 hour. 4-MEI blocked the stimulatory effects of human Chorionic Gonadotrophin (hCG) on testosterone secretion and prevented an expected increase in LH secretion after the 4-MEI-induced decrease in testosterone secretion. 4-MEI also reversed the effects of three other stimulants of testosterone secretion that presumably act through three different testicular regulatory systems: *N*-methyl-D,L-aspartate, an excitatory amino acid; NG-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor; and naltrexone, an opioid antagonist. The authors suggested that these results support the hypothesis that imidazoles inhibit testicular function and male reproductive function through inhibition of testosterone secretion, TIF formation, and LH secretion regulatory systems (Adams et al., 1998).

D.2.1 NTP studies on the toxicity and carcinogenicity of 4-MEI (text reproduced from NTP, 2004, and NTP 2007)

Groups of five male and five female rats and mice were fed diets containing 0, 300, 800, or 2500 mg/kg 4-MEI (equivalent to average daily doses of approximately 30, 80, or 220 mg/kg for rats and 65, 170, or 500 mg/kg for mice) for 15 days. All animals survived to the end of the studies, and there were no significant differences in mean body weights, clinical findings, organ weights, or gross or microscopic lesions between exposed and control groups (NTP, 2004).

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 625, 1250, 2500, 5000, or 10 000 mg/kg 2- or 4-MEI (equivalent to average daily doses of approximately 40, 80, 160, 300, or 560 mg/kg 2- or 4-MEI to rats; and 100, 165, 360, 780, or 1740 mg/kg 2-MEI or 100, 240, 440, 915, or 1840 mg/kg 4-MEI to male mice; and 90, 190, 400, 800, or 1860 mg/kg 2-MEI or 110, 240, 540, 1130, or 3180 mg/kg 4-MEI to female mice for 14 weeks. In the 14-week 4-MEI studies, one 10 000 mg/kg male mouse was found dead during week 4, and seven 10 000 mg/kg female mice were found dead during weeks 1 and 2. Mean body weights were significantly less than those of the controls for male rats exposed to 2500 mg/kg or greater, 5000 and 10 000 mg/kg female rats, male mice exposed to 1250 mg/kg or greater, and all exposed groups of female mice. Reduced feed consumption was observed in 5000 and 10 000 mg/kg male and female rats. Clinical findings included nasal/eye discharge, ruffled fur, thinness, ataxia, and abnormal breathing in rats, and ruffled fur and dull coats in female mice. On days 29 and 82, functional observations in 5000 and 10 000 mg/kg rats included labored or increased respiration, mild tremors, walking on tiptoes, hunched posture, piloerection, crouching over, impaired coordination of movement, ataxia, and pupillary constriction. 4-MEI induced a transient erythrocytosis and a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anaemia in male and female rats. Clinical chemistry evaluations generally showed a cholestatic effect in exposed male and female rats. At week 14, there was a significant decrease in total protein and albumin concentrations of female rats exposed to 5000 or 10 000 mg/kg (NTP, 2004).

In mice, 4-MEI induced a macrocytic, hyperchromic, responsive anemia and, particularly in males, increases in triiodothyronine concentrations and transient decreases in thyroxine concentrations. In the 4-MEI studies, the liver weights of male rats exposed to 2500 mg/kg or greater were significantly increased; spleen weights of female rats exposed to 2500 mg/kg or greater were decreased. The absolute liver weight was decreased in 10 000 mg/kg male mice, and relative weights were significantly increased in all exposed groups of mice. In female mice, there was a significant decrease in the absolute weights and increase in the relative weights of the heart, right kidney, and liver in

groups exposed to 2500 mg/kg or greater. The epididymal spermatozoal concentration was significantly increased in 5000 mg/kg male rats. Gross pathology observations included pale livers in male rats exposed to 2500 mg/kg or greater and small testes and uteri in 10 000 mg/kg male and female rats. Microscopic analysis identified significantly increased incidences of cytoplasmic hepatocyte vacuolization of the liver of male rats exposed to 2500 mg/kg or greater and 10 000 mg/kg female rats, hypospermia of the epididymis in 10 000 mg/kg male rats, atrophy and inflammation of the prostate gland in 10 000 mg/kg male rats, and degeneration of the testes in 5000 and 10 000 mg/kg male rats (NTP, 2004).

4-MEI was negative in the *S. typhimurium* mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 activation enzymes. *In vivo*, no consistent or significant increases in the frequencies of micronucleated erythrocytes were seen in the bone marrow of male rats or mice treated with 4-MEI by intraperitoneal injection, or in peripheral blood samples from male and female mice administered the compound in dosed feed for 14 weeks (NTP, 2007).

Groups of 50 male and 50 female rats were fed diets containing 0, 625, 1250, or 2500 mg/kg 4-MEI (males) or 0, 1250, 2500, or 5000 mg/kg 4-MEI (females) (equivalent to average daily doses of approximately 30, 55, or 115 mg 4-MEI/kg bw to males and 60, 120 or 260 mg 4-MEI/kg bw to females) for 106 weeks. Survival of all exposed groups of male and female rats was similar to that of the control groups. Mean body weights of males in the 1250 and 2500 mg/kg groups and females in the 2500 and 5000 mg/kg groups were less than those of the control groups throughout the study; mean body weights of 1250 mg/kg females were less after week 41. Feed consumption by 5000 mg/kg females was less than that by the controls. Clonic seizures, excitability, hyperactivity, and impaired gait were observed primarily in 2500 and 5000 mg/kg females. The incidence of mononuclear cell leukemia in 5000 mg/kg females was significantly greater than that in the controls, and the incidence exceeded the historical range in feed study controls. The incidences of hepatic histiocytosis, chronic inflammation, and focal fatty change were generally significantly increased in all exposed groups of male and female rats. The incidences of hepatocellular eosinophilic and mixed cell focus were significantly increased in 2500 mg/kg males and 5000 mg/kg females (NTP, 2007).

Groups of 50 male and 50 female mice were fed diets containing 0, 312, 625, or 1250 mg/kg 4-MEI (equivalent to average daily doses of approximately 40, 80, 170 mg 4-MEI/kg bw to males and females) for 106 weeks. Survival of all exposed groups of male and female mice was similar to that of the control groups. Mean body weights of males and females in the 1250 mg/kg groups were less than those of the control groups after weeks 17 and 12, respectively. Mean body weights of 312 and 625 mg/kg females were less after weeks 85 and 65, respectively. Feed consumption by exposed groups of male and female mice was generally similar to that by the controls. The incidences of alveolar/bronchiolar adenoma in all exposed groups of females, alveolar/bronchiolar carcinoma in 1250 mg/kg males, and alveolar/bronchiolar adenoma or carcinoma (combined) in 1250 mg/kg males and 625 and 1250 mg/kg females were significantly greater than those in the control groups. The incidence of alveolar epithelium hyperplasia was significantly increased in 1250 mg/kg females (NTP, 2007).

D.2.2 NTP conclusions on carcinogenicity of 4-MEI (NTP, 2007)

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of 4-MEI in male F344/N rats exposed to 625, 1250, or 2500 mg/kg. There was *equivocal evidence of carcinogenic activity* of 4-MEI in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of 4-MEI in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to 4-MEI resulted in non-neoplastic lesions in the liver of male and female rats and the lung of female mice and in clinical findings of neurotoxicity in female rats.

GLOSSARY AND/OR ABBREVIATIONS

ADI	Acceptable Daily Intake
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
APTT	Activated Partial Thromboplastin Time
BIBRA	British Industrial Biological Research Association
CAS	Chemical Abstracts Service
CE	Capillary Electrophoresis
CEF	EFSA Scientific Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary
CIAA	Confederation of the Food and Drink Industries of the EU
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
CP	Cyclophosphamide
CPY	Cytochrome P450
DB	Double positive
DBTC	Di-n-butyltin dichloride
DEAE	Diethylamino Ethyl
EC	European Commission
EFSA	European Food Safety Authority
EUTECA	European Technical Committee on Caramels
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FDA	Food and Drug Administration
GC	Gas chromatography
GS/MS	Gas Chromatography/Mass Spectrometry
HMCF	High Molecular weight Colour Fraction
HMF	5-hydroxymethyl-2-furfural
HPLC	High-performance liquid chromatography
IC ₅₀	50% cell growth inhibition concentration

IL-2	Interleukin-2
ITCA	International Technical Caramel Association
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KLH	Keyhole limpet haemocyanin
LD ₅₀	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
LGL	Large granular lymphocytes
LGL	Large granular lymphocytes
LH	Luteinizing Hormone
LMW	Low Molecular Weight
LOAEL	Low-Observed-Adverse-Effect-Level
MEI	Methylimidazole
MW	Molecular Weight
NCE/PCE	Normochromatic erythrocytes/Polychromatic erythrocytes
NK	Natural killer
ML	Maximum level
MLR	Mixed lymphocyte reaction
NOD	Non-Obese Diabetic
MRL	Maximum Reported Levels
MS	Mass spectrometry
NATCOL	Natural Food Colours Association
NOAEL	No-Observed-Adverse-Effect-Level
NTP	National Toxicology Programme
OECD	Organisation for Economic Co-operation and Development
PAPS	3'-phosphoadenosine-5'-phosphosulphate
PCR	Polymerase Chain Reaction
SCF	Scientific Committee on Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
SFFCC	Syndicat des Fabricants Français de Caramels Colorants
SMF	5-sulphoxymethyl-2-furfural

SP	Single positive
SP1	Sphingosine 1-phosphate
SULT	Sulphotransferase
UNEP	United Nations Environment Programme
UNESDA	Union of European Beverage Associations
TemaNord	Nordic Council of Ministers
THI	2-acetyl-4-tetrahydroxy-butylimidazole
TIF	Testicular Interstitial Fluid
WHO/FAO	World Health Organization/Food and Agriculture Organization

STATEMENT OF EFSA

Refined exposure assessment for caramel colours (E 150a, c, d)¹

European Food Safety Authority^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This EFSA statement is a refined exposure assessment of caramel colours (E 150a, E 150c and E 150d) taking into account additional information on its use in foods as consumed. The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted a scientific opinion on the re-evaluation of caramel colours (E 150a, E 150b, E 150c, E 150d) used as food additives in 2011. In that opinion, the Panel concluded that the anticipated dietary exposure of child and adult populations may exceed the Acceptable Daily Intake (ADI) for caramel colours E 150a, E 150c and E 150d, but exposure estimates to E 150b were below the ADI. Following this conclusion, new data from industry were submitted to EFSA providing updated use levels of caramel colours in foods ready to be consumed. A refined exposure assessment was performed for caramel colours that were exceeding the ADI in ANS Panel opinion (EFSA, 2011a) i.e. E 150a, E 150c and E 150d and concluded that the anticipated dietary exposure are considerably lower than in the previous exposure assessment. However, toddlers and adults have a higher exposure to caramel colour E 150c and could still exceed the ADI of 100 mg/kg bw/day for this caramel colour. The estimated combined exposure to the four caramel colours (E 150a, E 150b, E 150c, E 150d) are considerably lower and the group ADI of 300 mg/kg bw/day is not exceeded for any population group in the current review.

© European Food Safety Authority, 2012

KEY WORDS

Caramel colours, E 150a, E 150c, E 150d, exposure, EFSA Comprehensive European Food Consumption Database, food colours.

¹ On request from EFSA, Question No EFSA-Q- 2012-00742, approved on 3 December 2012.

² Correspondence: ans@efsa.europa.eu

³ Acknowledgement: EFSA wishes to thank the members of the Working Group on Exposure Assessment: Martine Bakker, Jürgen Koënik, Jean-Charles Leblanc, Oliver Lindtner, Christina Tlustos for the preparatory work on this scientific output and EFSA staff: Davide Arcella and Alexandra Tard for the support provided to this scientific output.

Suggested citation: European Food Safety Authority; Refined exposure assessment for caramel colours (E 150a, c, d). EFSA Journal 2012;10(12):3030. [39 pp.] doi:10.2903/j.efsa.2012.3030. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

This EFSA statement is a refined exposure assessment of caramel colours (E 150a, E 150c and E 150d) taking into account additional information on its use in foods as consumed.

Caramel colours are colouring substances authorised as food additives in the European Union (EU) for use in a number of foods according to the Commission Regulation (EU) N° 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) N°1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. The caramel colours are divided into four classes, Class I Plain caramel (E 150a), Class II Caustic sulphite caramel (E 150b), Class III Ammonia caramel (E 150c), and Class IV Sulphite ammonia caramel (E 150d), according to the reactants used in their manufacture. The four classes of caramel colours have been previously evaluated by the EU Scientific Committee for Food (SCF, 1975, 1984, 1989, 1997) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1970, 1972a, 1972b, 1978, 1986, 1987, 2001). The Nordic Council of Ministers has reviewed the caramel colours in a report which took into account the literature published on these substances up to the year 2000 (TemaNord, 2002).

In 2011, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted a scientific opinion on the re-evaluation of caramel colours (E 150a, E 150b, E 150c, E 150d) as food additives. The ANS Panel established a group ADI of 300 mg/kg body weight/day for four caramel colours (E 150a, E 150b, E 150c, E 150d) and an individual ADI of 100 mg/kg bw/day for E 150c (Class III Ammonia Caramel) due to immunotoxicity of one of its constituent. The ANS Panel concluded that, based on the data available, it was possible that the use of caramel colours in foods may lead to exposures in excess of the Acceptable Daily Intakes (ADIs): caramel colours E 150a and E 150d may exceed the group ADI of 300 mg/kg bw/day and E 150c may exceed the individual ADI of 100 mg/kg bw/day. On the opposite, caramel colour E 150b exposure estimates were below the group ADI of 300 mg/kg bw/day. The ANS Panel further noted that depending on the caramel colours, the main contributors to children's anticipated exposures were non-alcoholic beverages and within this category, flavoured drinks, fine bakery wares and desserts, including flavoured milk products; for adults, the main contributors were non-alcoholic beverages, beer and cider and confectionary. The ANS Panel also noted that the anticipated combined dietary exposure of both children and adults to all four caramel colours exceeded the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile, while the ADI is also exceeded by the combined mean exposure for children. In the case of children, this exceedance applied to the upper end of the exposure range only.

In 2012, data on the actual uses of caramel colours in foods as consumed were submitted to EFSA by industry. The present review provides a refined exposure assessment for caramel colours E 150a, E 150c, E 150d, based on these new data and consumption data available in the EFSA Comprehensive European Food Consumption Database.

Given that the anticipated exposure to caramel colour E 150b was below the group ADI of 300 mg/kg bw/day established by the ANS Panel in 2011 (EFSA, 2011a), the present statement does not include a refined dietary exposure for caramel colour E 150b.

EFSA concluded that in Europe the mean dietary exposures to caramel colour E 150a for toddlers (12-35 months old), children (3-9 years old) and adolescents (10-17 years old) range from 11 to 45 mg/kg bw/day, 15 to 39 mg/kg bw/day and 6 to 24 mg/kg bw/day, respectively. For caramel colour E 150c, the same population group exposures range from 10 to 60 mg/kg bw/day, 20 to 56 mg/kg bw/day and 9 to 33 mg/kg bw/day at the mean. For caramel colour E 150d, the mean dietary exposures for toddlers, children and adolescents range from 11 to 86 mg/kg bw/day, 19 to 62 mg/kg bw/day and 8 to 44 mg/kg bw/day, respectively.

At the 95th percentile, caramel colour E 150a exposure estimates range from 32 to 79 mg/kg bw/day for toddlers, from 36 to 81 mg/kg bw/day for children and from 16 to 56 mg/kg bw/day for

adolescents. For caramel colour E 150c, the anticipated high level exposures for toddlers, children and adolescents range from 36 to 106 mg/kg bw/day, from 43 to 97 mg/kg bw/day and from 20 to 86 mg/kg bw/day, respectively. The anticipated high level exposures for caramel colour E 150d range from 38 to 127 mg/kg bw/day for toddlers, from 49 to 136 mg/kg bw/day for children and from 21 to 122 mg/kg bw/day for adolescents.

For adults (18-64 years old) and the elderly (≥ 65 years old), the anticipated dietary exposure for caramel colour E 150a gives a mean range through all European countries, of respectively 6 to 18 mg/kg bw/day and 4 to 12 mg/kg bw/day; and of 16 to 42 mg/kg bw/day and 13 to 32 mg/kg bw/day at the 95th percentile. For caramel colour E 150c, the mean ranges from 10 to 43 mg/kg bw/day for adults and from 6 to 30 mg/kg bw/day for the elderly, with the 95th percentiles ranging from 27 to 151 mg/kg bw/day and from 14 to 82 mg/kg bw/day. For caramel colour E 150d, the mean ranges from 9 to 36 mg/kg bw/day for adults and from 6 to 22 mg/kg bw/day for the elderly; the 95th percentiles range from 26 to 101 mg/kg bw/day for adults and from 19 to 60 mg/kg bw/day for the elderly.

On the basis of the above estimates, high levels of dietary exposure to caramel colour E 150c for toddlers and adults could exceed the ADI of 100 mg/kg bw/day, respectively in one and five countries.

The anticipated combined exposures to caramel colours E 150a, E 150b, E 150c and E 150d give a mean range through all European countries, of respectively 19 to 105 mg/kg bw/day, 31 to 83 mg/kg bw/day, 12 to 56 mg/kg bw/day, 15 to 57 mg/kg bw/day, 9 to 35 mg/kg bw/day respectively for toddlers, children, adolescents, adults and the elderly. At the high levels, the estimated combined exposures range from 73 to 158 mg/kg bw/day, 68 to 160 mg/kg bw/day, 28 to 144 mg/kg bw/day, 41 to 161 mg/kg bw/day, 24 to 87 mg/kg bw/day for the same population groups. Thus, the estimated combined exposure to all four caramel colours is below the group ADI of 300 mg/kg bw/day for all population groups both at the mean and at the high level of exposure.

The results of the current exposure assessment for caramel colours E 150a, E 150c and E 150d and combined exposure to the four caramel colours are considerably lower than those of the original opinion (EFSA, 2011a).

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	4
Background as provided by EFSA	5
Terms of reference as provided by EFSA	5
Assessment	6
1. Introduction	6
2. Uses and use levels of caramel colours	7
2.1. Maximum Permitted Levels of use	8
2.2. Reported use levels of caramel colours	11
3. Food consumption	12
3.1. EFSA's Comprehensive European Food Consumption Database	12
3.2. Food items selected for the refined exposure assessment of caramel colours	13
4. Dietary exposure assessment	15
4.1. Exposure to caramel colours from their use as food additives	16
4.2. Main food categories contributing to exposure of caramel colours	17
4.3. Combined refined exposure to caramel colours	19
5. Results and discussion	21
6. Uncertainty analysis	24
Conclusions	25
Documentation provided to EFSA	27
References	27
Appendices	29
A. Rules defined by the Panel to deal with quantum satis (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated	29
B. Summary of use levels (mg/kg) of caramel colours (E 150a, E 150c and E 150d) used in the refined exposure assessment	31
C. Summary of total estimated exposure (using MPLs and use levels) per age class and survey*: mean and high level (mg/kg bw/day)	36
Glossary and abbreviations	39

BACKGROUND AS PROVIDED BY EFSA

In its letter of 26 May 2011 to the European Food Safety Authority (EFSA), the European Commission requested clarification on the outcomes of the exposure calculations undertaken by the ANS Panel in the opinions on the so-called Southampton colours (quinoline yellow⁴, sunset yellow⁵, ponceau 4R⁶). The Member States and stakeholders had informed the European Commission that the figures used in these exposure assessments required possibly some updating.

On 1 August 2011, EFSA responded by a letter indicating that following the discussions which took place on 27 May 2011 between EFSA, the Commission, and Member States representatives, where the possibility to make refined exposure assessments in the future was discussed, further exchanges between the Commission and EFSA have shown an interest for performing such refined assessments.

Once the necessary preparatory work to enable the realisation of the foreseen refined exposure assessments e.g. the establishment of a correspondence table between the food classification system (FCS) of the new European legislation (Regulation (EU) N° 1129/2012⁷) and of the EFSA Comprehensive food consumption database (FoodEx), had been finalised, by its letter of 26 April 2012, EFSA has requested information on the priorities set by the Commission.

On 23 May 2012, the European Commission sent a letter to EFSA setting the priorities for the refined exposure assessments of twelve food colours (Priority 1: caramel colours (E 150a, E 150c and E 150d); Priority 2: curcumin (E 100), amaranth (E 123), brown HT (E 155); Priority 3: azorubine/carmoisine (E 122), allura red AC (E 129), brilliant black BN (E 151); Priority 4: quinoline yellow (E 104), sunset yellow (E 110), ponceau 4R (E 124)) and indicating that revised data on use and use levels for food colours under priorities 2 and 3 were currently being collected by FoodDrinkEurope and should be provided to EFSA once they were available. Similar revised use data for the caramel colours (E 150a, E 150c and E 150d) have been provided by the Commission to EFSA.

TERMS OF REFERENCE AS PROVIDED BY EFSA

EFSA is to provide refined exposure assessments for food colours already re-evaluated taking into account the restrictions/exceptions listed in Regulation (EU) N° 1129/2011, especially in the case of main contributors.

Furthermore, it is requested that following the establishment of a correspondence table between the food classification system of Regulation (EU) N° 1129/2011 and of the EFSA Comprehensive food consumption database (FoodEx), EFSA will use the FoodEx system in order to provide refined exposure assessments and exclude non relevant food subgroups from the intake calculations. The list of priorities, as provided by the European Commission, is set as follows:

Priority 1 - caramel colours (E 150a, E 150c, E 150d)

Priority 2 - curcumin (E 100), amaranth (E 123), brown HT (E 155)

Priority 3 - azorubine/carmoisine (E 122), allura red AC (E 129), brilliant black BN (E 151)

Priority 4 - quinoline yellow (E 104), sunset yellow (E 110), ponceau 4R (E 124)

⁴ EFSA, 2009. Scientific Opinion on the re-evaluation of Quinoline Yellow (E 104) as a food additive, ON-1329.

⁵ EFSA, 2009. Scientific Opinion on the re-evaluation of Sunset Yellow FCF (E 110) as a food additive, ON-1330.

⁶ EFSA, 2009. Scientific Opinion on the re-evaluation of Ponceau 4R (E 124) as a food additive, ON-1328.

⁷ OJ L 295, 12.11.2011, p.1.

ASSESSMENT

1. Introduction

Caramel colours are colouring substances authorised as food additives in the EU. The caramel colours are divided into four classes, Class I Plain caramel (E 150a), Class II Caustic sulphite caramel (E 150b), Class III Ammonia caramel (E 150c), and Class IV Sulphite ammonia caramel (E 150d), according to the reactants used in their manufacture. The four classes of caramel colours have been previously evaluated by the EU Scientific Committee for Food (SCF, 1975, 1984, 1989, 1997) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1970, 1972a, 1972b, 1978, 1986, 1987, 2001). The Nordic Council of Ministers has reviewed the caramel colours in a report which took into account the literature published on these substances up to the year 2000 (TemaNord, 2002).

In 2011 the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) has re-evaluated caramel colours (E 150a, E 150b, E 150c and E 150d) as food additives (EFSA, 2011a). The safety in use of caramel colours was assessed on the basis of the uses and use levels authorised in the legislation⁸ and the reported use levels, as provided by industry.

The ANS Panel established a group ADI of 300 mg/kg bw/day for four caramel colours (E 150a, E 150b, E 150c, E 150d) and within this group ADI, established an individual ADI of 100 mg/kg bw/day for caramel colour E 150c due to concern regarding the immunotoxicity of a constituent of this caramel colour (2-acetyl-4-tetrahydroxybutylimidazole (THI), found in E 150c only).

The ANS Panel noted that the anticipated dietary exposure of the adult population at the 97.5th percentile to caramel colour E 150a exceeds the group ADI of 300 mg/kg bw/day proposed for the caramel colours, with a 97.5th percentile exposure estimate of 429 mg/kg bw/day. Similarly, the anticipated dietary exposure of the adult population at the 97.5th percentile to caramel colour E 150d exceeds this group ADI (high level exposure estimate of 369 mg/kg bw/day). For children, the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for caramel colour E 150a exceed the group ADI of 300 mg/kg bw/day (exposures range respectively from 77 to 427 mg/kg bw/day at the mean and from 180 to 882 mg/kg bw/day at the 95/97.5th percentile). Similarly, for children, the upper end of both the mean intake ranges and also the 95th/97.5th percentile intakes for caramel colour E 150d exceed the group ADI of 300 mg/kg bw/day (exposures range respectively from 23 to 506 mg/kg bw/day at the mean and from 130 to 1480 mg/kg bw/day at the 95/97.5th percentile).

The main contributors to the total anticipated exposure to caramel colour E 150a were non-alcoholic flavoured drinks both for adults and children. For children, fine bakery wares, desserts including flavoured milk products and sauces, seasoning and pickles were also main contributing food categories. For adults, beer and cider and soups were also main contributors. The two main contributors to the anticipated exposures of children and adults to caramel colour E 150d were soft drinks and confectionary.

⁸ Commission Regulation (EU) N° 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) N° 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011, p. 1–177

Table 1: Overview of exceedance of group or individual ADI in previous exposure assessment of caramel colours (EFSA, 2011a)

Caramel colour	Children		Adults	
	Mean	High level	Mean	High level
E 150a	> group ADI	> group ADI	> group ADI	> group ADI
E 150b	< group ADI	< group ADI	< group ADI	< group ADI
E 150c	> individual ADI	> individual ADI	> individual ADI	> individual ADI
E 150d	> group ADI	> group ADI	> group ADI	> group ADI
Combined exposure	> group ADI	> group ADI	> group ADI	> group ADI

The anticipated dietary exposure to caramel colour E 150b for both adults and children was below the group ADI of 300 mg/kg bw/day: from 22 to 110 mg/kg bw/day for adults respectively at the mean and high level and between 9 to 35 mg/kg bw/day at the mean and 18 to 117 mg/kg bw/day at high level for children.

For caramel colour E 150c, the upper end of the mean intake range for children exceeds the individual ADI of 100 mg/kg bw/day established for this colour within the group ADI (estimated mean range between 22 and 302 mg/kg bw/day), while the 97.5th percentile anticipated dietary exposures of both the child and adult populations are above this ADI of 100 mg/kg bw/day (high level exposure respectively range from 108 to 757 mg/kg bw/day for children and estimated at 295 mg/kg bw/day for adults). The food groups contributing the most to the anticipated exposure for children were fine bakery wares, desserts and sauces. For adults, food groups contributing most were beer and cider and sauces, seasoning and pickles.

Anticipated combined dietary exposures of both adults and children to all caramel colours exceed the group ADI of 300 mg/kg bw/day at the 95th/97.5th percentile (respectively of 474 mg/kg bw/day for adults and between 225 and 1672 mg/kg bw/day for children), while the ADI is also exceeded by the combined mean intake for children. In the case of children, this exceedance applies to the upper end of the exposure range only (range from 84 to 698 mg/kg bw/day).

The aim of the present statement is to provide refined exposure assessments for the caramels colours E 150a, E 150c and E 150d, from their uses as food colouring substances, taking into consideration updated information provided by industry in June, September and October 2012 on use levels in foods ready to be consumed.

Given that the anticipated exposure to caramel colour E 150b was below the group ADI of 300 mg/kg bw/day established by the ANS Panel in 2011 (EFSA, 2011a), the present statement does not include a refined dietary exposure for caramel colour E 150b.

Information on the minor constituents of caramel colours (4-MEI, THI, and sulphur dioxide) was presented by the ANS Panel previously (EFSA, 2011a) and considered that the outcome of the exposure estimates for these three minor constituents of caramel colours did not give rise to public health concern and that maximum levels have been established for THI, 4-MEI and sulphur dioxide in the specifications of the relevant caramel colours (Commission Regulation (EU) N° 231/2012⁹) the present statement does not present any new exposure estimates for these constituents.

2. Uses and use levels of caramel colours

The main use of the caramel colours is to impart a brown colour and associated characteristic caramel flavour to the foods to which they are added. They are used in a wide range of foodstuffs including

⁹ Commission Regulation (EU) N° 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295

non-alcoholic flavoured drinks (especially cola-type), alcoholic drinks such as beer and cider, spirits (e.g. whiskey), cereals and bakery wares, soups, sauces and gravies, decorations and coatings, confectionary, meat and fish analogues.

In addition, caramel colours are used in cosmetics and pharmaceutical products as well as in feed.

2.1. Maximum Permitted Levels of use

Maximum Permitted Levels (MPLs) of use for the caramel colours have been defined in the Commission Regulation (EU) N° 1129/2011¹⁰ on food additives for use in foodstuffs.

Caramel colours are authorised as food colours at quantum satis¹¹ (QS) level in the EU. In addition, caramel colours are included in the Group II of food additives authorised for use at QS, as defined in the Commission Regulation (EU) N° 1129/2011 and may therefore also be used in the food categories in which Group II food additives are allowed.

Table 2 summarises the food categories that are permitted to contain caramel colours in accordance to Commission Regulation (EU) N° 1129/2011.

Table 2: Food categories in which caramel colours (E 150a, E 150b, E 150c, E 150d) are authorised to be used as food additives at QS level according to the Commission Regulation (EU) N° 1129/2011

Category No	Food categories	E Number/ Group	Name/ Group name	Restrictions/exception
1.4	Flavoured fermented milk products including heat treated products	Group II	Colours	
1.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	Colours	except unflavoured products
1.6.3	Other creams	Group II	Colours	only flavoured creams
1.7.1	Unripened cheese excluding products falling in category 16	Group II	Colours	only flavoured unripened cheese
1.7.3	Edible cheese rind	Group II	Colours	
1.7.4	Whey cheese	Group II	Colours	
1.7.5	Processed cheese	Group II	Colours	only flavoured processed cheese
1.7.6	Cheese products (excluding products falling in category 16)	Group II	Colours	only flavoured unripened products
1.8	Dairy analogues, including beverage whiteners	Group II	Colours	
3.	Edible ices	Group II	Colours	
4.2.1	Dried fruit and vegetables	E 150a/b/c/d	Caramels	only preserves of red fruit
4.2.2	Fruit and vegetables in vinegar, oil, or brine	E 150a/b/c/d	Caramels	only preserves of red fruit
4.2.2	Fruit and vegetables in vinegar, oil, or brine	E 150a/b/c/d	Caramels	only vegetables (excluding olives)
4.2.3	Canned or bottled fruit and vegetables	E 150a/b/c/d	Caramels	only preserves of red fruit
4.2.4.1	Fruit and vegetable preparations excluding compote	Group II	Colours	only mostarda di frutta

¹⁰ Commission Regulation (EU) N° 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) N°1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. . OJ L 295, 12.11.2011, p. 1–177. The Panel noted that the Commission Regulation (EC) N° 1129/2011 of 11 November 2011 will enter into force on June, 1st 2013 but confirms the approved uses of caramels as food additives as described in Council Directive N° 94/36/EC of 30 June 1994 on colours for use in foodstuffs.

¹¹ According to the Regulation (EC) N°1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (OJ L 354, 31.12.2008, p. 16–33), ‘quantum satis’ shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled.

Category No	Food categories	E Number/ Group	Name/ Group name	Restrictions/exception
4.2.4.1	Fruit and vegetable preparations excluding compote	E 150 a/b/c/d	Caramels	only preserves of red fruit
4.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	E 150a/b/c/d	Caramels	except chestnut puree
4.2.5.3	Other similar fruit or vegetable spreads	Group II	Colours	except <i>crème de pruneaux</i>
5.2	Other confectionery including breath freshening microsweets	Group II	Colours	
5.3	Chewing gum	Group II	Colours	
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Group II	Colours	
6.3	Breakfast cereals	Group II	Colours	only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals
6.3	Breakfast cereals	E 150c	Ammonia caramel	only extruded puffed and or fruit flavoured breakfast cereals
6.5	Noodles	Group II	Colours	
6.6	Batters	Group II	Colours	
6.7	Pre-cooked or processed cereals	Group II	Colours	
7.1.	Bread and rolls	E 150a/b/c/d	Caramels	only Malt bread
7.2	Fine bakery wares	Group II	Colours	
8.1.2	Meat preparations as defined by Regulation (EC) No 853/2004	E 150a/b/c/d	Caramels	only <i>breakfast sausages</i> with a minimum cereal content of 6 % and <i>burger meat</i> with a minimum vegetable and/or cereal content of 4 % mixed within the meat; In these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance
8.2.1	Non heat treated processed meat	E 150a/b/c/d	Caramels	only sausages
8.2.2	Heat treated processed meat	E 150a/b/c/d	Caramels	only sausages, patés and terrines
8.2.3	Casings and coatings and decorations for meat	Group II	Colours	except edible external coating of pasturmas
9.2.	Processed fish and fishery products including molluscs and crustaceans	Group II	Colours	only surimi and similar products and salmon substitutes.
9.2.	Processed fish and fishery products including molluscs and crustaceans	E 150a/b/c/d	Caramels	only fish paste and crustacean paste
9.2.	Processed fish and fishery products including molluscs and crustaceans	E 150a/b/c/d	Caramels	only precooked crustacean
9.3	Fish roe	Group II	Colours	except Sturgeons' eggs (Caviar)
12.2.2	Seasonings and condiments	Group II	Colours	only seasonings, for example curry powder, tandoori
12.3	Vinegars	E 150a/b/c/d	Caramels	
12.4	Mustard	Group II	Colours	
12.5	Soups and broths	Group II	Colours	
12.6	Sauces	Group II	Colours	excluding tomato-based sauces
12.7	Salads and savoury based sandwich spreads	Group II	Colours	
12.9	Protein products, excluding products covered in category 1.8	Group II	Colours	

Category No	Food categories	E Number/ Group	Name/ Group name	Restrictions/exception
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II	Colours	products in this category can also use additives that are allowed in the corresponding food counterparts categories
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II	Colours	
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009	Group II	Colours	products in this category can also use additives that are allowed in the corresponding food counterparts categories. In addition, all additives in the gluten containing counterparts are authorised.
14.1.4	Flavoured drinks	Group II	Colours	excluding chocolate milk; malt products
14.2.1	Beer and malt beverages	E 150a/b/c/d	Caramels	only beer
14.2.3	Cider and perry	Group II	Colours	excluding <i>cidre bouché</i>
14.2.3	Cider and perry	E 150a/b/c/d	Caramels	only <i>cidre bouché</i>
14.2.4	Fruit wine and made wine	Group II	Colours	
14.2.5	Mead	Group II	Colours	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	Colours	except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà.
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	E 150a/b/c/d	Caramels	except: fruit spirits, spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà. Whisky, whiskey can only contain E 150a.
14.2.7.1	Aromatised wines	Group II	Colours	except <i>americano</i> , <i>bitter vino</i>
14.2.7.1	Aromatised wines	E 150a/b/c/d	Caramels	
14.2.7.1	Aromatised wines	E 150a/b/c/d	Caramels	only <i>americano</i> , <i>bitter vino</i>
14.2.7.2	Aromatised wine-based drinks	Group II	Colours	except <i>bitter soda</i> , <i>sangria</i> , <i>claria</i> , <i>zurra</i>
14.2.7.2	Aromatised wine-based drinks	E 150a/b/c/d	Caramels	only <i>bitter soda</i>
14.2.7.3	Aromatised wine-product cocktails	Group II	Colours	
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group II	Colours	
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II	Colours	
15.2	Processed nuts	Group II	Colours	
16	Desserts excluding products covered in category 1, 3 and 4	Group II	Colours	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	Group II	Colours	

Category No	Food categories	E Number/ Group	Name/ Group name	Restrictions/exception
17.2	Food supplements supplied in a liquid form	Group II	Colours	
17.3	Food supplements supplied in a syrup-type or chewable form	Group II	Colours	

2.2. Reported use levels of caramel colours

In the EU most food additives are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. For those additives where no MPL is set and which are authorised at QS, information on actual use levels is required. When no use levels are made available, the ANS Panel defined some rules to deal with QS (Appendix A, Figure 2). As a first step, it implies using the levels proposed by the Codex Alimentarius in its General Standard for Food Additives database (GSFA database). This database provides MPLs for caramel colours E 150c and E 150d; no maximum levels are set forth for caramel colour E 150a. It has to be mentioned that in the previous ANS Panel opinion (EFSA, 2011a), MPLs from the GSFA database were not considered. However, to re-evaluate food additives authorised at QS, it appeared that other data from regulatory sources should be used. Therefore, the levels of caramel colours used for the current exposure estimates could be higher than the ones used in the previous ANS Panel opinion (EFSA, 2011a) because coming from Codex's MPLs: e.g. for aromatised wines, food supplements, mustard. The second step is to use available values for the same food categories among other colours which are authorised in Commission Regulation (EU) N° 1129/2011.

Updated information on the actual use levels of the caramel colours in foods was made available to EFSA for several food categories of finished products by FoodDrinkEurope and the European Technical Caramel Association (EUTECA) (Appendix B). No analytical data from other sources (Member States, scientific literature) were collected. Appendix B also shows the levels used for the refined exposure assessment identified by EFSA using rules defined by the ANS Panel, based either on the data provided by industry or from the rules set to deal with QS authorisation, as indicated in Appendix A (Figures 1 and 2).

Summarised data on reported use levels of caramel colours in foods from industries

The data provided cover the main food categories in which caramel colours are authorised to be added: both non-alcoholic (e.g. flavoured drinks) and alcoholic beverages (e.g. beer), sauces and seasonings, confectionary, bakery wares and breakfast cereals, dairy products, snacks and desserts. Some clarifications with the data provider took place in September and October 2012 in order to ensure that the data were correctly used within the food additive nomenclature.

Unlike the previous data used in the 2011 ANS Panel opinion, information provided by industry is presented as “gathered from industries operating at the end of the supply chain”. Therefore the levels should reflect the actual levels of use in food products as consumed. For most of the food categories, two use levels were provided: a typical or usual use level and an extreme use level. The present exposure assessment follows a conservative approach, using the extreme reported use levels for the calculations. Some information could also be gained from the data provided relating to how representative some use levels were for the European market or with regards to the specific foods in which these use levels actually apply. Thus, extreme use levels reported for niche products (e.g. very specific products eaten in one country) were not taken into account in the exposure estimates.

For some food categories, no use levels were provided by industry i.e. for the three caramel colours E 150a, E 150c and E 150d for several fruit and vegetables categories (dried, in oil or brine, canned or bottled, preparations excluding compote), chewing gum, batters, processed cereals, fruit wine and made wine, mead, aromatised wines and aromatised wine-based drinks, aromatised wine-product cocktails and other alcoholic drinks incl. mixtures of alcoholic drinks with non-alcoholic drinks and

spirits, food supplements. Furthermore, no data were provided for caramel colour E 150a for processed meat, processed fish, vinegars, cider and perry, for caramel colour E 150c for processed cheese, mustard, salads and savoury spreads, protein products, dietary foods for weight control, for caramel colour E 150d for processed cheeses, noodles, vinegars, mustard, soups and broths, protein products, dietary foods for weight control, other alcoholic drinks and desserts.

For some of the products above mentioned, the MPLs from the Codex Alimentarius were considered too high due to technological issues which according to the industry, may include stability challenges (due to the interaction of caramel colours with milk proteins, or to their interaction with the salt in the food matrix, or for caramel colour E 150c to interaction in alcoholic drinks with the product due to its colloidal charge under acidic conditions) or because a high level of caramel colours in the food product would render its colour not acceptable (e.g. black/opaque). Therefore, EFSA used the levels provided for other caramel colours for the same food category as a conservative approach. This applies to the following food groups: whey and processed cheese, noodles, soups and broths, salads and savoury based sandwich spreads, other alcoholic drinks and desserts.

Use levels for the remaining food categories (i.e. aromatised wines, some fruit and vegetable products, meat and fish preparations, food supplements, mustard and vinegars) followed the rules defined by the ANS Panel as described above.

For some food categories, the use levels currently provided are far lower than the ones used in the previous exposure estimate (EFSA, 2011a). One of the major changes in the use levels is for seasonings and condiments, soups, sauces and vinegars, where the use levels provided in 2012 are lower by a factor of 5 to 200 according to the caramel colour and the food group. For the flavoured fermented milk products for the three caramel colours: the actual use levels are lower by a factor of 20 to 35. Lower use levels were also provided for bakery wares and breakfast cereals (by a factor of 2 to 10). Use levels of caramel colours E 150 a and E 150c provided for flavoured drinks were also lower by a factor of 2 to 7, and different use levels were provided for the caramel colours E 150d according to the kind of soft drinks (cola-type or not). Caramel colour E 150a use levels were lower than in the previous ANS Panel opinion for aromatised alcoholic drinks.

The use levels used in the previous ANS Panel opinion and in the present exposure estimate are presented in the Appendix B.

3. Food consumption

3.1. EFSA's Comprehensive European Food Consumption Database

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in each European country provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

Consumption records were codified according to the FoodEx classification system (EFSA, 2011c). Nomenclature from FoodEx classification system has been linked to the Food Classification System (FCS), as presented in the Commission Regulation (EU) N° 1129/2011, part D, to perform exposure estimates.

3.2. Food items selected for the refined exposure assessment of caramel colours

The food categories in which the use of caramel colours (E 150a, E 150c, E 150d) is authorised were selected from the nomenclature of the Comprehensive Database (FoodEx classification system codes), at a detailed level (up to FoodEx level 4) (EFSA, 2011b).

Some of the food categories in which the use of food colours is permitted at QS (Group II food additives) are very unlikely to contain caramel colours and therefore it was decided not to take them into account. Other food items were not referenced in the EFSA Comprehensive Database and therefore could not be taken into account in the present estimate. This results in an underestimation of the exposure estimates.

The food categories which were not taken into account in the present exposure assessment are described below (in ascending order of the Food Classification System codes):

- **1.5. Dehydrated milk as defined by Directive 2001/114/EC, except unflavoured products:** this food category corresponds to dried or condensed milk, products which are unlikely to contain colours such as caramel colours. Only unflavoured dehydrated milks are included in the corresponding category in FoodEx.
- **1.7.4. Whey cheese:** this food category corresponds to cheeses such as ricotta and mizithra which are unlikely to contain caramel colours.
- **1.7.6. Cheese products (excluding products falling in category 16), only flavoured unripened products:** all flavoured cheeses or analogues are gathered under "processed cheese" (category number 1.7.5), no food items correspond to this category in the FoodEx category.
- **4.2.4.1. Fruit and vegetable preparations excluding compote, only *mostarda di frutta*:** this item is not referenced in the FoodEx nomenclature, even at the original food name level. Therefore, it was not taken into account. Considering that this is a niche product, this leads to a minor underestimation of the total exposure.
- **5.4. Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4:** This category covers any confectionery product generally used for decorating and filling any foodstuff e.g. fine bakery wares, edible ices, candy and confections. This food category is not available in the FoodEx nomenclature, but foodstuffs that are likely to be filled or decorated (e.g. bakery wares) are included in the assessment.
- **6.6. Batters:** as for decorations and coatings, batters represent a part of a composite food and are not referenced in the FoodEx nomenclature. This category was not taken into account in the present estimates.
- **6.7. Pre-cooked or processed cereals:** this category covers pre-cooked and processed rice products, including rice cakes (Oriental type only) and other pre-cooked cereals such as quick-cook rice, *polenta* and *Semmelknödelteig*. No information on the processed status (cooked, treated) of cereals is available in the FoodEx nomenclature. Therefore, this food category was not taken into account in the present assessment.
- **7.1. Bread and rolls, only malt bread:** Malt bread is a niche product almost only consumed in UK. This food item is not referenced in the FoodEx nomenclature and is not taken into account in the present evaluation.
- **8.2.3. Casings and coatings and decorations for meat, except edible external coating of *pasturmas*:** as for decorations and coatings, these represent a part of composite foods (such as sausages, category number 8.2.1). Moreover, casings and coatings for meat are not referenced

in the FoodEx nomenclature. This category was not taken into account in the present estimates.

- **13.4. Foods suitable for people intolerant to gluten:** this category was not taken into account in this estimate. Since people who require such foods do not follow the same diet as the general population, exposure estimates specifically for people intolerant to gluten are not in the scope of the present statement.
- **14.2.4. Fruit wine and made wine:** this category includes wine made from fruits other than grapes and apples and from other agricultural products, including grains; it may be still or sparkling (e.g. rice wine, sake). No such category of wines is present in the FoodEx nomenclature.

Other limitations of the current exposure estimates, which are due to the linkage between the FoodEx classification system and the Food Classification System (Commission Regulation (EU) N° 1129/2011), are listed below (in ascending order of FCS codes). It results in overestimations, most often minor, of the exposure estimate:

- **1.7.3. Edible cheese rind:** no consumption data for cheese rind were available in the Comprehensive Database. However, in order to take this category into account, it was assumed that cheese rind corresponds to 10% of ripened cheese, and that consumers always eat the rind, assumptions which are conservative.
- **4.2.5.2. Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC, except chestnut purée, as well as 4.2.5.3. Other similar fruit or vegetable spreads, except crème de pruneaux:** the exception of the specific products chestnut purée or crème de pruneaux could not be taken into account, therefore all jam, jellies and marmalades or purée and other spreads made from fruits and vegetables were included in the present estimate.
- **8.1.2. Meat preparations, only breakfast sausages:** all sausages were included in the estimation taking as a reference the use level of non-heated treated processed meat (category number 8.2.1).
- **9.2. Processed fish and fishery products including molluscs and crustaceans, only surimi and similar products and salmon substitutes, only fish paste and crustacean paste:** all processed fish products (such as fish paste, fish fingers) were included in the present estimate.
- **9.2. Processed fish and fishery products including molluscs and crustaceans, only pre-cooked crustaceans:** since no information on the processing of food items is available within the Comprehensive Database, all crustaceans were included in the present estimate.
- **14.1.3. Cider and perry:** no distinction was possible between cider and *cidre bouché*, therefore, the category was accounted for as a whole.
- **14.1.4. Flavoured drinks, excluding chocolate milk, malt products:** One of the main uses of caramel colours is in non-alcoholic beverages (soft drinks). Within this food category, caramel colours are mostly used in cola-flavoured carbonated drinks. Caramel colours are also used at lower levels in some other soft drinks. With the help of ingredients databases (Irish¹² and German¹³ databases), a list of non cola-type soft drinks that may contain caramel colours (ginger-ale, ice-tea, energy-drinks, blackcurrant soft drinks) was made. The correspondent food items in the FoodEx nomenclature were selected. No blackcurrant flavour soft drink item

¹² Irish Universities Alliance (2008-2010) Irish National Food Ingredients Database Version 3 (NANS).

¹³ BfR. Database on food additives as labelled on foods reported in German consumption surveys for children. Database was developed by BfR subcontracting University of Paderborn. Publication is foreseen for 2013.

exists as such in the FoodEx nomenclature, but some could be classified under the parental food category as unclassified soft drinks, therefore all non-classified soft drinks were taken into account. Then, the use level specifically reported for cola-type drinks was applied to beverages classified as cola-type in the FoodEx nomenclature, whereas another use level reported for other type of soft drinks (lower than for cola-type drinks) was applied to the other selected drinks within the non-alcoholic beverages category. Chocolate milk, malt products were not taken into account.

- **14.2.5. Mead:** it is not referenced as such in the FoodEx nomenclature. Mead is included in the cider food item and was taken into account within this food item.
- **17.1/17.2/17.3. Food supplements:** no distinction between the forms of the food supplements is possible within the FoodEx nomenclature, therefore these three food categories were considered as a whole and the highest reported use levels were taken into account.

For food categories not mentioned above, foods from the Comprehensive Database were chosen in accordance with the legislation (Table 2).

4. Dietary exposure assessment

Dietary exposure to caramel colours from their use as food colours was estimated based on the consumption data available within the Comprehensive Database as presented in Section 3.2., and with the limitations described below.

For calculation of chronic exposure, intake statistics have been calculated based on individual consumption over the total survey period, excluding surveys with only one day per subject, considered as not adequate to assess repeated dietary exposure, as suggested by the EFSA Working group on Food Consumption and Exposure (EFSA, 2011b).

Chronic exposure to caramel colours (E 150a, E 150c and E 150d) was calculated for the following population groups: toddlers, children, adolescents, adults and the elderly. For the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries, as mentioned in Table 3.

Table 3: Population groups considered for the exposure estimates of caramel colours

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, Netherlands, Spain
Children ¹⁴	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly ¹⁴	Older than 65 years	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

¹⁴ The terms “children” and “the elderly” correspond respectively to “other children” and the merge of “elderly” and “very elderly” in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011b).

High level consumption was only calculated for those foods and population groups where the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011b). Therefore, in the present estimate, high levels for toddlers from Belgium, Italy and Spain were not included.

In summary, FoodEx food codes were matched to the food categories reported in Table 2 and subsequently each individual exposure was calculated by using the corresponding individual body weight and based on the assumption that caramel colours are present in the food at the levels shown in Appendix B. The average and high percentile exposures were calculated for the total population, for the five population groups described in Table 3.

4.1. Exposure to caramel colours from their use as food additives

Table 4 summarises the anticipated refined estimated exposure to caramel colours from their use as food additives for all five population groups.

Table 4: Summary of refined exposure to caramel colours (E 150a, E 150c, E 150d) in five population groups (mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (> 65 years)
Caramel colour E 150a					
• Mean	11-45	15-39	6-24	6-18	4-12
• High level (95 th percentile)	32-79	36-81	16-56	16-42	13-32
Caramel colour E 150c					
• Mean	10-60	20-56	9-33	10-43	6-30
• High level (95 th percentile)	36-106	43-97	20-86	27-151	14-82
Caramel colour E 150d					
• Mean	11-86	19-62	8-44	9-36	6-22
• High level (95 th percentile)	38-127	49-136	21-122	26-101	19-60

As a reminder, Table 5 presents the dietary exposures to caramel colours E 150a, E 150c and E 150d as estimated in ANS Panel opinion in 2011 for the two population groups: children and adults.

Table 5: Summary of anticipated exposure to caramel colours E 150a, E 150c and E 150d in children and adult populations as calculated in the previous ANS Panel opinion (EFSA, 2011a) (mg/kg bw/day)

	Children (1-14 years)	UK Adults (> 18 years)
Caramel colour E 150a		
• Mean	77-427	137
• High level (97.5 th percentile)	180-882	429
Caramel colour E 150c		
• Mean	22-302	61
• High level (97.5 th percentile)	108-757	295
Caramel colour E 150d		
• Mean	23-506	89
• High level (97.5 th percentile)	130-1480	369

4.2. Main food categories contributing to exposure of caramel colours

Table 6: Main food categories contributing to mean exposure to caramel colour E 150a (> 5% of total exposure) using reported use levels and number of surveys in which each food category is a main contributor.

Cat N°	Foods*	% contribution to total exposure (Number of Surveys)**				
		Toddlers	Children	Adolescents	Adults	The elderly
7	Bakery wares	5.4-67.3 (7)	5.7-63.9 (15)	4.8-52.6 (12)	11.0-42.4 (13)	5.6-41.8 (6)
12	Salts, spices, soups, sauces, salads and protein products	5.6-41.7 (6)	6.3-33.1 (14)	6.4-24.2 (10)	6.9-53.5 (14)	7.3-57.9 (7)
1	Dairy products and analogues	10.9-31.4 (6)	5.4-14.1 (8)	5.5-6.0 (2)	7.7 (1)	5.1 (1)
14.1	Non-alcoholic beverages	7.8-28.5 (3)	5.4-43.3 (15)	13.3-51.1 (12)	6.7-46.3 (15)	8.0 -39.2 (2)
6	Cereals and cereal products	5.8-8.6 (3)	5.3-12.3 (6)	11.1 (1)	7.8-9.4 (2)	10.5 (1)
16	Desserts excluding products covered in category 1, 3 and 4	6.5-23.2 (3)	6.9-14.5 (5)	5.1-7.9 (2)	5.8-5.9 (2)	7.2 (1)
15	Ready-to-eat savouries and snacks	6.1-8.4 (2)	5.0-6.3 (3)	5.2-9 (5)		
5.2	Other confectionery including breath refreshing microsweets	7.7 (1)	5.2-16 (8)	5.0-14.1 (5)	7.4-9.4 (2)	
3	Edible ices	5.9-18.2 (2)	5.0-15.4 (9)	6.4-7.3 (2)	5.2 (1)	
4	Fruit and vegetables	5.1 (1)			5.0 (1)	5.2-10.1 (6)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts			5.6-6.4 (3)	9.3-44.6 (15)	6.1-34.4 (7)

* Food groups in bold exceed 5% contribution to total exposure in population groups.

** Total number of surveys may be greater than total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific age range.

Table 7: Main food categories contributing to mean exposure to caramel colour E 150c (> 5% of total exposure) using reported use levels and number of surveys in which each food category is a main contributor.

Cat N°	Foods*	% contribution to total exposure (Number of Surveys)**				
		Toddlers	Children	Adolescents	Adults	The elderly
7	Bakery wares	6.5-55.7 (7)	5.6-60.6 (15)	15.7-47.6 (11)	6.8-28.5 (13)	11.6-34.2 (2)
1	Dairy products and analogues	6.4-25.8 (7)	5.3-14 (11)	5.1-30.4 (10)	5.1-13.5 (10)	5.1-24.8 (7)
8	Meat	5.2-26.2 (6)	5.6-29.3 (14)	7.7-17.2 (10)	5.4-28.9 (11)	5.2-28.8 (6)
12	Salts, spices, soups, sauces, salads and protein products	7.3-40.4 (6)	8.8-40.4 (13)	6.4-41.4 (11)	5.2-37.3 (14)	11.1-30.1 (6)
5.2	Other confectionery including breath refreshing microsweets	6.3-8.3 (2)	6.3-19.8 (2)	5.4-17.9 (8)	5.8-6.2 (2)	
15	Ready-to-eat savouries and snacks	5.2 (1)		6.2 (1)		
6	Cereals and cereal products	12.6 (1)	5.1-11.2 (5)	10.4 (1)	6.2 (1)	8.6 (1)
14.1	Non-alcoholic beverages	7.1 (1)	5.1-12.6 (4)	5.1-15.1 (7)	8.4 (1)	6.3 (1)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts			6.5-24.3 (6)	23-73.2 (15)	11.8-71.9 (7)
16	Desserts excluding products covered in category 1, 3 and 4	9.0-26.7 (3)	7.0-21 (5)	5.1-10.1 (3)		5.4 (1)
3	Edible ices	5.0 (1)				
4	Fruit and vegetables	9.6-18.9 (2)	6.2 (1)			
7	Fish and fisheries products	5.0 (1)	10.3 (1)	7.2 (1)		

* Food groups in bold exceed 5% contribution to total exposure in population groups.

** Total number of surveys may be greater than total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific age range.

High level exposures for toddlers may slightly exceed the ADI of 100 mg/kg bw/day for caramel colour E 150c. The food categories contributing to this exceedance are desserts and bakery wares, i.e. the fine bakery wares (pastries and cakes, biscuits and cereal bars) within the later food category.

For adults, the ADI is exceeded in 5 countries out of 15: from 105% to 151% (Appendix C). The food category which contributes the most leading to an exceedance of the ADI is alcoholic beverages with a contribution from 23 to 73% in all countries. In the 5 countries exceeding the ADI, fine bakery wares also contribute to more than 5% (up to 11%) of the total mean exposure to caramel colour E 150c; salts, spices, soups, sauces, salads and protein products food category, and within this food category especially sauces, and soups and broth, were also contributing to more than 5% in 4 countries (up to 12%).

Table 8: Main food categories contributing to mean exposure to caramel colour E 150d (>5% of total exposure) using reported use levels and number of surveys in which each food category is a main contributor

Cat N°	Foods*	% contribution to total exposure (Number of Surveys)**				
		Toddlers	Children	Adolescents	Adults	The elderly
12	Salts, spices, soups, sauces, salads and protein products	10.5-31.8 (7)	10.8-45.7 (15)	10.5-47.1 (11)	10.4-55.7 (15)	9.7-44.7 (7)
1	Dairy products and analogues	5.8-26.2 (7)	5.1-13.8 (11)	5.3-34.8 (8)	5.3-15.2 (13)	5.7-22.0 (7)
7	Bakery wares	6.4-27.7 (6)	7.8-32.8 (13)	6.3-19.1 (11)	5.1-14.8 (11)	6.3-16.4 (5)
5.2	Other confectionery including breath refreshing microsweets	6.5-19.0 (5)	5.1-31.2 (12)	8.4-29.8 (7)	5.7-20.2 (5)	8.6-8.7 (2)
14.1	Non-alcoholic beverages	5.5-39.3 (4)	8.5-36.2 (15)	14.0-51.8 (12)	7.9-44.7 (15)	5.7-44.0 (4)
3	Edible ices	8.0-27.5 (4)	5.4-22.0 (14)	5.4-12.5 (8)	6.0-8.6 (3)	7.2 (1)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts			5.9-8.7 (2)	6.0-37.3 (10)	11.6-30.1 (4)
16	Desserts excluding products covered in category 1, 3 and 4	6.3-37.9 (3)	5.3-17.6 (6)	5.7-9.7 (3)	5.4 (1)	6.4 (1)
4	Fruit and vegetables	10.7-18.2 (2)	6.5 (1)	5.0 (1)	5.4-21.0 (3)	12.8-26.7 (2)
8	Meat	5.8-11.1 (3)	5.5-7.7 (6)	5.7-7.3 (5)	5.7-10.9 (4)	6.1-11.7 (3)
17	Food supplements as defined in Directive 2002/46/EC ¹⁵ excluding food supplements for infants and young children					6.3 (1)

* Food groups in bold exceed 5% contribution to total exposure in population groups.

** Total number of surveys may be greater than total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific age range.

4.3. Combined refined exposure to caramel colours

A refined combined dietary exposure estimate was also calculated for the four caramel colours (E 150a, E 150b, E 150c, E 150d), by selecting within each food category, the highest use level among the use levels reported for the four caramel colours. Caramel colour E 150b use levels used in the combined refined exposure are the ones used in the previous ANS Panel opinion (EFSA, 2011a)

This scenario represents an additional uncertainty in the combined exposure estimates, by assuming, on one hand, that caramel colours are present in each food category at the highest use level reported and on the other hand by not taking into account that more than one caramel colours may be added in the same food product.

¹⁵ Directive 2002/46/EC of the European Parliament and Council of 10 June 2002 on the approximation of the laws of Member States relating to food supplements. OJ L OJ L 183, 12.7.2002, p. 51-57.

Table 9: Summary of anticipated refined combined exposure to all four caramel colours in the five population groups (mg/kg bw/day).

Combined exposure to caramel colours E 150a, E 150b, E 150c, E 150d	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (> 65 years)
• Mean	19-105	31-83	12-56	15-57	9-35
• High level (95 th percentile)	73-158	68-160	28-144	41-165	24-87

As a reminder, Table 9 presents the combined dietary exposures to caramel colours as estimated in ANS Panel opinion in 2011 for the two population groups: children and adults.

Table 10: Summary of anticipated combined exposure to all four caramel colours in children and adult populations as calculated in the previous ANS Panel opinion (EFSA, 2011a) (mg/kg bw/day)

Combined exposure to caramel colours E 150a, E 150b, E 150c, E 150d	Children (3-9 years)	Adults (18-64 years)
• Mean	83-698	195
• High level (95 th percentile)	225-1672	474

Table 11: Main food categories contributing to the mean combined exposure to caramel colour E 150a, E 150b, E 150c and E 150d using reported use levels and number of surveys in which each food category is a main contributor.

Cat N°	Foods*	% contribution to total exposure (Number of Surveys)**				
		Toddlers	Children	Adolescents	Adults	The elderly
1	Dairy products and analogues	10.1-25.1 (7)	5.2-11.1 (13)	5.0-23.8 (7)	5.1-10.3 (6)	5.4-16.5 (4)
12	Salts, spices, soups, sauces, salads and protein products	6.4-24.4 (7)	7.1-34.1 (15)	8.5-34.7 (11)	5.6-34.5 (15)	18.7-31.5 (6)
7	Bakery wares	11.4-41.3 (6)	11.7-44.6 (13)	10.6-28.5 (11)	6.3-17.7 (12)	9.0-21.3 (5)
8	Meat	7.0-17.2 (5)	5.6-13.5 (13)	5.3-12.6 (9)	5.3-17.8 (8)	6.8-18.9 (3)
5.2	Other confectionery including breath refreshing microsweets	6.1-14.2 (4)	5.7-25.4 (10)	6.4-23.5 (7)	5.1-10.8 (3)	5.4 (1)
14.1	Non-alcoholic beverages	5.8-31.9 (3)	5.5-27.8 (15)	9.7-40.3 (12)	6.0-27.5 (14)	5.9-19.1 (2)
3	Edible ices	8.4-26.3 (4)	5.3-23.2 (15)	5.0-14.2 (10)	5.8-7.9 (3)	7.8 (1)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts			8.3-15.8 (4)	17.9-63.9 (15)	8-56.1 (7)
16	Desserts excluding products covered in category 1, 3 and 4	5.1-23.1 (3)	5.9-13.5 (5)	7.0 (1)		
4	Fruit and vegetables	8.1-11.5 (2)	5.6 (1)		14.9 (1)	10.3-19.3 (2)
6	Cereals and cereal products	6.6 (1)	7.6 (1)	8.1 (1)		5.6 (1)
9	Fish and fisheries products		6.5 (1)			

* Food groups in bold exceed 5% contribution to total exposure in population groups.

** Total number of surveys may be greater than total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific age range.

5. Results and discussion

EFSA has performed a refined exposure assessment for caramel colours (E 150a, E 150c, E 150d) taking into consideration newly submitted reported use levels provided by industry (FoodDrinkEurope, 2012; FoodDrinkEurope-EUTECA, 2012).

The present refined exposure estimates have been performed using the maximum reported use levels, or, where these were not reported, the MPLs from the Codex Alimentarius. When Codex Alimentarius MPLs were not available, values defined by decision rules for QS usages (Appendix A, figure 2) were used (Appendix B).

The results of the present exposure assessment to caramel colours E 150a, E 150b and E 150d are considerably lower than those from the previous exposure assessment performed by the ANS Panel in 2011 (EFSA, 2011a).

For caramel colour E 150a, toddlers and children have higher dietary exposures than adolescents, adults and the elderly. The mean dietary exposures for toddlers and children range respectively from 11 to 45 mg/kg bw/day and from 15 to 39 mg/kg bw/day; the 95th percentile anticipated exposures range respectively from 32 to 79 mg/kg bw/day and from 36 to 81 mg/kg bw/day. For adolescents, adults and the elderly, the anticipated dietary exposure to caramel colour E 150a gives a range through all European countries of 4 to 24 mg/kg bw/day at the mean and of 13 to 56 mg/kg bw/day at the 95th percentile. Compared to the previous ANS Panel opinion (EFSA, 2011a), exposures of children are 5 to 11 times lower both at the mean and at the high level. Current exposures for adults are around 10 to 20 times lower than the estimated exposures from previous ANS Panel opinion.

The main contributors (contributing > 5% of the total exposure in all or several countries) to the total mean exposure to caramel colour E 150a for toddlers are bakery wares (> 5 % in all surveys: from 5 to 67%); for children and adolescents, bakery wares are also a main contributor together with non-alcoholic beverages (> 5 % in all surveys). For adults, beverages (alcoholic and non-alcoholic) are the main contributing food categories (> 5 % in all surveys). For the elderly, salt, spices, soups and sauces and alcoholic beverages are the main contributing food categories. The same foods groups contributed the most in the previous exposure estimates (EFSA, 2011a) both for children and adults.

For caramel colour E 150c, anticipated exposures of toddlers and children are quite similar: estimates range from 10 to 60 mg/kg bw/day and from 36 to 106 mg/kg bw/day for toddlers, and for children from 20 to 56 mg/kg bw/day and from 43 to 97 mg/kg bw/day, for mean and high levels respectively. The anticipated exposures to E 150c for adolescents and the elderly are also similar: from 6 to 33 mg/kg bw/day at the mean and from 14 to 86 mg/kg bw/day at the 95th percentile. The anticipated exposures for adults range from 10 to 43 mg/kg bw/day at the mean and from 27 to 151 mg/kg bw/day at the 95th percentile. Compared to the previous ANS Panel opinion, the results of the current exposures are 5 times lower at the mean and from 2 to 10 times lower for the high level exposure estimates for both children and adults.

For caramel colour E 150c, the main contributors to the total mean exposure for toddlers are bakery wares and dairy products (> 5 % in all surveys). For children, bakery wares are also the food category contributing the most (> 5 % in all surveys). In the previous ANS Panel opinion, fine bakery wares were also the main contributing food category, together with desserts, and sauces and seasonings. Alcoholic beverages are a major contributing food category for adults and the elderly (> 5 % in all surveys). Dairy products are the second food category contributing most for the elderly. Beer and cider were the food category contributing most in the previous exposure estimates for adults, sauces with seasoning being the second one.

Mean anticipated exposures of toddlers, children and adolescents to caramel colour E 150d range respectively from 11 to 86 mg/kg bw/day, from 19 to 62 mg/kg bw/day and from 8 to

44 mg/kg bw/day at the mean. At the 95th percentile, exposures range from 38 to 127 mg/kg bw/day, 49 to 136 mg/kg bw/day and 21 to 122 mg/kg bw/day, for toddlers, children and adolescents, respectively. Anticipated exposure to caramel colour E 150d for adults ranges from 9 to 36 mg/kg bw/day at the mean, and from 26 to 101 mg/kg bw/day at the high level. For the elderly, exposures range from 6 to 22 mg/kg bw/day at the mean and from 19 to 60 mg/kg bw/day at the 95th percentile. Compared to the previous ANS Panel opinion exposures for children are at least 6 times lower at the mean and between 3 and 11 times lower at the high level. Current exposures for adults are around 2 to 10 times lower at the mean and up to 14 times lower at the high level, than the estimated exposures from the previous ANS Panel opinion.

For caramel colour E 150d, salt, spices, soups and sauces are the main contributing food category for toddlers, children, adults and the elderly (> 5 % in all surveys). Non-alcoholic beverages contribute to > 5% in all surveys for children, adolescents and adults. Dairy products also are contributing to > 5% in all surveys for toddlers and the elderly. In the previous ANS Panel opinion, soft drinks, confectionary and fine bakery wares were the main contributing food categories. For adults, confectionary and soft drinks were the main contributors to the total mean exposure in the previous estimates (EFSA, 2011a). Newly submitted reported use levels are much lower than those used in the previous ANS Panel opinion (lower levels by factor 12 to 15) may explained that confectionary is no more a main contributing food group.

The combined mean exposures to the four caramel colours range from 19 to 105 mg/kg bw/day for toddlers, from 31 to 83 mg/kg bw/day for children, from 12 to 56 mg/kg bw/day for adolescents and from 15 to 57 mg/kg bw/day for adults. At high levels, anticipated combined exposures range from 73 to 158 mg/kg bw/day for toddlers, from 68 to 160 mg/kg bw/day for children, from 28 to 144 mg/kg bw/day for adolescents and from 41 to 165 mg/kg bw/day for adults. The estimated exposures for the elderly are a bit lower with a mean combined intake ranging from 9 to 35 mg/kg bw/day and from 24 to 87 mg/kg bw/day at the 95th percentile. Compared to the previous ANS Panel opinion, current exposures for children are between 2 to 12 times lower at the mean and between 3 to 12 times lower at the high level. Current exposures for adults are around 3 to 13 times lower than the estimated exposures from previous ANS Panel opinion both at the mean and at the high level.

The main contributors to the combined mean exposure to caramel colours E 150a, E 150b, E 150c and E 150d are dairy products and salt, spices, soups and sauces for toddlers. The main contributors for children are salt, spices, soups and sauces, non-alcoholic beverages as well as edible ices. Non-alcoholic beverages contribute also to > 5 % in all surveys for children. Alcoholic beverages are the main contributors for adults and the elderly. For adults, salt, spices, soups and sauces contribute also for > 5% in all surveys. In the previous ANS Panel opinion, the main contributing food categories for children and adults were non-alcoholic beverages and confectionary. Beer and cider were the third greatest contributing food category for adults.

The current total mean and high level anticipated dietary exposure to caramel colour E 150a is below the group ADI of 300 mg/kg bw/day for all population groups at both mean and high exposure level while in the previous opinion of the ANS Panel (EFSA, 2011a), the estimated total mean and high (95th percentile) dietary exposures to caramel colour E 150a were above the ADI for children at the upper end of the ranges, as well as for adults for high exposures (97.5th percentile).

The refined estimated dietary exposure to caramel colour E 150c is below the ADI of 100 mg/kg bw/day at the mean for all population groups, while previously the upper end of the range of the mean anticipated exposure of children was above the ADI. At the high level of exposure, the ADI is still slightly exceeded at the upper end of the range estimates for toddlers and the upper end of the range estimates for adults exceed the ADI by 50%. In the previous assessment, the total estimated high intakes (95/97.5th percentile) of caramel colour E 150c were above the ADI for both children and adults.

For toddlers, the ADI is exceeded in one country at the high level of exposure (Appendix C), due to desserts, bakery wares and soups and sauces. In the previous ANS Panel opinion, children exceeded the ADI with the same main contributing food groups. It should be noted that the use levels used in the present estimate are the same as used previously for desserts while use levels in bakery wares were approximately half of previous values and those in sauces, soups, spices and seasonings were generally lower by a factor of 10.

For adults, the ADI is exceeded at the high levels (95th percentile) in 5 countries out of 15 (Appendix C). The exceedance is due to alcoholic beverages which is the main food category contributing to total mean exposure to caramel colour E 150c. It should be noted that the newly submitted use levels are lower for beer and cider but for spirit drinks and other alcoholic drinks (including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with < 15% of alcohol), the use levels used are now far higher than the ones used in the previous ANS Panel opinion for caramel colour E 150c (by a factor up to 50 times in spirits). No use levels were provided for some of the alcoholic beverages (aromatised wines, aromatised wines-based drinks) and MPLs from the Codex Alimentarius were used (as described in the rules defined by the ANS Panel). These MPLs are also higher than the use levels from the previous ANS Panel opinion (EFSA, 2011a).

For caramel colour E 150d, the refined estimated dietary exposure is below the group ADI at the mean as well as at the high level for all populations. In the previous estimates, adults were exceeding the group ADI at the high level and children at the upper end of the range of exposures at both mean and high level.

The combined exposure to caramel colours E 150a, E 150b, E 150c and E 150d both at the mean and at the high level are below the group ADI of 300 mg/kg bw/day for all population groups. In the previous opinion of the ANS Panel, mean and high level of anticipated intake for children was above 300 mg/kg bw/day at the upper end of the range; for adults, high level anticipated exposure was above the group ADI.

Table 12: Overview of exceedance of group or individual ADI in the present refined assessment of caramel colours

Caramel colour	Toddlers		Children		Adolescents		Adults		The elderly	
	mean	high level	mean	high level	mean	high level	mean	high level	mean	high level
E 150a	< group ADI		< group ADI		< group ADI		< group ADI		< group ADI	
E 150c	< individual ADI	> individual ADI	< individual ADI		< individual ADI		< individual ADI	> individual ADI	< individual ADI	
E 150d	< group ADI		< group ADI		< group ADI		< group ADI		< group ADI	
Combined exposure	< group ADI		< group ADI		< group ADI		< group ADI		< group ADI	

The exposure estimates of the current refined assessment are considerably lower than those of the previous ANS Panel opinion published in 2011. This is due both to the consumption data and to the occurrence data used. This refined exposure estimate is based on more consumption surveys for adults allowing a range of exposure estimates. No summary statistics were used and consumption data were used at the individual level which permit to decrease the conservativeness of the exposure output. As far as possible, foods were selected at detailed level from the FoodEx nomenclature. For example, non-alcoholic beverages which may contain caramel colours (cola-type beverages) were differentiated from other soft drinks and different use levels were applied for cola-type drinks and other soft drinks; this contributed to reducing the current exposure estimates. With respect to the previously reported use levels, lower levels as described in Appendix B, were provided for several food categories by food

industry (dairy products, sauces, soups and broths, beer, fine bakery wares, confectionary, fish and meat products, seasonings and condiments, vinegar), which also accounts for a major reduction of the total exposure.

As discussed before, several sources of uncertainty affect the exposure estimates. The Comprehensive Database gathers food consumption data from dietary surveys conducted in several Member States and collected through different methodologies, thus harmonisation of all databases in one results in loss of detailed information. Information on how representative the products are regarding use levels in the European market (market share data for European countries), and more occurrence data (monitoring data as well as usage data coming from more food industries) on the food categories could allow more refinements.

6. Uncertainty analysis

According to the guidance provided by the EFSA opinion (EFSA, 2006), the following sources of uncertainties have been considered. These were already presented and discussed in the sections above and are summarised below:

Assessment objective

The objective of the assessment was clearly specified in the terms of reference. EFSA carried out a refined exposure assessment using the FoodEx system and excluding non relevant subgroups from intake calculations.

- Input data

- Food consumption data: the data used for the exposure estimates were data reported at the individual level in several different dietary surveys which applied different methodologies. Under-reporting and/or misreporting often represent a bias in dietary surveys.
- Food nomenclature: the food nomenclatures used in the dietary surveys considered and then the FoodEx nomenclature used in the Comprehensive Database are different from the Food Classification System developed for the definition of food additives uses in the Commission Regulation (EU) N° 1129/2011. Therefore, linking between different food nomenclatures leads to uncertainties.
- Reported use levels: the estimates calculated are considered as being conservative because they are based on the assumption that all products within a given food category contain the caramel colours at the maximum reported use level. Typical use levels were used on a case by case basis when more information on the representativeness of use levels was made available.

Table 13: Qualitative evaluation of influence of uncertainties

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies / representativeness / under reporting / misreporting / no portion size standard	+/-
Extrapolation from food consumption survey of few days to estimate chronic exposure	+
Linkage between reported use levels and food items in the consumption database: uncertainties on which precise types of food the use levels refer.	+/-
Occurrence data: maximum reported use levels within a food category, exposure calculations based on the maximum reported use levels (use of typical use levels when available on a case by case basis)	+
Exposure model: uncertainty in possible national differences in use levels of food categories, data set not fully representative of foods on the EU market	+/-
Combined exposure to all four caramel colours:	+/-

(a) +: uncertainty with potential to cause over-estimation of exposure;
 -: uncertainty with potential to cause under-estimation of exposure.

EFSA considered the impact of the uncertainties in the exposure assessment to caramel colours and concluded that overall uncertainty should lead to an overestimation of the calculated exposure estimates.

CONCLUSIONS

The current exposure estimates differ considerably from those of the previous opinion of the ANS Panel in 2011 (EFSA, 2011a). For caramel colour E 150a, the present estimates are 5 to 20 times lower compared to the earlier exposure estimates. For caramel colour E 150c, the current estimates are up to 5 times lower at the mean and from 2 to 10 times lower at the high levels for both children and adults. The mean exposure estimate for caramel colour E 150d is lower by a factor of 6 for children, and up to 10 times lower for adults; high level exposure estimates are up to 14 times lower. Compared with the previous ANS Panel opinion the combined exposure to all four caramel colours is lower by a factor of 3 to 13 for children and adults.

These differences in the outcomes of the exposure estimates are due to the new consumption data used and a refined selection of food items within the FoodEx nomenclature as well as to lower use levels provided by industry which represent use levels in products ready to be consumed.

EFSA concluded that the mean dietary exposure for the three populations of European children (toddlers, children and adolescents) ranges from 6 to 45 mg/kg bw/day for caramel colour E 150a, from 9 to 60 mg/kg bw/day for caramel colour E 150c and from 8 to 86 mg/kg bw/day for caramel colour E 150d. At the 95th percentile, estimates range from 16 to 81 mg/kg bw/day for caramel colour E 150a, from 20 to 106 mg/kg bw/day for caramel colour E 150c, and from 21 to 136 mg/kg bw/day for caramel colour E 150d.

For adults and the elderly, the anticipated dietary exposure for caramel colour E 150a gives a mean range through all European countries, of 4 to 18 mg/kg bw/day and of 13 to 42 mg/kg bw/day at the 95th percentile. For caramel colour E 150c, the mean ranges from 6 to 43 mg/kg bw/day and the 95th percentile ranges from 14 to 151 mg/kg bw/day. For caramel colour E 150d, the mean ranges from 6 to 36 mg/kg bw/day and the 95th percentile ranges from 19 to 101 mg/kg bw/day.

The anticipated combined mean exposures to the four caramel colours range from 12 to 105 mg/kg bw/day for toddlers, children, adolescents and adults, and between 28 and

165 mg/kg bw/day at the 95th percentile. For the elderly, the combined exposure to caramel colours E 150a, E 150b, E 150c and E 150d ranges from 9 to 35 mg/kg bw/day at the mean and from 24 to 87 mg/kg bw/day at the 95th percentile.

Based upon the above estimates, it is concluded that the ADI might be exceeded for caramel colour E 150c at the upper end of the range of high level exposure estimates for toddlers and adults. The combined exposure estimates of the four caramel colours do not exceed the group ADI for any population group.

DOCUMENTATION PROVIDED TO EFSA

1. FoodDrinkEurope (Europe Food and Drink Industry), 2012. FoodDrinkEurope's compilation on usage data on caramels colours. (FoodDrinkEurope's 2009 compilation of concentration data with 2011-2012 updates, February 2012)
2. FoodDrinkEurope (Europe Food and Drink Industry), EUTECA (European Technical Caramel Association), letters to the European Commission, September and October 2012.

REFERENCES

- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006, 438, 1-54.
- EFSA (European Food Safety Authority), 2011a. Opinion of the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) on the re-evaluation of caramel colours (E 150a,b,c,d) as food additives. EFSA Journal, 9(3):2004, 103 pp.
- EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal, 9(3):2097, 34 pp.
- EFSA (European Food Safety Authority), 2011c. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal, 9(3):1970, 27 pp.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1970. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. 13th Report of JECFA. WHO Technical Report Series, No. 445, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972a. Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological evaluations and specifications and a review of the technological efficacy of some antioxidants. 14th Report of JECFA. WHO Technical Report Series, No. 488, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972b. Toxicological evaluation of some enzymes, modified starches, and certain other substances. 15th Report of JECFA. WHO Food Additives Series, No 1.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1978. Evaluation of certain food additives. 21st report of JECFA. WHO Technical Report Series 617. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1986. Evaluation of certain food additives and contaminants. 29th report of JECFA. WHO Technical Report Series, No 733, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1987. Toxicological evaluation of certain food additives and contaminants. 29th Meeting of JECFA. WHO Food Additives Series, No 20.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Evaluation of certain food additives and contaminants. 55th report of JECFA. WHO Technical Report Series, No 901, 8-10.
- SCF (Scientific Committee on Food), 1975. Reports of the Scientific Committee for Food, 1st series. Opinion expressed 31 December 1975, 17-20.
- SCF (Scientific Committee on Food), 1984. Reports of the Scientific Committee for Food, 14th series. EUR 8752. Luxembourg: Office for Official Publications of the European Communities, 1983, ISBN 92-825-3893-1. Opinion expressed 1983, 56-57.
- SCF (Scientific Committee on Food), 1989. Reports of the Scientific Committee for Food, 21st series. EUR 11617. Luxembourg: Office for Official Publications of the European Communities, 1998. ISBN 92-826-0823-9. Opinion expressed 1987, 3-8.

SCF (Scientific Committee on Food), 1997. Reports of the Scientific Committee for Food, 36th series. Opinion of the SCF on Caustic sulphite caramel and Ammonia caramel. Luxembourg: Office for Official Publications of the European Communities, 1997, ISBN 92-827-9580-2. 1-8.

TemaNord, 2002. Food additives in Europe 2000; Status of safety assessments of food additives presently permitted in the EU. TemaNord 2002, 560, 145-148.

A. RULES DEFINED BY THE PANEL TO DEAL WITH QUANTUM SATIS (QS) AUTHORISATION, USAGE DATA OR OBSERVED ANALYTICAL DATA FOR ALL REGULATED FOOD ADDITIVES TO BE RE-EVALUATED

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

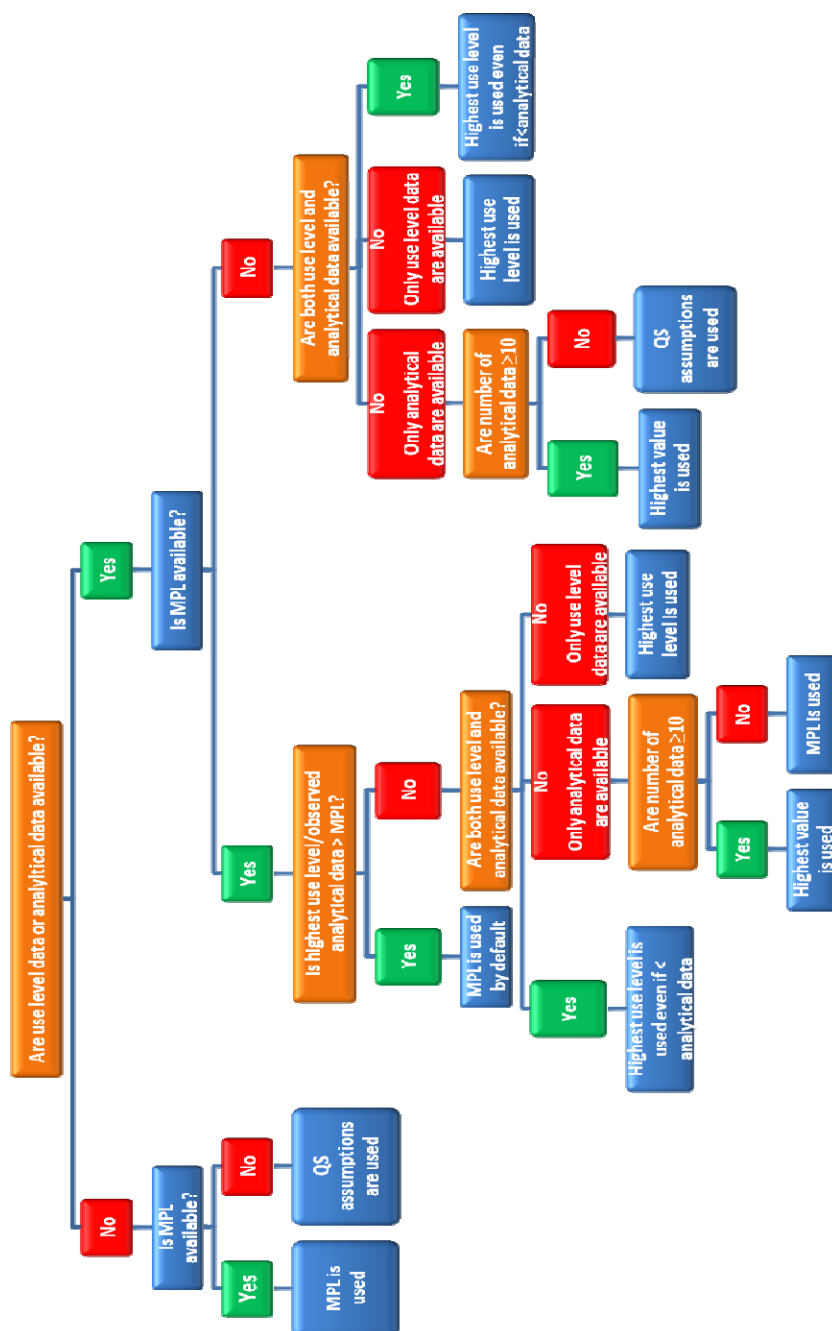
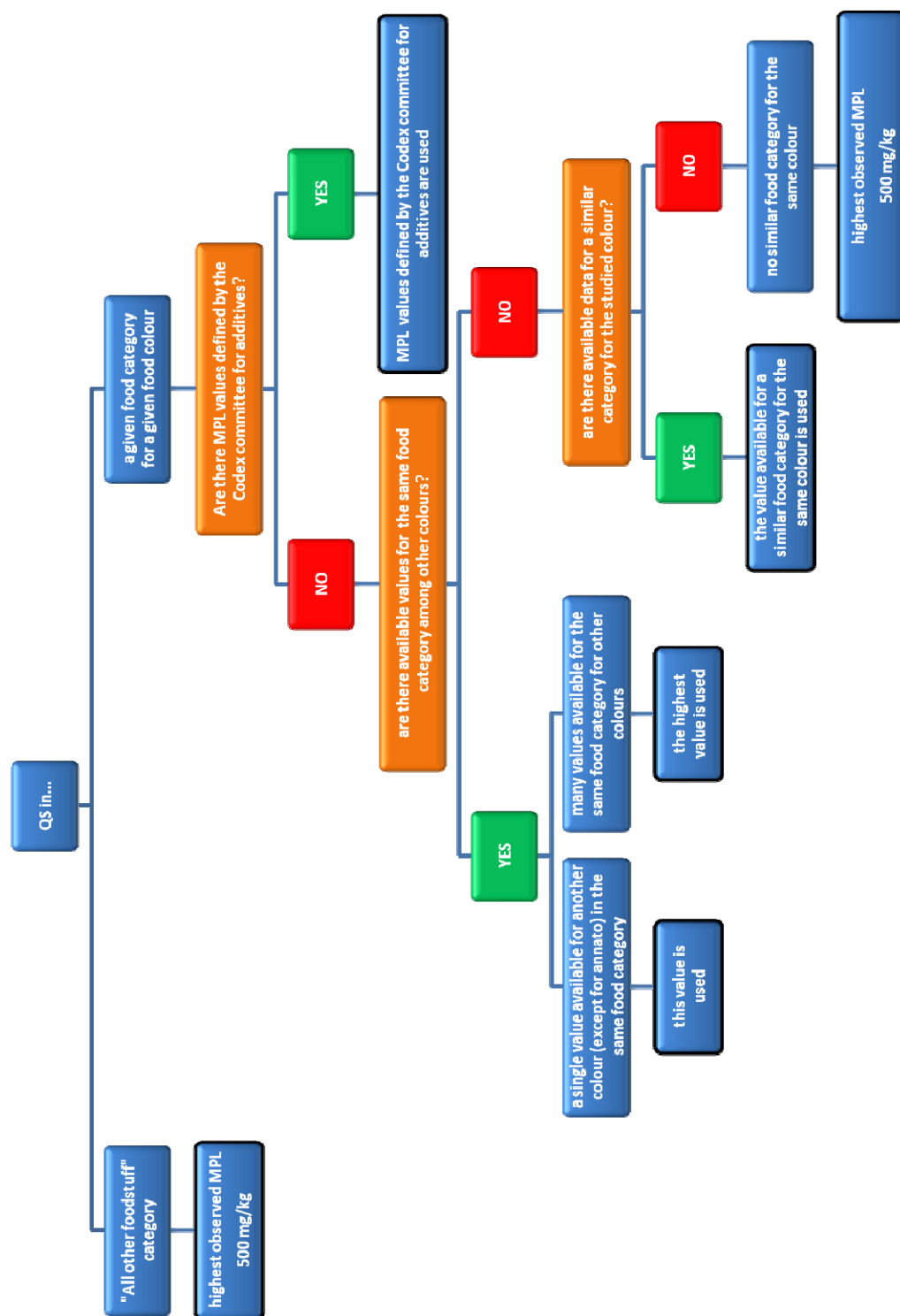


Figure 2: Rules defined by the Panel to deal with quantum satis (QS) authorisation



B. SUMMARY OF USE LEVELS (MG/KG) OF CARAMEL COLOURS (E 150A, E 150C AND E 150D) USED IN THE REFINED EXPOSURE ASSESSMENT

Cat N°	Foods	E Number / Group	Restrictions /exception	Caramel colour E 150a				Caramel colour E 150c					Caramel colour E 150d				
				FDE*	QS rules **	used levels	2011 Opinion levels	FDE	Codex MPLs ***	QS rules	used levels	2011 Opinion levels	FDE	Codex MPLs	QS rules	used levels	2011 Opinion levels
1.4	Flavoured fermented milk products including heat treated products	Group II		750		750	15 000	60			60	5120	60			60	1000
1.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	except unflavoured products	570		570	-	60			60	-	570			570	-
1.6.3	Other creams	Group II	only flavoured creams	570		570	-	60			60	-	570			570	-
1.7.1	Unripened cheese excluding products falling in category 16	Group II	only flavoured unripened cheese	570		570	-	570			570	-	570			570	-
1.7.3	Edible cheese rind	Group II			100	100	100		50 000		50 000	100		50 000		50 000	100
1.7.4	Whey cheese	Group II		570		570	-	570			570	-	570			570	-
1.7.5	Processed cheese	Group II	only flavoured processed cheese	570	100	570	100	570			570	-	570			570	-
1.7.6	Cheese products (excluding products falling in category 16)	Group II	only flavoured unripened products	570		570	100	570			570	-	570			570	-
1.8	Dairy analogues, including beverage whiteners	Group II			5	5	-		1000		1000	-		1000		1000	-
3	Edible ices	Group II		1990		1990	5000		1000		1000	1360	5200			5200	5200
4.2.1	Dried fruit and vegetables	E 150a-d	only preserves of red fruit		200	200	200			200	200	200			200	200	200
4.2.2	Fruit and vegetables in vinegar, oil, or brine	E 150a-d	only preserves of red fruit	-	200	200	200		200		200	200		7500		7500	200
4.2.2	Fruit and vegetables in vinegar, oil, or brine	E 150a-d	only vegetables (excluding olives)	-	200	200	500		500		500	500		7500		7500	500
4.2.3	Canned or bottled fruit and vegetables	E 150a-d	only preserves of red fruit		200	200	200		200		200	200		7500		7500	200
4.2.4.1	Fruit and vegetable preparations excluding compote	Group II	only mostarda di frutta		200	200	200		7500		7500	2050		7500		7500	200
4.2.4.1	Fruit and vegetable preparations excluding compote	E 150a-d	only preserves of red fruit		200	200	200		7500		7500	200		7500		7500	200
4.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	E 150a-d	except chestnut puree	4000		4000	4000	400			400	100	400			400	100
4.2.5.3	Other similar fruit or vegetable spreads	Group II	except crème de pruneaux	4000		4000	4000	400			400	100	400			400	100

Cat N°	Foods	E Number / Group	Restrictions /exception	Caramel colour E 150a				Caramel colour E 150c					Caramel colour E 150d				
				FDE*	QS rules **	used levels	2011 Opinion levels	FDE	Codex MPLs ***	QS rules	used levels	2011 Opinion levels	FDE	Codex MPLs	QS rules	used levels	2011 Opinion levels
5.2	Other confectionery including breath refreshing microsweets	Group II		7000		7000	10 000	10 000			10 000	8000	25 000			25 000	300 000
5.3	Chewing gum	Group II			10 000	10 000	10 000		20 000		20 000	8000		20 000		20 000	300 000
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Group II		297		297	40	6050			6050	3400	14 000			14 000	500
6.3	Breakfast cereals	Group II	only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals	2200		2200	-	3000			3000	-	41			41	-
6.3	Breakfast cereals	E 150c	only extruded puffed and or fruit flavoured breakfast cereals	2200		2200	10 010	3000			3000	2040	41			41	500
6.5	Noodles	Group II			500	500		510			510		510			510	
6.6	Batters	Group II															
6.7	Pre-cooked or processed cereals	Group II															
7.1.	Bread and rolls	E 150a-d	only Malt bread	5000		5000	30 000	2000			2000	2040	5000			5000	5000
7.2	Fine bakery wares	Group II		4900		4900	15 000	5500			5500	11 500	2500			2500	5000
8.1.2	Meat preparations as defined by Regulation (EC) No 853/2004	E 150a-d	only <i>breakfast sausages</i> with a minimum cereal content of 6 % and <i>burger meat</i> with a minimum vegetable and/or cereal content of 4 % mixed within the meat; In these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance	-	100	100	100	5000			5000	100	2000			2000	100
8.2.1	Non heat treated processed meat	E 150a-d	only sausages		100	100	8000	5000			5000	5000	2000			2000	5000
8.2.2	Heat treated processed meat	E 150a-d	only sausages, patés and terrines		100	100	8000	5000			5000	5000	2000			2000	5000
8.2.3	Casings and coatings and decorations for meat	Group II	except edible external coating of pasturmas		500	500	500	5000			5000	500	2000			2000	500
9.2.	Processed fish and fishery products including molluscs and crustaceans	Group II	only surimi and similar products and salmon substitutes.		500	500	500	5000			5000	500	2000			2000	500

Cat N°	Foods	E Number / Group	Restrictions /exception	Caramel colour E 150a				Caramel colour E 150c					Caramel colour E 150d				
				FDE*	QS rules **	used levels	2011 Opinion levels	FDE	Codex MPLs ***	QS rules	used levels	2011 Opinion levels	FDE	Codex MPLs	QS rules	used levels	2011 Opinion levels
9.2.	Processed fish and fishery products including molluscs and crustaceans	E 150a-d	only fish paste and crustacean paste		100	100	100	5000			5000	100	2000			2000	100
9.2.	Processed fish and fishery products including molluscs and crustaceans	E 150a-d	only precooked crustacean		250	250	250	5000			5000	250	2000			2000	250
9.3	Fish roe	Group II	except Sturgeons' eggs (Caviar)		300	300	300	5000			5000	300	2000			2000	300
12.2.2	Seasonings and condiments	Group II	only seasonings, for example curry powder, tandoori	21 000		21 000	100 000	5000			5000	100 000	20 000			20 000	20 000
12.3	Vinegars	E 150a-d		-	500	500	6000	5000			5000	100 000		50 000		50 000	100 000
12.4	Mustard	Group II		25 000	300	25 000	300		50 000		50 000	2000		50 000		50000	300
12.5	Soups and broths	Group II		1332		1332	50 000	3000			3000	3000	3000			3000	4000
12.6	Sauces	Group II	excluding tomato-based sauces	500		500	100 000	10000			10 000	100 000	10 000			10 000	20 000
12.7	Salads and savoury based sandwich spreads	Group II		5		5	-	5			5	-	5			5	-
12.9	Protein products, excluding products covered in category 1.8	Group II		16 000		16 000	40 000			1000 0	10 000	10 000			1000	1000	2000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II	Products in this category can also use additives that are allowed in the corresponding food counterparts categories		50	50	50	9500			9500	9500	615			615	1000
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II		2500		2500	7000		20 000		20 000	50		20 000		20 000	50
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009	Group II	Products in this category can also use additives that are allowed in the corresponding food counterparts categories. In addition, all additives in the gluten containing counterparts are authorised.		500	500	-			500	500	-			500	500	-
14.1.4	Flavoured drinks	Group II	excluding chocolate milk; malt products/ FOR COLAS	1500		1500	10 000	500			500	1360	4950			4950	5000
14.1.4	Flavoured drinks	Group II	excluding chocolate milk; malt	1500		1500		500			500		2000			2000	

Cat N°	Foods	E Number / Group	Restrictions /exception	Caramel colour E 150a				Caramel colour E 150c					Caramel colour E 150d				
				FDE*	QS rules **	used levels	2011 Opinion levels	FDE	Codex MPLs ***	QS rules	used levels	2011 Opinion levels	FDE	Codex MPLs	QS rules	used levels	2011 Opinion levels
			products														
14.2.1	Beer and malt beverages	E 150a-d	only beer	1000		1000	10 000	6000			6000	8000	0			0	500
14.2.3	Cider and perry	Group II	excluding <i>cidre bouché</i>		200	200	6000	60			60	680	70			70	600
14.2.3	Cider and perry	E 150a-d	only <i>cidre bouché</i>		200	200	2000	60			60	2040	70			70	500
14.2.4	Fruit wine and made wine	Group II			200	200	6000		1000		1000	680	560			560	560
14.2.5	Mead	Group II			200	200			1000		1000			1000		1000	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà.	15 000		15 000	5000	10 000			10 000	680	10 000			10 000	5000
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	E 150a-d	except: fruit spirits, spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà. Whisky, whiskey can only contain E 150a.	15 000		15 000	15 000	10000			10 000	200	10 000			10 000	5000
14.2.7 .1	Aromatised wines	Group II	Except <i>americano</i> , <i>bitter vino</i>	-	200	200	10 000		50 000		50 000	680		50 000		50 000	200
14.2.7 .1	Aromatised wines	E 150a-d		-	200	200	5000		50 000		50 000	680		50 000		50 000	5000
14.2.7 .1	Aromatised wines	E 150a-d	only <i>americano</i> , <i>bitter vino</i>	-	100	100	5000		50 000		50 000	100		50 000		50 000	1000
14.2.7 .2	Aromatised wine-based drinks	Group II	except <i>bitter soda</i> , <i>sangria</i> , <i>claria</i> , <i>zurra</i>		200	200	5000		50 000		50 000	680		50 000		50 000	5000
14.2.7 .2	Aromatised wine-based drinks	E 150a-d	only <i>bitter soda</i>		100	100	100		50 000		50 000	100		50 000		50 000	100
14.2.7 .3	Aromatised wine-product cocktails	Group II			200	200	10 000		50 000		50 000	680		50 000		50 000	200

Cat N°	Foods	E Number / Group	Restrictions /exception	Caramel colour E 150a				Caramel colour E 150c					Caramel colour E 150d				
				FDE*	QS rules **	used levels	2011 Opinion levels	FDE	Codex MPLs ***	QS rules	used levels	2011 Opinion levels	FDE	Codex MPLs	QS rules	used levels	2011 Opinion levels
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	Group II			200	200	30	4950			4950	30	4950			4950	30
15.1	Potato, cereal, flour or starch-based snacks	Group II		3000		3000	3000	3000			3000	2040	1490			1490	1000
15.2	Processed nuts	Group II		3000		3000	3000	3000			3000	2040	16			16	1000
16	Desserts excluding products covered in category 1, 3 and 4	Group II		2774		2774	15000	5120			5120	5120	5120			5120	1000
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	Group II			300	300	300		20 000		20 000	300		20 000		20 000	300
17.2	Food supplements supplied in a liquid form	Group II			100	100	15 000		20 000		20 000	100	1500			1500	620
17.3	Food supplements supplied in a syrup-type or chewable form	Group II			300	300	-		20 000		20 000	-		20 000		20 000	-

*Data from FoodDrinkEurope/EUTECA provided to EFSA between June and October 2012.

**Data from Quantum Satis rules (see Appendix A)

***Maximum Permitted Levels as mentioned in the Codex Alimentarius GSFA

C. SUMMARY OF TOTAL ESTIMATED EXPOSURE (USING MPLS AND USE LEVELS) PER AGE CLASS AND SURVEY*: MEAN AND HIGH LEVEL (MG/KG BW/DAY)

	E 150a		E 150c		E 150d		Combined exposure	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
Toddlers								
Belgium (Regional_Flanders)	44.6	-	59.7	-	85.6	-	105.3	-
Bulgaria (Nutrichild)	22.5	54.1	30.5	65.5	27.9	64.3	41.1	91.8
Finland (DIPP)	10.7	32.3	9.9	35.6	11.1	38.4	18.9	73.4
Germany (Donald 2006_2008)	12.9	34.3	23.6	58.6	22.3	62.1	35.9	88.6
Italy (INRAN_SCAI_2005_06)	16.2	-	19.8	-	19.5	-	28.5	-
The Netherlands (VCP_Kids)	39.9	78.5	53.2	106.3	58.2	126.5	77.5	158.4
Spain (enKid)	17.3	-	27.7	-	19.5	-	32.0	-
Children								
Belgium (Regional_Flanders)	39.3	80.7	48.0	91.6	62.1	135.9	80.9	160.3
Bulgaria (Nutrichild)	26.9	61.4	34.3	74.6	34.9	85.3	51.5	119.0
Czech Republic (SISP04)	32.4	70.7	32.0	69.5	41.2	98.5	58.1	129.3
Denmark (Danish Dietary Survey)	23.6	43.4	26.9	48.2	43.1	81.1	52.3	92.6
Finland (DIPP)	19.3	36.3	22.7	50.0	35.3	86.3	49.2	109.3
Finland (STRIP)	37.5	64.4	56.0	97.2	62.2	116.3	83.2	136.4
France (INCA 2)	29.1	54.6	40.5	73.4	39.0	76.9	56.1	104.5
Germany (Donald 2006_2008)	19.4	39.5	30.6	57.1	35.5	78.2	51.9	105.9
Greece (Regional_Crete)	19.3	42.0	22.9	51.2	19.2	49.2	31.1	69.3
Italy (INRAN_SCAI_2005_06)	15.5	37.2	19.9	43.1	20.3	50.9	31.4	68.0
Latvia (EFSA_TEST)	23.3	54.6	42.6	93.3	37.7	83.5	50.6	112.4
The Netherlands (VCP_Kids)	37.1	74.2	47.3	93.5	56.6	122.5	74.0	146.4
Spain (enKid)	18.0	44.9	28.9	62.7	25.0	61.4	38.7	82.4
Spain (Nut_Ink05)	17.2	35.6	30.6	63.2	26.8	66.7	40.0	80.9
Sweden (NFA)	31.8	62.1	43.8	82.3	55.3	115.2	75.6	144.5

	E 150a		E 150c		E 150d		Combined exposure	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
Adolescents								
Belgium (Diet_National_2004)	20.0	43.3	33.2	86.3	43.8	121.9	56.4	143.8
Cyprus (Childhealth)	6.4	16.5	9.4	20.3	8.2	20.7	12.1	27.8
Czech Republic (SISP04)	24.3	56.3	26.9	60.5	31.6	74.4	44.9	106.7
Denmark (Danish Dietary Survey)	18.4	37.8	20.7	47.3	31.1	66.5	39.4	82.6
France (INCA 2)	15.4	34.6	22.2	45.3	23.2	52.4	32.1	69.6
Germany (National_Nutrition_Survey_II)	15.4	45.0	29.1	73.3	32.8	92.3	43.3	125.5
Italy (INRAN_SCAI_2005_06)	11.1	36.0	11.5	28.8	17.0	50.8	23.0	61.1
Latvia (EFSA_TEST)	15.0	36.4	27.9	58.3	24.6	52.0	33.3	69.4
Spain (AESAN_FIAB)	10.4	22.3	15.2	34.2	15.9	40.9	24.0	52.1
Spain (enKid)	12.2	30.4	20.4	46.7	19.2	43.5	27.7	57.1
Spain (Nut_Ink05)	11.6	27.6	19.9	39.5	21.2	56.3	29.1	68.1
Sweden (NFA)	19.3	42.5	26.1	54.0	37.2	88.9	47.3	101.9

	E 150a		E 150c		E 150d		Combined exposure	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
Adults								
Belgium (Diet_National_2004)	15.5	37.9	35.9	104.5	35.6	100.6	50.9	139.1
Czech Republic (SISP04)	15.9	42.1	43.3	151.3	14.4	33.4	50.0	165.4
Denmark (Danish_Dietary_Survey)	14.4	33.7	26.4	75.5	19.8	47.2	37.7	90.4
Finland (FINDIET_2007)	7.7	23.0	16.7	60.4	12.7	39.2	23.8	76.7
France (INCA2)	10.3	24.1	25.1	74.0	23.6	72.6	31.8	85.8
Germany (National_Nutrition_Survey_II)	12.5	32.2	31.1	93.5	22.3	63.4	39.5	111.7
Hungary (National_Repr_Surv)	7.1	15.9	13.0	41.8	13.7	36.2	21.1	58.4
Ireland (NSIFCS)	12.9	33.0	38.3	139.3	15.6	41.0	44.8	147.9
Italy (INRAN_SCAI_2005_06)	6.4	19.0	9.6	27.4	9.1	26.0	15.1	41.4
Latvia (EFSA_TEST)	9.3	22.7	21.9	54.9	14.7	31.6	23.8	60.7
The Netherlands (DNFCS_2003)	17.9	40.8	41.0	137.3	36.1	99.9	57.4	148.2
Spain (AESAN)	8.8	25.1	17.4	62.0	16.0	52.3	27.7	84.7
Spain (AESAN_FIAB)	8.1	20.7	13.5	37.3	13.9	43.3	21.9	58.3
Sweden (Riksmaten_1997_98)	14.7	29.8	30.2	65.2	20.2	46.1	39.3	82.7
United Kingdom (NDNS)	12.3	29.0	38.9	134.3	24.3	70.1	46.1	145.2
The elderly								
Belgium (Diet_National_2004)	11.6	25.8	30.0	81.9	22.2	60.3	34.5	87.1
Denmark (Danish_Dietary_Survey)	12.4	31.7	25.8	68.2	14.8	43.3	34.0	83.2
Finland (FINDIET_2007)	4.8	13.6	7.9	24.4	7.6	22.0	12.1	35.7
France (INCA2)	7.7	18.9	18.8	52.0	15.9	45.4	22.7	58.7
Germany (National_Nutrition_Survey_II)	10.2	25.9	26.3	80.3	13.2	30.7	29.5	83.3
Hungary (National_Repr_Surv)	5.7	13.4	9.7	27.3	9.6	19.6	14.8	34.4
Italy (INRAN_SCAI_2005_06)	4.1	12.8	5.6	13.9	6.3	18.5	9.0	24.3

*The different methodologies of European dietary surveys included in the EFSA Comprehensive Database are fully described in the Guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a). A summary is available p.11, Table 1 of the guidance.

GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
ANS Panel	Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	Body weight
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EUTeca	European Technical Committee on Caramels
FCS	Food Categorisation System (food nomenclature) presented in the Commission Regulation (EU) N° 1129/2011
GSFA	Codex General Standard for Food Additives. Online Database available here: http://www.codexalimentarius.net/gsfonline/index.html
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MPL	Maximum Permitted Level
QS	Quantum satis
SCF	Scientific Committee for Food
TemaNord	Nordic Council of Ministers

CARAMEL COLOURS

EXPLANATION

In the period since 1972, when the fifteenth report of the Committee was published (Annex 1, reference 26), caramel colours have been classified into 4 classes which differ in their method of manufacture, composition, functional properties, and application.

1. Caramel Colour I (synonyms: plain caramel, caustic caramel, and spirit caramel); this class is prepared by the controlled heat treatment of carbohydrates with alkali or acid.
2. Caramel Colour II (synonyms: caustic sulfite process caramel); this class is prepared by the controlled heat treatment of carbohydrates with sulfite-containing compounds.
3. Caramel Colour III (synonyms: ammonia caramel, ammonia process caramel, closed-pan ammonia process caramel, open-pan ammonia process caramel, bakers' caramel, confectioners' caramel, and beer caramel); this class is prepared by the controlled heat treatment of carbohydrates with ammonium compounds.
4. Caramel Colour IV (synonyms: ammonia sulfite process caramel, sulfite ammonia caramel, sulfite ammonia process caramel, acid-proof caramel, beverage caramel, and soft-drink caramel); this class is prepared by the controlled heat treatment of carbohydrates with ammonium-containing and sulfite-containing compounds.

Caramel colours were reviewed at the eighth, thirteenth, fifteenth, sixteenth, eighteenth, twenty-first, and twenty-fourth meetings of the Committee (Annex 1, references 8, 19, 26, 30, 35, 44, & 53). The thirteenth meeting concluded that a toxicological distinction between caramels produced commercially and caramel formed in cooked foods or when sucrose is heated is unwarranted except with caramel prepared by processes using ammonia or ammonium salts. This conclusion was endorsed by the fifteenth meeting and an ADI "not limited" was allocated to caramel colours prepared by processes other than those involving ammonia or ammonium salts; a temporary ADI of 0-100 mg/kg b.w. was allocated to caramel colours produced by the ammonia process. The sixteenth meeting reviewed further information on the composition of caramel colours produced by the ammonia process, including the 4-methylimidazole content. Revised specifications were prepared and the previously-established temporary ADI was maintained. The specifications for this class were further revised by the eighteenth meeting and the temporary ADI was extended pending the

results of long-term and reproduction studies on caramel colours prepared by the ammonia or ammonia sulfite process.

The twenty-first meeting of the Committee noted that the specifications for caramel colour (ammonia process) were ambiguous, since they appeared to cover caramel colours manufactured by the ammonia sulfite process as well. Separate specifications were prepared for caramel colour (ammonia process) and caramel colour (ammonia sulfite process), which have since been designated caramel colours III and IV, respectively. The two classes have been shown to differ in toxicity. The principal toxic effect of caramel colour III is depression of circulating lymphocytes and leucocytes, and a no-effect level could not be determined; accordingly, the temporary ADI was revoked. The temporary ADI of 0-100 mg/kg b.w. was retained for caramel colour IV pending the submission of reports of adequate carcinogenicity/teratogenicity studies.

The twenty-fourth meeting extended the temporary ADI for caramel colour IV and confirmed that caramel colour II had no ADI since it was not included in the ADI for caramel colour I nor the temporary ADI for caramel colour IV.

Since the previous evaluations, additional data have become available and are summarized and discussed in the following monographs. Previously-published monographs have been expanded and are reproduced in their entirety under the class of caramel colour to which they relate.

CARAMEL COLOUR I

EXPLANATION

At the thirteenth and fifteenth meetings (Annex 1, references 19 & 26), the Committee concluded that caramel colour I is a natural constituent of the diet and is acceptable as an additive. An ADI "not limited" was allocated at the fifteenth meeting.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special study on mutagenicity

Two samples of caramel colour I with different colour intensities were subjected to the Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538. Caramel colour I was neither mutagenic nor cytotoxic, either with or without activation by rat liver S-9 fraction, at concentrations up to 20 µl per plate (Richold & Jones, 1980a,b).

Acute toxicity

No information available.

Short-term studies

Rats

Caramel colour I was administered to groups of 20 weanling female Wistar rats at dietary levels of 0, 15, or 30% for 8 weeks followed by a 4-week recovery period. Diarrhoea was observed in the treated animals and food efficiency was decreased, but the growth rate was normal. Haematological indices, in particular leucocyte counts, were normal throughout the study. The relative caecal weights were increased after eight weeks, but returned to normal by the end of the 4-week recovery period. Discolouration of the mesenteric lymph nodes was observed in animals of both treatment groups after eight weeks, but the discolouration diminished during the recovery period. No other gross or microscopic pathological changes were reported (Sinkeldam & van der Heyden, 1976).

Long-term studies

No information available.

Observations in man

No information available.

Comments

Caramel colour I is free of the heterocyclic compounds associated with convulsant activity or depressed lymphocyte counts which occur in caramels prepared using ammonia or ammonium salts, and displays a low order of short-term toxicity. None of the data suggest a need to revise earlier JECFA recommendations.

EVALUATION

Estimate of acceptable daily intake for man

ADI "not specified".

REFERENCES

Richold, M. & Jones, E. (1980a). Ames metabolic activation test to assess the potential mutagenic effect of ETA-38-2H. Unpublished report No. FDC 8/80359 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.

Richold, M. & Jones, E. (1980b). Ames metabolic activation test to assess the potential mutagenic effect of ETA-38-1W. Unpublished report No. FDC 8/80361 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J. & van der Heyden, C.A. (1976). Short-term feeding test with three types of caramels in albino rats. Unpublished report No. R4789 from CIVO/TNO, Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

CARAMEL COLOUR II

EXPLANATION

The twenty-fourth meeting of the Committee (Annex 1, reference 53) drew attention to the fact that caramel colour II has no ADI since it is not included in the ADI of caramel colour I. This material has not previously been evaluated by the Committee.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on mutagenicity

Caramel colour II at concentrations of 2.5-20 µl/plate was neither mutagenic nor cytotoxic in the Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, with or without metabolic activation by rat liver S-9 fraction (Richold & Jones, 1980).

In a similar test, caramel colour II was neither mutagenic nor

cytotoxic at concentrations of 50-5000 µg/plate (Richold et al., 1984).

Caramel colour II was tested for potential mutagenic activity based on induction of DNA repair (unscheduled DNA synthesis) in cultured human epithelial (HeLa 53) cells. Caramel colour II was incorporated in the culture medium at concentrations of 25-51,200 µg/ml and the test was performed on 2 occasions both in the presence and absence of rat liver S-9 mix. In both tests, in the absence of the S-9 mix a small but statistically significant increase in the number of silver grains over nuclei was observed at a concentration of 25,600 µg/ml; no significant increases were seen at higher concentrations in this test nor at any concentration in the repeat test (Allen & Proudlock, 1984).

Caramel colour II was not clastogenic to cultured Chinese hamster ovarian cells at concentrations of 500, 2500, or 5000 µg/ml either in the presence or absence of rat liver S-9 mix (Allen et al., 1984).

Acute Toxicity

No information available.

Short-term studies

Rats

Five groups of 20 male and 20 female weanling Fischer F-344 rats were given caramel colour II in drinking water at dose levels of 0, 4, 8, 12, or 16 g/kg b.w./day for 90 days. Body weights and food and water intakes were recorded weekly. Haematological examinations, blood biochemical studies, and urinalysis were performed during the seventh week and at termination on all animals. Animals were fasted overnight prior to bleeding from the orbital sinus under anaesthesia; the animals were also fasted during the 24-hour urine collection period. At necropsy, organs were weighed and all animals were examined macroscopically. Histopathological examinations were conducted on all animals in the control and high-dose groups and on animals from the low- and mid-dose groups that died or were sacrificed in extremis.

All animals showed a weight loss and reduced food intake between weeks 7 and 8, presumably due to the fasting prior to bleeding and during urine collection. In addition, caramel colour II caused a dose-related reduction in body-weight gain and in food and fluid intake in both sexes. Males treated with 8 g caramel colour II per kg b.w., and females treated with 4 and 8 g/kg b.w., had significantly lower food consumption than controls for 4 of 13 weeks, 3 of 13 weeks, and 6 of 13 weeks, respectively; mean food consumption, of both males and

females treated with 12 and 16 g/kg b.w., was significantly lower than food consumption of their respective controls. All treated groups had lower mean fluid intake than their respective controls, usually significantly lower. Males receiving 12 and 16 g/kg b.w. had significantly lower mean body weights than controls from weeks 7-13 and weeks 4-13, respectively. Females treated with 8 g/kg b.w. had significantly lower mean body weights than controls from week 11-13 while, beginning at week 7, females receiving 12 and 16 g/kg b.w. had lower mean body weights than controls.

Occasional statistically-significant changes were seen in some haematological and clinical chemical parameters, but the mean values were within the normal range for Fischer 344 rats and were not considered to be of toxicological significance. There was a dose-related reduction in urinary volume and pH, and an increase in urine specific gravity.

At necropsy, treatment-related increases were observed in kidney weights and in full and empty caecum weights, but no significant histopathological changes were observed in these or other tissues. Dose-related staining of the gastrointestinal tract and mesenteric lymph nodes was noted, and deposits of yellow pigment were seen histopathologically in the caecal submucosa and mesenteric lymph nodes of the top-dose groups (the only treated animals examined comprehensively). Reactive hyperplasia was not associated with the pigment deposition.

The investigators concluded that the reduced body weights, food and fluid intakes, and increased kidney weights were due to water imbalance reflecting poor palatability of the drinking solution rather than toxic effects of caramel colour II per se (MacKenzie, 1985).

Comments

The above data are insufficient to evaluate caramel colour II for an ADI, but the substance has a low sub-chronic toxicity and no frankly pathological effects were seen in the 90-day study.

EVALUATION

Estimate of acceptable daily intake for man

No ADI allocated.

REFERENCES

Allen, J.A., Brooker, P.C., Birt, D.M., & McCaffrey, K.J. (1984).
Analysis of metaphase chromosomes obtained from CHO cells

cultured in vitro and treated with caramel colour (II).
Unpublished report No. ITC 3A/84965 from Huntingdon Research
Centre, Huntingdon, England. Submitted to WHO by International
Technical Caramel Association.

Allen, J.A., & Proudlock, R.J. (1984). Autoradiographic assessment of
DNA repair in mammalian cells after exposure to caramel colour
II. Unpublished report No. ITC 2A/84750/2 from Huntingdon
Research Centre, Huntingdon, England. Submitted to WHO by
International Technical Caramel Association.

MacKenzie, K.M. (1985). 90-day toxicity study of caramel color
(caustic sulfite process) in rats. Vols. I & II. Unpublished
report No. 6154-105 from Hazleton Laboratories America Inc.,
Madison, WI, USA. Submitted to WHO by International Technical
Caramel Association.

Richold, M. & Jones, E. (1980). Ames metabolic activation test to
assess the potential mutagenic effect of ETA-38-3N. Unpublished
report No. FDC 8/80356 from Huntingdon Research Centre,
Huntingdon, England. Submitted to WHO by International Technical
Caramel Association.

Richold, M., Jones, E., & Fenner, L.A. (1984). Ames metabolic
activation test to assess the potential mutagenic effect of
caramel colour II. Unpublished report No. ITC 1A/84708 from
Huntingdon Research Centre, Huntingdon, England. Submitted to WHO
by International Technical Caramel Association.

CARAMEL COLOUR III

EXPLANATION

In earlier evaluations, the presence of 4-methylimidazole in
caramel colour III was noted, particularly since this compound was
likely to be the causal agent of convulsions in cattle and sheep fed
ammonia-treated molasses. However, at its twenty-first meeting, the
Committee (Annex 1, reference 44) no longer considered this a cause
for concern since the introduction of chemical specifications limits
the concentration of 4-methylimidazole in caramel colour III. The
twenty-first meeting identified the principal toxic effect of
ammoniated caramels as the depression of circulating lymphocytes and
total leucocytes for which a no-effect level had not been determined;
consequently, the temporary ADI for caramel colour III was revoked.

BIOLOGICAL DATA

Biochemical aspects

Absorption, distribution, and excretion

In groups of 2-4 rats, the absorption of the colour-giving components of caramel was determined by faecal extraction. Recoveries varied widely for the 10 or 20% caramel solutions examined despite pre-treatment for 100 days before testing. About one-third of the colour-giving components appeared to be absorbed, but no conclusions could be drawn regarding the absorption of colourless components (Haldi & Wynn, 1951).

Toxicological studies

Special study on 4-methylimidazole (4-MeI)

4-MEI has been shown to be the most likely toxic component in ammoniated molasses, its being a convulsant to rabbits, mice, and chicks at oral doses of 360 mg/kg b.w. (Nishie *et al.*, 1969).

Mice

Male albino mice (20-25 g) were used to determine the median convulsive dose (CD_{50}) and the median lethal dose (LD_{50}) of a few imidazoles. The results are given in the following table:

Convulsant and lethal effects of imidazoles

	$CD_{50} \pm SE$ in mg/kg		$LD_{50} \pm SE$ in mg/kg b.w.	
	b.w	i.p oral	b.w. i.p	oral
4-methylimidazole	155 $\pm$ 5	360 $\pm$ 18	165 $\pm$ 3	370 $\pm$ 15
1-methylimidazole	380 $\pm$ 8.2	1,400 $\pm$ 79	380 $\pm$ 8.2	1,400 $\pm$ 79
2-methylimidazole	500 $\pm$ 12	1,300 $\pm$ 70	480 $\pm$ 18	1,400 $\pm$ 114
imidazole	560 $\pm$ 34	1,800 $\pm$ 45	610 $\pm$ 7.4	1,880 $\pm$ 45

All the imidazoles tested produced varying degrees of tremor, running, restlessness, diarrhoea, Straub tail, opisthotonus and tonic extensor seizure that ended in death (Nishie *et al.*, 1970).

Chickens

The CD₅₀ and the LD₅₀ of 4-MEI by i.p. injection in 1-day-old chicks were 174±10 mg/kg b.w. and 210±15 mg/kg b.w., respectively. Orally, the CD₅₀ was 580±30 mg/kg b.w. and the LD₅₀ was 599±50 mg/kg b.w. Doses of 100 mg/kg b.w. i.p. caused tremors, peeping, and spreading of the wings. Doses over 150 mg/kg b.w. i.p. caused opisthotonus, prostration with clonic leg movements, and terminal tonic extensor seizure (Nishie *et al.*, 1970).

Special studies on reduction of total lymphocyte counts

Following the request in the twenty-first report of the Committee for information on the factor(s) responsible for the haematological effects of caramel colour III, a series of studies have been performed to investigate the mechanism(s) underlying these effects and the agent(s) responsible.

Rats

The effects of vitamin E, folic acid, pyridoxine and choline on the capacity of caramel colour III to reduce total lymphocytes in the blood of rats fed caramel colour III were examined. Four groups of 10 male weanling rats were fed Spratt's diet containing 8% caramel colour III and supplemented with vitamin E (100 mg/kg), folic acid (10 mg/kg), pyridoxine hydrochloride (10 mg/kg), or choline chloride (1000 mg/kg). Groups on Spratt's diet alone or on Spratt's diet with 8% caramel colour III without any supplement were used as controls. After 12 days, there was a marked reduction in total white blood cells and lymphocytes in rats fed the diet containing 8% caramel colour III and neutrophil counts were increased. Dietary supplementation with vitamin E, folic acid, or choline did not noticeably affect lymphocyte counts. However, rats receiving the diet supplemented with pyridoxine had white blood cells and lymphocytes in numbers similar to those fed the basal diet without caramel colour III. The basal diet was found to contain 2.3 mg/kg pyridoxine.

In a further study using CIVO stock diet (pyridoxine concentration 3 mg/kg), rats receiving 8% caramel colour III in the diet showed a reduction in total white blood cell and lymphocyte numbers; these changes were ameliorated by the addition of 10 mg/kg pyridoxine in the diet. In this study, neutrophils were not affected by the caramel colour III treatment, but the plasma pyridoxal phosphate levels were reduced by treatment (Sinkeldam *et al.*, 1984).

In order to quantify the relationship between dietary pyridoxine and reduction of lymphocyte counts by caramel colour III, and to study the effects of age, two 14-day studies were performed, one in

weanling, and the second in mature, Wistar rats; in other respects the protocols were identical. Groups of 10 male animals were fed diets containing approximately 2.5, 6, 12, or 24 ppm of pyridoxine. At each dietary level groups were given caramel colour III¹ in the drinking water at levels of 0, 1, 4, or 8%. A clear inverse dose relationship between the severity of lymphocyte depression and the pyridoxine level of the diet was observed. In weanling rats fed a diet containing 2.5 ppm pyridoxine, statistically significant lymphocyte reduction occurred at all caramel colour III levels on days 6 and 13. Groups fed diets containing 6, 12, or 24 ppm pyridoxine did not show statistically significant reductions in lymphocyte counts on day 6. However, on day 13, groups receiving 4 or 8% caramel colour III and 6 ppm pyridoxine in the diet had a statistically significant reduction in lymphocytes. On day 13 at a dietary level of 12 ppm pyridoxine only the 8% caramel colour III group had a statistically significant reduction in lymphocytes. There were no significant reductions in lymphocyte counts in animals fed 24 ppm pyridoxine at any level of caramel colour III intake. In mature rats fed 4 and 8% caramel colour III a statistically significant reduction in lymphocytes occurred at dietary pyridoxine levels of 12 ppm and lower on day 6. On day 13 lymphocytes were significantly decreased at caramel colour III doses 1, 4, and 8% in animals fed a diet containing 2.5 ppm pyridoxine. There were no significant decreases in lymphocytes at these dose levels of caramel colour III in animals fed 6, 12, or 24 ppm pyridoxine (Sinkeldam, 1981; 1982a).

¹ This sample of caramel colour III contained 107 mg/kg 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI) on a colour equivalent basis, 204 mg THI/kg on an 'as is' basis, and 295 mg THI/kg on a solids basis.

Special studies on 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI)

An isolation procedure was developed and a fraction of caramel colour III that contained the lymphocyte-depressing activity was isolated. The single component of this fraction that was responsible for the activity was identified as THI (Kroplien *et al.*, 1984).

Rats

THI was administered to groups of 10 male weanling Wistar rats at levels of 0, 2, 5, or 20 ppm in drinking water for 7 days; a fifth group received a 1% solution of caramel colour III in drinking water as a positive control. THI produced a marked depression of lymphocyte counts at all dose levels and a dose-dependent increase in

neutrophils. The lymphocyte-depressing potency of 2 ppm THI was comparable to that of 1% caramel colour III in the drinking water, indicating a level of approximately 200 mg/kg THI in the caramel sample. This compares with the result of chemical analysis of a sample of this batch of caramel colour III, which indicated a value of 204 mg/kg THI on an 'as is' basis (Sinkeldam, 1982b; Kroplien, 1984).

Special studies on carcinogenicity (see also long-term studies)

Mice

Three groups of 50 male and 50 female B₆C₃F₁ mice were given drinking water containing 0, 1.25, or 5% caramel colour III for 96 weeks followed by 8 weeks of drinking water without caramel colour; the animals were fed CFR diet ad libitum. The caramel colour III used in this study contained less than 25 mg/kg THI.

The animals were observed daily for abnormalities and mice showing signs of ill-health were isolated to be returned to the group if their condition improved but otherwise killed and autopsied. Individual body weights were recorded weekly for the first 14 weeks, then every-other-week. Food and water consumption were recorded over a 2-day period before each weighing. During week 104, fresh urine samples were obtained from all survivors and analysed for pH, protein, glucose, bilirubin, ketones, occult blood, and urobilinogen. At termination the animals were killed by exsanguination under ether anaesthesia and haematological examinations were performed, which consisted of measurements of haemoglobin concentrations, haematocrit values, erythrocyte counts, leucocyte counts, platelet counts, and differential leucocyte counts. At autopsy, gross findings were recorded and the following organs weighed: brain, heart, liver, spleen, adrenals, and testes or ovaries. Samples of these organs and of the salivary gland, trachea, lungs, thymus, lymph nodes, stomach, small intestine, pancreas, urinary bladder, pituitary, thyroid, prostate, seminal vesicle, uterus, mammary gland, skeletal muscle,

eye, Harderian glands, spinal cord, sciatic nerve, and any other tissues of abnormal appearance were examined histologically (haematoxylin- and eosin-stained). Histopathological examinations were also performed on mice that died and on those that were killed in moribund condition. During the in-life phase no consistent differences were noted between the test and control groups with respect to growth or water intake.

The cumulative mortality of males given 5% caramel colour III was higher than that of controls from week 100 to the end of the experiment, but there were no clear pathological differences in any

organs and no treatment-related abnormalities in urinalyses.

The only statistically significant differences in haematological parameters between control and treated groups were elevations of the total leucocyte counts in males of both treatment groups, but the observed values were within the range encountered for the strain of B₆C₃F₁ mice used in the study. No treatment-related gross pathology was noted during or at the end of the experiment. Malignant lymphoma/leukaemia, hepatocellular carcinoma, and sub-cutaneous fibrosarcoma and/or malignant fibrous histiocytoma were frequent, but no significant differences were found in their incidences between treated and control groups. Adenomas and adenocarcinomas of the lungs, hyperplastic nodules of the liver, and fibroma of the sub-cutis were frequent in males, but their incidences were similar in treated and untreated mice.

The authors concluded that, under the conditions used, caramel colour III was not carcinogenic for B₆C₃F₁ mice (Hagiwara *et al.*, 1983).

Rats

Three groups of 50 male and 50 female F344 rats were given caramel colour III in drinking water at levels of 0, 1, or 4% for 104 weeks followed by drinking water without caramel for 9 weeks. The animals were fed *ad libitum* basal diet that contained 11-12 mg/kg pyridoxine, and the caramel colour III used contained less than 25 mg/kg THI. During the experimental period, all animals were observed daily, and clinical signs and mortality were recorded. Body weights were recorded weekly during the first 13 weeks of the study and then every 4 weeks. Moribund or dead animals and animals sacrificed at termination were autopsied and examined for the development of tumours in the following organs and tissues: brain, pituitary, thyroid (including parathyroid), thymus, lungs, trachea, heart, salivary glands, liver, spleen, kidneys, adrenals, tongue, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads, accessory genital organs, mammary gland, skin, musculature, peripheral nerve, spinal cord, sternum, femur, eyes, ear duct, and nasal cavity. These tissues were also examined histologically (haematoxylin- and eosin-stained).

No treatment-related differences in growth or survival rates were noted. No dose-related effects were found either in the incidence or induction time of tumours in the various organs and tissues except in the pituitary gland of males of the top-dose group in which the incidence of tumours was significantly higher than that in controls. However, pituitary tumours are among the most common spontaneous

tumours in F344 rats that occur with variable incidence; most of the tumours were microscopic and there were no significant differences in their induction times compared with controls. The authors concluded that the higher incidence of pituitary tumours was not related to caramel administration, but could be explained by the variability of spontaneous tumour incidence (Maekawa et al., 1983).

Special studies on mutagenicity

Caramel colour III (a blend of 3 commercial samples) was evaluated by the Ames Salmonella/microsome plate test and the Saccharomyces/microsome plate test. The Salmonella test organisms used were TA98, TA100, TA1535, TA1537, and TA1538; the Saccharomyces was strain D4, and the tests were conducted in the presence and absence of liver S-9 mix from Aroclor-induced rats. The dose range employed was 1-50 mg/plate for TA100 and 1-20 mg/plate for all other test strains. Caramel colour III was non-mutagenic to the test organisms under these conditions (Jagannath & Brusick, 1978a).

In a similar study using the same Salmonella tester strains, caramel colour III was non-mutagenic in a range of concentrations up to 20 µl/plate with or without metabolic activation (Richold & Jones, 1980).

Caramel colour III was non-mutagenic in the Ames test against Salmonella strains TA98 and KTA100 at concentrations of 0-1 mg/plate, with or without metabolic activation. Acidic and basic organic extracts of the caramel were also non-mutagenic (Ashoor & Monte, 1983).

Thirteen commercial caramel colours (not identified) were examined for mutagenicity in the Ames test, using Salmonella strains TA98 and TA100 with and without metabolic activation, and for DNA-damaging effects in E. coli (Wild/pol A⁻, Wild/rec A⁻); none of the samples tested was active under the test conditions (Kawana et al., 1980).

Five samples of commercial caramel colour (not identified) were tested for mutagenicity in the Ames test using Salmonella strains TA98 and TA100. All samples gave equivocal results with TA100 and 2 of the 5 samples were equivocal with TA98 (Kawachi et al., 1980).

Five samples of caramel colour were tested in the Ames Salmonella plate assay using strains TA98, TA100, and TA1537 without metabolic activation but with a 20-minute preincubation step. The same samples were used in chromosome aberration tests performed on a cultured Chinese hamster lung fibroblast cell line, both in the presence and absence of S-9 fraction. All samples of caramel colour

were designated as positive both in the Ames assay and in the chromosome aberration test (aberrations in 20% of metaphase cells) (Ishidate & Yoshikawa, 1980).

The same series of caramel colour samples was tested by Jagannath and Brusick, and all were found to be non-mutagenic in the Ames test using Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, and Saccharomyces strain D4, in the presence or absence of S-9 mix. One of the samples in this study and in the Ishidate & Yoshikawa (1980) study was caramel colour III (Jagannath & Brusick, 1978b).

The mutagenicity of a series of samples taken at various stages in the manufacture of caramel colour III were assayed in the Ames test using Salmonella strains TA98, TA100, and TA1535, with and without metabolic activation. No mutagenicity was seen with any sample against all three tester strains in the presence of S-9 fraction but an increased number of revertants was found with strain TA100 in the absence of S-9 fraction. Mutagenic activity was associated with samples taken late in the process (Jensen et al., 1983).

A sample of caramel colour III was non-mutagenic in the Ames test against Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 both in the presence and absence of rat hepatic S-9 fraction at caramel colour concentrations up to 5 mg/plate (Richold et al., 1984).

The same sample as that used by Richold et al. (1984b) was tested for potential mutagenic activity based on induction of unscheduled DNA synthesis in cultured human epithelial (HeLa 53) cells both in the presence and the absence of rat liver S-9 fraction. The test was performed on two occasions at caramel colour III concentrations of 25-51,200 µg/ml in the culture medium. In both tests, caramel colour III caused a significant increase in the number of silver grains found over cell nuclei at concentrations of 6,400 and 12,800 µg/ml in the absence of S-9 mix; significant increases were not observed at higher or lower concentrations in the absence of S-9 mix nor at any of the concentrations tested in the presence of S-9 mix. The authors concluded that, although reproducible effects on unscheduled DNA synthesis were demonstrated in the test, this effect may be moderated by metabolism (Allen & Proudlock, 1984).

Caramel colour III was not clastogenic to cultured Chinese hamster ovarian cells at concentrations of 500, 2500, or 5000 µg/ml either in the presence or absence of rat liver S-9 mix (Allen et al., 1984).

The clastogenicity of caramel colour III was evaluated in an in vitro cytogenetic assay using cultured Chinese hamster ovarian

cells. A significant increase in chromosome aberrations was observed at concentrations of 3 mg/ml or higher in the absence of metabolic activation; similar findings were reported for sodium ascorbate at 2 mg/ml (Galloway & Brusick, 1981a).

These tests were repeated in the presence of rat liver S-9 fraction, and neither caramel colour III at a concentration up to 5 mg/ml nor sodium ascorbate at a concentration of 5 mg/ml was active (Galloway & Brusick, 1981b).

In an in vivo mouse micronucleus test, caramel colour III was administered by gavage to 5 males and 5 females at doses of 0, 1.05, or 3.5 g/kg b.w. (2 doses 24 hours apart). Caramel colour III did not increase the incidence of micronuclei in polychromatic erythrocytes obtained from marrow and was considered by the authors not to exhibit clastogenic activity under the conditions of the test (Cimino & Brusick, 1981).

Special studies on teratogenicity

Teratogenicity studies were carried out on caramel colour III in mice, rats, and rabbits. The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. in all three species.

Mice

Caramel colour III was administered by gavage to groups of 22 or 23 pregnant CD1 mice at the above doses beginning on day 6 and continuing through day 15 of gestation. On day 17, all dams were subjected to Caesarian section under anaesthesia and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The urogenital tract of each dam was examined for anatomical abnormalities and all fetuses were examined grossly for external abnormalities. One-third of the fetuses from each litter were examined for visceral abnormalities using the Wilson technique, and the remaining two-thirds were examined for skeletal defects after clearing and staining with Alizarin Red. Caramel colour III had no clearly discernible effects on nidation nor on maternal or foetal survival. The number of abnormalities of either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974).

Rats

Caramel colour III was administered by gavage to groups of 21-24 pregnant Wistar rats at the above doses beginning on day 6 and continuing daily through day 15 of gestation. On day 20, all dams were subjected to Caesarian section under anaesthesia and the numbers of

implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of live pups were also recorded. All fetuses were examined grossly for external abnormalities. One-third of the fetuses from each litter were examined for visceral abnormalities using the Wilson technique, and the remaining two-thirds were examined for skeletal defects after clearing and staining with Alizarin Red. Caramel colour III had no clearly discernible effect on nidation nor on maternal or foetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974).

Rabbits

Caramel colour III was administered by gavage to groups of 11 or 12 pregnant Dutch-belted female rabbits at the above doses beginning on day 6 and continuing daily through day 18 of pregnancy. On day 29, all does were subjected to Caesarian section under anaesthesia and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. The live fetuses from each litter were then placed in an incubator for 24 hours for evaluation of neonatal survival. Surviving pups were sacrificed and all pups examined for visceral abnormalities by dissection. All fetuses were then cleared and stained with Alizarin Red and examined for skeletal defects. Caramel colour III treatment had no effect on nidation nor on maternal or foetal survival. The number of abnormalities in either soft or skeletal tissues of the test groups did not differ from those occurring spontaneously in controls (Morgareidge, 1974).

Acute toxicity

Species	Route	LD ₅₀ (mg/kg b.w.)	Reference
Rat	oral	> 2.3 ml approx. or eq. 1,900	Foot et al., 1958
Rat	oral	> 25 ml approx. or eq. 17,500	Chacharonis, 1960
Rat	oral	> 30 ml approx. or eq. 20,400	Chacharonis, 1963

No treatment-related effects were detected during the observation of animals for 14 days after administration of single doses of 12 different caramels manufactured with ammonia or sulphate ammonia

catalysts (Foote et al., 1958; Chacharonis, 1960; 1963). Single doses of caramel colour III of up to 10 g/kg b.w. in mice and 15 g/kg b.w. in rabbits did not cause convulsions or others signs of distress (Sharratt, 1971).

Short-term studies

Mice

In a 4- to 6-week study, albino Swiss mice (10 males and 10 females/group) were fed caramel colour III containing 830 ppm 4-methylimidazole at concentrations of 0, 1, 2, 4, 8, or 16% in the diet. No influence of caramel colour III on appearance, behaviour, or food intake was observed. Growth was decreased, especially in the third and fourth weeks, in the groups fed 16% caramel colour III. The faeces of the animals fed the higher dose levels were soft, tarry in appearance, poorly-formed, and sticky or pasty in consistency.

In the males fed 16% caramel colour III, an increase of neutrophilic leucocytes and a decrease in lymphocytes were observed. The mean relative weights of the caeca (full and empty) were increased at the 4, 8, and 16% dietary levels. No other remarkable findings were observed on gross examination; histopathological examinations were not conducted. No information is available on the THI content of the sample of caramel colour III used in this study, nor on the dietary levels of pyridoxine (Procter, 1976).

In a pilot study to select the dose levels for a carcinogenicity study, B₆C₃F₁ mice were fed caramel colour III for 13 weeks. No details of this study were reported (Hagiwara et al., 1983).

Rats

Four groups of 10 male and 10 female rats received either 0 or 10% of 2 different samples of caramel colour III in their diet for 90 days. Weight gains showed slight reductions compared with controls, but food consumption was normal in all groups. No abnormalities were noted regarding haematology, urinalysis, gross pathology, or histopathology. No information is available on the THI content of the samples of caramel colour III used in this study (Charcharonis, 1963).

Four groups of rats received 0, 4, 8, or 16% caramel colour III in their diet for 3 months. No convulsions or other behavioural abnormality or signs of neurological damage were seen. No macroscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971).

Six groups of 15 rats (CFE strain) of each sex were fed diets containing 4, 8, or 16% caramel colour III produced by either an "open" or a "closed" pan process for 13 weeks; a group of 25 rats of each sex served as controls. Body-weight gain was decreased at all dietary levels of both caramel colours. Haemoglobin concentrations were reduced at the highest dietary levels at week 6, while at the lower levels this effect was less clear. After 13 weeks, the males at all dose levels had significantly decreased haemoglobin

concentrations, but this effect occurred in females only at the 8 and 16% levels. In some groups there was a less consistent decrease in the total number of red blood cells at 6 and 13 weeks. The total number of leucocytes was significantly decreased and a lymphocytopenia was present at all dose levels at 6 and 10 weeks; at 13 weeks these changes were observed only in males. At necropsy, decreased weights of the thymus and spleen were observed at the 8 and 16% dose levels. The caecal weights were increased at the 8 and 16% dose levels compared with controls. Increased relative liver and kidney weights suggested an effect on these organs. Changes in the weights of other organs were considered to be related to the differences of body weight between the groups. The volumes of urine excreted during a 6-hour period without water or in the 2-hour period after a water load were lower than the control values. The latter differences were accompanied by higher values for the specific gravity of the urine. The histopathological study did not reveal treatment-related changes. No details of the THI content of the caramel colours used in this study were available (Gaunt *et al.*, 1975).

Seven groups of 20 female Wistar rats were fed diets containing 0, 15, or 30% of 3 types of caramel colour, one of which was a sample of caramel colour III, for 8 weeks followed by a 4-week recovery period. The diets containing caramel colour III caused dose-related decreases in body weights and food efficiency, and also caused diarrhoea at the 30% dietary level. Leucocyte counts at 4 weeks were significantly increased at the highest dose level whereas at 8, 10, and 12 weeks no differences were observed between test groups and controls. The relative weights of the caeca of animals receiving caramel colour III, both filled and empty, were increased at 4 and 8 weeks but reverted to normal after the 4-week recovery period.

Gross examination at autopsy after 8 weeks revealed a slight, dose-related brown discolouration of the mesenteric lymph nodes in a few animals of each test group. After recovery periods of 2 or 4 weeks the discolouration was less intensive, but still visible. Microscopically, the lymph nodes of the test rats showed accumulation of pigment-laden macrophages, which was not noticeably diminished after withdrawal of the caramel for 2 or 4 weeks. No details of the THI content of the caramel colour III used in this study were

available (Sinkeldam & van der Heyden, 1976a).

In a 10-week feeding study, groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing caramel colour III at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 15.0%. In the animals receiving caramel colour III at levels of 5% or higher, the faeces became soft within two weeks and the water content of the faeces was higher than the faeces of controls. Body-weight gains were generally reduced in animals fed caramel colour III, particularly during the last 2-4 weeks of the study.

In rats fed caramel colour III, there were no changes in haemoglobin levels or erythrocyte counts; a significant reduction in lymphocyte counts and a coincident increase in the number of segmented neutrophils was observed at all dose levels. No macroscopic evidence of abnormal pigmentation of the mesenteric lymph nodes was found. An increase in empty caecal weight was consistently evident in animals of both sexes given caramel colour III at the 5, 10, and 15% levels.

Histopathological examination did not reveal changes in the structure of the ileal or caecal mucosa nor in the reticuloendothelial components of the central or peripheral systems. No abnormal pigmentation of the lymph nodes was found. No details of the THI content of the caramel colour used in this study were available (Procter et al., 1976).

Groups of 10 male and 10 female Wistar rats were fed diets containing caramel colour III at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 15.0% for 10 weeks. Caramel colour III caused loose stools, particularly at the 5% dietary level and body weights were slightly decreased in both sexes at this level. Leucocyte (lymphocyte) counts were decreased in males and in females fed 15% caramel colour III; at lower dose levels this effect occurred only in females. The relative weight of the caecum (both full and empty) was increased by feeding caramel colour III. Minimal amounts of pigment were observed in mesenteric lymph nodes of several rats fed 1.25% and higher levels of caramel colour III. From this experiment it appeared that caramel colour III was more active than two other types of caramel tested concurrently in regard to growth depression, enlargement of the caecum, and decrease in leucocyte counts. No details of the THI content were available (Sinkeldam & van der Heyden, 1976b).

In a 10-week study, weanling Wistar rats were fed caramel colour III in the diet at levels of 0, 0.5, 1.0, 2.0, 4.0, or 16%. There were 15 males and 15 females in each group except for the control group, which had 60 animals of each sex, and the highest dose group, which had 10 animals of each sex. Food intake and growth rates were recorded, and haematological examinations and histopathological

studies were carried out. In particular, the lymph nodes, thymus, spleen, and caecum were examined for distribution of pigment.

Another 2 groups of 10 rats were given basal diet or diet containing 16% caramel colour III for 10 weeks followed by a 28-day period during which the basal diet was fed (recovery experiment). Five rats of each sex were killed after 7 days and the remainder after 28 days of the recovery period.

Caramel colour III depressed body-weight gain at dietary levels greater than 1%. Total leucocyte counts were decreased in males in the groups receiving 2, 4, and 16% caramel colour III and in females receiving 4 and 16% caramel colour III. However, the lymphocyte/

neutrophil ratio was significantly decreased in both sexes at all dose levels. Relative liver weights were increased at dietary levels of 2% and higher levels of caramel colour III, and reduced spleen weights were observed at the highest (16%) dose level. Increased relative kidney weights were observed in both sexes at 2% and higher dietary levels of caramel colour III. Increased caecal weights were observed at the 16% dietary level; microscopically, pigment was observed in the mesenteric lymph nodes of male and female rats at this dose level.

During the recovery phase, white cell counts, cell ratios, and total numbers of lymphocytes rapidly returned to normal; recovery was complete in both sexes by 7 days. The caecal weights had returned to normal by 7 days and the relative liver and spleen weights returned to normal during the recovery phase, while kidney weights partially recovered. No details of the THI content of the caramel nor of the pyridoxine content of diet were available (BIBRA, 1977).

Test groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing 10 or 15% caramel colour III for 4 weeks; a control group of 20 rats of each sex received basal diet. During the course of the study, the rats fed diets containing caramel colour III had soft dark-coloured faeces, particularly at the highest dietary level. There were no consistent differences in body-weight gain or food consumption and no mortality occurred. Haematological studies were conducted prior to feeding caramel colour III and after 2 and 4 weeks. In males, total white cell and lymphocyte counts were unaffected by treatment at either sampling interval or dose level. In females, total white cell and lymphocyte numbers were significantly depressed after 4 weeks but not at 2 weeks at both dose levels. However, significant differences in differential white cell counts were observed in both sexes. In males, the differential lymphocytes (%) were significantly depressed at both dose levels and treatment intervals, and there was a concomitant increase in segmented neutrophils. In females, the differential counts of lymphocytes and segmented neutrophils were

similarly affected after 4 weeks.

At necropsy, increased caecal weights were observed in both sexes and a statistically significant increase in relative weights of the thymus in male rats fed both doses of caramel colour III was noted. Histopathological studies were not conducted. Details of the THI content of the caramel sample used were not available (Procter, 1977).

In a 13-week toxicity study, caramel colour III was administered in drinking water at concentrations of 0, 4, 6, 8, or 10% to groups of 10 male and 10 female weanling Wistar rats. The caramel colour III sample used was analysed and found to contain 78 mg/kg THI on an 'as is' basis, 105 mg/kg THI on a solids basis. The diet fed to the rats contained approximately 13 mg/kg pyridoxine. The general condition and behaviour of the animals was checked at frequent intervals, individual body weights were measured weekly, food consumption was measured (on a

cage basis) over weekly periods during the whole experimental period, and fluid consumption was measured daily (on a cage basis). Samples of blood for haematology were collected from the tip of the tail of all rats initially and at days 29/30, 57/58, and 84/85. Urinalysis was performed on individual urine samples from all animals during the last 16 hours of a 24-hour period of deprivation of food and water at day 87. Tail tip blood collected at day 87 was examined for glucose and urea nitrogen. Blood was obtained from the aorta under ether anaesthesia at termination (day 91 for males, 92 for females), and the following assays performed on the plasma; alkaline phosphatase, GPT, GOT, lactate dehydrogenase, total protein, and albumin. Pyridoxal phosphate was measured in plasma from EDTA-treated blood samples. At autopsy the following organs were weighed: thyroid, adrenals, testes/ovaries, kidneys, thymus, brain, spleen, heart, liver, and caecum (full and empty). Histological examinations were carried out on all animals of the control and top-dose groups and, in addition to the weighed organs, the following organs/tissues were examined: aorta, axillary and mesenteric lymph nodes, cervix, colon, oesophagus, stomach, duodenum, ileum, jejunum, lungs, epididymides, pituitary, prostate, skeletal muscle, skin, sternum, pancreas, trachea, urinary bladder, uterus, and all gross lesions.

There was a dose-related decrease in fluid consumption, decreased urinary output of more concentrated urine, decreased food consumption, and decreased body-weight gain in both sexes. These changes were related to the palatability of the drinking fluid. No outstanding differences were observed in red blood cell analyses between test groups and controls. Total lymphocyte counts were relatively low in all test groups of both sexes. The differences from control values were statistically significant in all male treatment groups after 29/30 days, in the female 8% group after 29/30 days, and in the male 4

and 8% groups after 57/58 days. However, these differences did not show a clear dose-dependent relationship and at 13 weeks there were no significant differences among any of the treatment groups compared to controls.

At necropsy, the relative weights of the kidneys and caecum were increased in several groups receiving caramel colour III. The increase in kidney weight was dose-dependent, but no treatment-related histopathological changes were seen in any of the organs examined and the enlargement of the kidneys was attributed to the decreased fluid consumption by the treated rats. No effects on the relative weights of spleen or thymus were observed and these tissues were histologically normal (Sinkeldam *et al.*, 1980b).

A 13-week toxicity study was conducted with caramel colour III given in the drinking water at concentrations of 0, 5, 10, 15, or 20% to groups of 10 male and 10 female weanling Wistar rats. The caramel colour III sample used in this study contained 0-3 mg/kg THI on an 'as is' basis. The diet fed to the rats contained 13.5 mg/kg pyridoxine.

The protocol used was similar to that described in the previous study except that blood samples were collected at days 30/31, 57/58, and 80/81, and urinalysis was performed at day 85.

Water intake (fluid intake corrected for caramel colour solids content) showed a dose-related decrease in both sexes. Food intake was also generally lower in all treated groups. Body-weight gains of males were decreased in a dose-related manner, while body-weight gains of females were comparable to controls. Urine volumes were decreased in rats treated with caramel colour III and the urines were more concentrated in treated groups than in controls. These changes were attributed to the decreased water intake. The urine was darker-coloured at the 15 and 20% dose levels; however, urine composition was essentially normal. Lymphocyte counts were in general relatively low in all test groups in both sexes but the differences only attained statistical significance in males of the top-dose group after 30 days and males of all treatment groups after 57 days. No significant differences in lymphocyte counts were observed in males of any group after 80 days, nor in females at any dose level at any time interval. Mean neutrophil counts were significantly increased in females receiving 20% caramel colour III for 81 days.

Although the relative weights of the caecum, liver, brain, kidneys, and testes were increased in several test groups, there were no pathological changes in any of these organs. Treatment-related microscopic changes consisting of increased numbers of macrophages containing a yellow-brown PAS-positive pigment were found in the mesenteric lymph nodes. These were the only treatment-related changes

noted (Sinkeldam et al., 1980a).

Groups of 10 male and 10 female weanling F344 rats were given caramel colour III in the drinking water at concentrations of 0, 0.5, 1.0, 2.0, 4.0, or 8.0% for 4 weeks. The sample of caramel colour III used was found to contain 70 mg/kg THI on an 'as is' basis and the NIH 07 Open Formula Mouse and Rat diet used in this study contained 17 mg/kg pyridoxine.

No differences in body-weight gain were noted for any of the test groups, although food consumption of the males was significantly lower than controls throughout the study. Transient decreases in lymphocyte counts were noted among the treatment groups at the midpoint of the study, but no significant differences between control and treated groups were noted at 1 month. Other haematological and clinical chemistry parameters were normal for all groups. At necropsy no differences in organ weights were noted between treated and control groups and there were no gross or microscopic pathological changes related to treatment (Heidt and Rao, 1981).

Groups of 10 male and 10 female F344 rats were given caramel colour III in the drinking water at concentrations of 0, 1.25, 2.5, 5.0, 10.0, or 20.0% for 13 weeks in order to select doses for long-term carcinogenicity/chronic toxicity studies. Rats were given 20 ml/day of these solutions and basal diet (CRF-1, Charles River Japan, Inc.) was available ad libitum. The caramel colour III used contained 78 mg/kg THI on an 'as is' basis and the diet contained 11-12 mg/kg pyridoxine. During the experimental period, all animals were observed daily and clinical signs were recorded. Body weights were measured every other week and haematological examinations were performed every 4 weeks in the control and 20% groups. At the end of the study, all survivors were sacrificed for gross and microscopic examination.

Weight gains were less in all experimental groups than in the control group from the first experimental week, and in week 13 the weight gains of the 1.25, 2.5, 5.0, 10.0, and 20.0% groups were 89, 94, 84, 76, and 76%, respectively, of that of controls for males and 96, 98, 80, 84, and 92%, respectively, for females. Except in the male 2.5% group and the female 1.25, 2.5, and 20.0% groups, the differences in weight gain from the controls were significant. No haematological changes were observed either during or at the end of the experimental period. At necropsy, no pronounced macroscopic changes were observed in any animals, although a few rats in the experimental groups were very emaciated. No histological changes related to caramel colour III administration were found in any experimental groups.

From these results, the authors concluded that 1 and 4% caramel

colour III in drinking water were the appropriate dose levels for a carcinogenicity study (Maekawa et al., 1983).

In a 90-day toxicity study, 2 samples of caramel colour III were used, one containing approximately 15 mg/kg THI on a solids basis (batch A) and the other containing 295 mg/kg THI on a solids basis (batch B). Groups of 20 male and 20 female weanling F344 rats were given caramel colour III in drinking water at dose levels of 0, 10, 15, or 20 g/kg b.w. of batch A and 20 g/kg b.w. of batch B. The NIH 07 Open Formula Mouse and Rat diet used in this study contained more than 10 mg/kg pyridoxine. During the study, body weights and food intake were recorded weekly. Fluid consumption was recorded 3 times per week and concentrations of the test material were adjusted on the basis of body weight and fluid intake to give the required dose. Haematological analyses were performed at 2 and 6 weeks and at termination, and clinical chemistry analyses were carried out at 6 weeks and termination. At necropsy, all animals were examined macroscopically and selected organ weights were determined. Histopathological examinations were conducted on the 20 g/kg test groups and on the control animals.

Although the findings were not always consistent, the animals treated at the higher dose levels (15 and 20 g/kg) of batch A and those treated with batch B generally had decreased body weights. All treated groups had significantly decreased food and fluid intake. If fluid intake values are corrected for solid contents of the caramel colours, the values for water intake were markedly below those of the controls. Because of the rather constant fluid intake of the treated groups, it was necessary to increase progressively the caramel colour concentration to maintain a constant intake of the caramel colour on a body-weight basis. It is likely that the effects on body-weight gain and food consumption were due to the reduced water intake of the rats, reflecting the poor palatability of the drinking solution rather than toxic effects of the caramel colour per se.

The haematology studies revealed decreased lymphocyte counts in the male and female rats fed batch B at 2 weeks and in the male rats fed batch B at 6 weeks. All lymphocyte values in these groups were normal at the termination of the study. No decreases in lymphocyte counts occurred in male or female groups fed batch A at any of the dose levels. There were no consistent changes in clinical chemistry values, with the exception of slightly increased values in blood urea nitrogen in the rats treated with batch B. Urinalysis revealed decreased urinary volume and increased specific gravity in rats of both sexes treated with either batch of caramel colour III at 6 weeks. At termination, these differences were only significant in male rats in the top-dose groups (both batches). Treatment-related increases were observed in the absolute and relative weights of the caecum (full

and empty) in animals of both sexes at all dose levels. Dose-related increases occurred in absolute and relative kidney weights and were considered to reflect compensatory hypertrophy as a consequence of reduced water intake; there were no histopathological changes in the kidneys of any of the test groups. A dose-related decrease in the absolute weight of the thymus was observed, which reached statistical significance in the top-dose group males with both samples of caramel colour III and top-dose group females with batch B; this decrease was not evident when the thymus weight was expressed relative to body weight. Other differences in absolute and relative organ weights appeared to be a consequence of the dose-related reduction in body weight.

The only treatment-related microscopic changes noted were minimal to moderate accumulation of pigment in the tissues of the intestinal tract and mesenteric lymph nodes without pathologic alteration.

Although statistically-significant changes were identified in some clinical and anatomical parameters, the authors did not consider them to be toxicologically important. On this basis, the no-adverse-effect level for ammonia caramel III was considered to be 20 g/kg b.w. (MacKenzie, 1985).

A paired-feeding study was conducted to determine whether poor palatability is the mechanism underlying the decreased body-weight gains frequently noted in toxicity studies of caramel colour III. A group of 10 male Wistar rats were fed 12% caramel colour III in the drinking water, and similar groups were permitted a limited intake of food or water equivalent to that consumed by the caramel colour III group. Body-weight gain and food and fluid intake were decreased in the group fed caramel colour III. A similar decrease in body-weight gain was noted in the groups of rats restricted to the equivalent intake of either food or water. The author concluded that the growth depression observed in rats when caramel colour III is fed in the drinking water is the result of decreased fluid and food intake. Poor palatability of drinking fluid containing caramel colour III was the probable cause of these changes (Sinkeldam, 1979).

Long-term studies

Rats

Four groups of 48 male and 48 female Wistar rats were given diets containing 0, 1, 3, or 6% caramel colour III (ammonia catalysed "half open-half closed pan" caramel; no indication about the presence of 4-methylimidazole was given) for 2 years. Food and water intake, growth, mortality, and organ weights were measured; haematological examinations, urinalyses, kidney function tests, and histopathological

examinations also were carried out.

A decrease in growth, which was significant in the males, was observed at all dose levels. This effect was accompanied by a reduction in the cumulative food intake. A significant reduction in white cell number (in the 6% group) was associated in the early part of the study with a lymphocytopenia, which was present until week 80 in the male rats fed a diet containing 3 or 6% caramel. In the female animals the lymphocytopenia was present until week 52 in these 2 groups (the 1% group was not tested).

Spleen weights were reduced in a dose-related manner. The relative weights of the (full) caeca were clearly increased at all dose levels. No changes were found in the pancreata of the control animals, while in the test groups (not dose-related) a total of 10 hyperplastic changes were found. However, the number of tumours of the pancreas showed no relation to the administration of caramel. There was no evidence of a carcinogenic effect.

The authors concluded that a no-observed-effect level could not be established for the caramel used in this particular study (Evans et al., 1976).

Observations in man

In a number of animal studies with caramel colour III, a decrease in the total number of leucocytes associated with a decrease in the number of lymphocytes was noted. A pilot study in humans was carried out in which 1.5 g of caramel colour III (prepared by a closed-pan process) was ingested daily by 9 volunteers for 21 days. Total circulating leucocytes, lymphocytes, and erythrocytes, together with haemoglobin concentrations, were measured prior to and during the treatment. No changes were found that could be attributed to treatment with caramel colour III. In this experiment 6 of the subjects showed no differences from normal in stool frequency or condition. Three volunteers occasionally had soft stools; no control groups were used (BIBRA, 1976).

Comments

The temporary ADI for caramel colour III was revoked at the twenty-first meeting of the Committee due to its effects on circulating total leucocytes and lymphocytes. Since that time, a component of caramel colour III, 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI), has been identified and shown to cause a depression of lymphocyte counts; the lymphocyte depression caused by caramel colour III has been shown to be due largely, if not solely, to this minor component. Comparison of the lymphocyte-

depressing activity of pure THI with a batch of caramel colour III containing a known level of THI indicated that other components of this batch had an insignificant activity. The lymphocyte depression was largely ameliorated by dietary pyridoxine. In studies on samples of caramel containing 10 mg/kg THI, no effects on lymphocyte counts were observed with adequate dietary levels of pyridoxine.

Long-term studies in rats and mice indicate that caramel colour III is not carcinogenic at dose levels of up to 4% in drinking water, which was also the no-effect level in these long-term studies.

EVALUATION

Level causing no toxicological effect

As it was not possible to include caramel colour III at higher levels than 4% in drinking water in long-term studies, and as the effect of most concern, i.e. lymphopenia, could best be evaluated from short-term studies, the Committee based its evaluation on the no-effect level of 20 g/kg b.w./day in a 90-day study in rats using caramel colour III which contained approximately 15 ppm THI on a solids basis (10 ppm on an 'as is' basis).

Estimate of acceptable daily intake for man

0-200 mg/kg b.w. (0-150 mg/kg b.w. on a solids basis).

Further work or information

Desired

Analytical data to confirm that the sample on which the evaluation is based is representative of current commercial samples.

Studies to confirm that THI is the sole component of caramel colour III that has lymphocyte-depressing activity and to establish a no-effect level for THI.

REFERENCES

- Allen, J.A., Brooker, P.C., Birt, D.M., & McCaffrey, K.J. (1984). Analysis of metaphase chromosomes obtained from CHO cells cultured in vitro and treated with caramel colour III. Unpublished report No. ITC 3B/84966 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.
- Allen, J.A. & Proudlock, R.J. (1984). Autoradiographic assessment of

DNA repair in mammalian cells after exposure to caramel colour III. Unpublished report from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.

Ashoor, S.H. & Monte, W.C. (1983). Mutagenicity of commercial caramels. Cancer Letters 18, 187-190.

BIBRA (1976). A study of the haematological effects of caramel in human volunteers. Unpublished report No. 1/172/76 from the British Industrial Biological Research Association, Carshalton, Surrey, England.

Chacharonis, P. (1960). Acute and chronic toxicity studies on caramel colours A and B. Unpublished report No. S.A. 54219 from Scientific Associates Inc., St. Louis, MO, USA.

Chacharonis, P. (1963). Acute oral toxicity study in rats on caramel colorings 25A-1, 30B-0, and 30F-1. Unpublished report No. S.A. 79105 from Scientific Associates Inc., St. Louis, MO, USA.

Cimino, M.C. & Brusick, D.J. (1981). Mutagenicity evaluation of ETA 48-IH caramel color in the mouse micronucleus test. Unpublished report No. 22129 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.

Evans, J.G., Butterworth, K.R., Gaunt, I.F., & Grasso, P. (1976). Long-term toxicity study in the rat of a caramel produced by the "half-open-half closed pan" ammonia process. Unpublished report No. 6/1976 from the British Industrial Biological Research Association, Carshalton, Surrey, England.

Foote, W.L., Robinson, R.F., & Davidson, R.S. (1958). Toxicity of caramel color products. Unpublished report of Battelle Memorial Institute, Columbus, OH, USA.

Galloway, S.M. & Brusick, D.J. (1981a). Mutagenicity evaluation of ETA-48-IH in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.

Galloway, S.M. & Brusick, D.J. (1981b). Mutagenicity evaluation of ETA-48-IH in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Part II. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical

Caramel Association.

- Gaunt, I.F., Lloyd, A.G., Grasso, P., Gangolli, S.P., & Butterworth, K.R. (1975). Toxicological investigations of caramels. I. A short-term study in the rat with two caramels produced by variations of the ammonia process. Unpublished report No. 14 from the British Industrial Biological Research Association, Carshalton, Surrey, England.
- Hagiwara, A., Shibata, M., Kurata, Y., Seki, K., Furushima, S., & Ito, N. (1983). Long-term toxicity and carcinogenicity test of ammonia-process caramel colouring given to B₆C₃F₁ mice in the drinking water. Fd. Chem. Toxicol. 21, 701-706.
- Haldi, J., & Wynn, W. (1951). A study to determine whether or not caramel has any harmful physiological effect. I. Unpublished report from Emory University, Atlanta, GA, USA.
- Heidt, M. & Rao, G.N. (1981). Subchronic toxicity study of ammonia caramel color type AC2 in rats. Unpublished report No. 80036 from Raltech Scientific Services, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.
- Ishidate, M. Jr. & Yoshikawa, K. (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation - a comparative study on mutagens and carcinogens. Arch. Toxicol. Suppl. 4, 41-44.
- Jagannath, D.R. & Brusick, D. (1978a). Mutagenicity evaluation of ETA 4-10, ETA 4-11, ETA 4-15 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Jagannath, D.R. & Brusick, D. (1978b). Mutagenicity evaluation of ETA 4-8, ETA 4-9, ETA 4-12, ETA 4-13, ETA 4-14 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Jensen, N.J., Willumsen, D., & Knudsen, I. (1983). Mutagenic activity at different stages of an industrial ammonia caramel process detected in Salmonella typhimurium TA 100 following pre-incubation. Fd. Chem. Toxicol. 21, 527-530.
- Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M., & Sugiyama, T. (1980). Cooperative programme on short-term

assays for carcinogenicity in Japan. In Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Scientific Publication No. 27, 323-330. International Agency for Research on Cancer, Lyon, France.

Kawana, K., Akema, R., Nakaoka, T., Ikeda, H., Takimoto, T., & Kawauchi, S. (1980). Studies on mutagenicities of natural food additives. II. Mutagenicities and antibacterial activities of caramels. Eisei Kagaku 26, 259-263.

Kroplien, U. (1984). Letter dated 17 December 1984 to International Technical Caramel Association, submitted to WHO.

Kroplien, U., Rosdorfer, J., van der Greef, J., Long, R.C. Jr., & Goldstein, J.H. (1985). 2-Acetyl-4(5)-(tetrahydroxybutyl)-imidazole: Detection in commercial caramel colour III and preparation by a model browning reaction. J. Org. Chem. 50, 1131-1133.

MacKenzie, K.M. (1985). 90-day toxicity study of caramel color (ammonia process) in rats. Vols. I & II. Unpublished report No. 6154-107 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.

Maekawa, A., Ogiu, T., Matsuoka, C., Onodera, H., Furuta, K., Tanigawa, H., Hayashi, Y., & Odashima, S. (1983). Carcinogenicity study of ammonia-process caramel in F344 rats. Fd. Chem. Toxicol. 21, 237-244.

Morgareidge, K. (1974). Teratologic evaluation of FDA 71-82 (caramel, bakers and confectioners) in mice, rats and rabbits. Unpublished report No. PB 234-870 from Food and Drug Research Laboratories Inc., Waverly, NY; USA.

Nishie, K., Waiss, A.C., & Keyl, A.C. (1969). Toxicity of methylimidazoles. Toxicol. Appl. Pharmacol. 14, 301-307.

Nishie, K., Waiss, A.C., & Keyl, A.C. (1970). Pharmacology of alkyl and hydroxyalkylpyrazines. Toxicol. Appl. Pharmacol. 17, 1.

Procter, B.G. (1976). A preliminary evaluation of the potential toxicological effects of ammonia caramel in mice. Unpublished report No. 5705 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

Procter, B.G. (1977). A preliminary evaluation of the potential toxicological effects of ammonia caramel (U.S. origin) in rats.

Unpublished report No. 5617 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada. Submitted to WHO by International Technical Caramel Association.

Procter, B., Berry, G., & Chappel, C.I. (1976). A toxicological evaluation of various caramels fed to albino rats. Unpublished report No. 4244 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

Richold, M. & Jones, E. (1980). Ames metabolic activation test to assess the potential mutagenic effect of ETA-48-1H. Unpublished report No. FDC 8/80358 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.

Richold, M., Jones, E., & Fenner, L.A. (1984). Ames metabolic activation test to assess the potential mutagenic effect of caramel colour III. Unpublished report No. ITC 1B/84709 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by International Technical Caramel Association.

Sharratt, M. (1971). Unpublished report.

Sinkeldam, E.J. (1979). Paired feeding test in rats with caramel (AC₃) in drinking water. Unpublished report No. R 6157 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J. (1981). Quantitative relationship between dietary pyridoxine content and lymphocyte counts in rats fed ammonia caramel. Unpublished report No. V81.390/211704 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J. (1982a). Quantitative relationship between dietary pyridoxine content and lymphocyte counts in mature rats fed ammonia caramel. Unpublished report No. V82.102/220102 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J. (1982b). Short-term (7 day) bioassay in rats with three dose levels of 2-acetyl-4(5)-tetrahydroxybutylimidazole (13-A-82). Unpublished report No. V82.291/221153 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The

Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J., Bruyntjes, J.P., & Kuper, C.F. (1980a). Sub-chronic (13-week) oral toxicity study with a modified ammonia caramel (ETA-26-1) in rats. Unpublished report No. R 6483 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J., Roverts, W.G., & Kuper, C.F. (1980b). Sub-chronic (13-week) oral toxicity study with ammonia caramel (AC₂) in rats. Unpublished report No. R 6121 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands. Submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J., de Groot, A.P., van den Berg, H., & Chappel, C.I. (1984). The effect of vitamin B₆ on the number of lymphocytes in blood of rats fed caramel colour (ammonia process). Unpublished report submitted to WHO by International Technical Caramel Association.

Sinkeldam, E.J. & van der Heyden, C.A. (1976a). Short-term feeding test with three types of caramels in albino rats. Unpublished report No. R 4789 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.

Sinkeldam, E.J. & van der Heyden, C.A. (1976b). Short-term (10 week) feeding study in rats with three different ammonia caramels. Unpublished report No. R 5120 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.

CARAMEL COLOUR IV

EXPLANATION

The report of the twenty-fourth meeting of the Committee (Annex 1, reference 53) drew attention to the need for adequate specifications for caramel colour IV and for a long-term study of carcinogenicity. The temporary ADI of 0-100 mg/kg b.w. was extended pending the results of long-term toxicity studies.

BIOLOGICAL DATA

Biochemical Aspects

Absorption, distribution, and excretion

Pigmentation of tissues of the lymphoreticular system, particularly of the mesenteric lymph nodes, has been a frequent observation in rats fed high levels of caramel colour. A study was undertaken to determine the distribution of the colour component of caramel colour in rats and to determine whether accumulation of caramel colour was the cause of pigmentation in the mesenteric lymph nodes.

Experimental batches of unlabelled and ^{14}C -labelled caramel colour IV were prepared and a concentrate of the higher molecular-weight colour components was made by ultrafiltration. The absorption, tissue distribution, and excretion of this fraction was studied in male F344 rats after a single oral gavage of 2.5 g/kg b.w. ^{14}C -Labelled material was administered to naive animals and to animals which had received 2.5 g/kg b.w. unlabelled material in drinking water daily for 13 days prior to administration of ^{14}C -labelled material.

Differences between the results in naive and pretreated animals were small. Most of the colour components were not absorbed, but instead were excreted in the faeces, mainly within 48 hours. By 96 hours after dosing, 99.7-102.4% of the administered dose was excreted in the faeces, with only 1-2% in urine and insignificant amounts ($< 0.1\%$) as $^{14}\text{CO}_2$ in expired air.

Groups of 4 rats were killed at intervals of 4, 8, 12, 24, and 96 hours after receiving the single oral dose of ^{14}C -labelled material, and radioactivity was measured in the following tissues/organs; blood, brain, heart, lungs, liver, kidneys, spleen, thymus, mesenteric and cervical lymph nodes, gastrointestinal tract (contents and tissues), and carcass. Most of the radioactivity was located in the gastrointestinal tract, and only low levels of radioactivity were found in blood and tissues. The specific activity in the thymus, mesenteric lymph nodes, spleen, kidneys, and liver exceeded that in

blood, but with the exception of the mesenteric lymph nodes, the radioactivity was cleared rapidly over the 96-hour study period. With the exception of the gastrointestinal tract, the highest tissue levels attained, in the liver and kidneys, never exceeded 0.02% and 0.01% of the administered dose.

The results demonstrate that, after administration of large doses of the coloured components of caramel colour IV, only a small fraction was absorbed, distributed in lymphoreticular tissue, and eventually excreted in the urine; retention by the mesenteric lymph nodes

appeared to account for the pigmentation observed in this tissue (Selim, 1982).

Toxicological studies

Special studies on carcinogenicity (See also long-term studies)

Mice

A carcinogenicity study of caramel colour IV was performed on B₆C₃F₁ mice using 5 groups of 50 male and 50 female mice in each group. Two groups of each sex served as controls and the 3 treatment groups received caramel colour IV in the drinking water at dose levels of 2.5, 5.0, or 10.0 g/kg b.w./day for 104 weeks. All animals were observed twice daily and palpated for tissue masses weekly from week 27. Body weights and food intake were recorded weekly, and fluid intake was measured on the first, third, fifth, and seventh days of the first 12 weeks and over a 48-hour period each week thereafter.

A complete necropsy was performed on all animals dying on test or sacrificed in a moribund condition, and on all survivors at termination. Histology (haematoxylin and eosin staining) was performed on all gross lesions and on the following organs/tissues of all animals: adrenals, brain (three sections), bone, bone marrow, epididymis, oesophagus, eyes, gall bladder, heart, intestine (duodenum, ileum, caecum and colon), kidneys, liver, lymph nodes (cervical, mesenteric, and thoracic), mammary glands, ovaries, pancreas, parathyroid, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord, spleen, stomach, testes, thymus, thyroids, trachea, urinary bladder, and uterus.

Four animals were found in the wrong cages during week 15 and had probably been misplaced 9 weeks earlier. These animals were removed from the study during week 31 and were not included in subsequent examinations.

Although during the course of this study there were sporadic significant differences in mean body weights of treated male and female groups compared to controls, caramel-colour feeding did not have consistent effects on body weights or body-weight gains in either sex. Males of the 10 g/kg b.w.-dose group had lower mean food

consumption than the control groups for 67 of the 104 weeks and males receiving 2.5 g/kg b.w. had lower mean food consumption for 21 of the 104 weeks. There were no consistent differences in food intake of males at the 5 g/kg b.w. dose level nor of any of the females. Decreased fluid intake was noted, particularly in males at the higher

dose levels; mean fluid intakes of treated females were usually equal to or only slightly lower than controls. There were no treatment-related differences in survival rates, which at termination were 65-75% for the male groups and 67-79% for the female groups. At necropsy, there were dose- and/or treatment-related effects on the gastrointestinal tract and mesenteric lymph nodes, including dark gastrointestinal contents, staining of the mucosa, and diffusely red and congested mesenteric lymph nodes. These changes were not considered to be toxicologically important and there were no other changes of toxicological significance. There was no evidence of treatment-related neoplastic lesions in any organs (MacKenzie, 1985b).

Rats

A long-term toxicity and carcinogenicity study of caramel colour IV was conducted on F344 rats. In the carcinogenicity portion of the study, 5 groups of 50 male and 50 female weanling rats were selected randomly; 2 groups of each sex served as controls and the 3 treatment groups received caramel colour IV in drinking water at dose levels of 2.5, 5.0, or 10 g/kg b.w./day for 24 months. In the chronic toxicity portion of the study, groups of 25 male and 25 female rats received caramel colour IV in drinking water at doses of 0, 2.5, 5.0, 7.5, or 10 g/kg b.w./day for 1 year. The following parameters were monitored: mortality, clinical observations including ophthalmic changes, body weight, and food and fluid consumption. In the chronic toxicity portion clinical chemistry and haematological studies were done at intervals of 6 and 12 months and necropsies were performed at 12 months.

In the carcinogenicity portion of the study, a complete necropsy was performed on all animals dying on test or sacrificed in moribund condition, and on all survivors at termination. Histology (haematoxylin and eosin staining) was performed on all gross lesions and on the following organs/tissues of all animals: adrenals, brain (three sections), bone, bone marrow, epididymis, oesophagus, eyes, heart, intestine (duodenum, ileum, caecum, and colon), kidneys, liver, lung, lymph nodes (cervical, mesenteric, and thoracic), mammary gland, ovaries, pancreas, parathyroid, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, and uterus.

The feeding of caramel colour IV did not affect survival in either the chronic toxicity or carcinogenicity sections of this study and, other than dark-stained and soft faeces, there were no treatment-related antemortem observations. During the course of both studies body weights were reduced for both males and females at the 5 and 10 g/kg b.w. dose levels. These effects on body weights were correlated with reduced water and food consumption at these dose

levels and reflect the reduced palatability of the drinking fluid.

Clinical chemistry and haematological studies at 6 and 12 months in the chronic toxicity study did not reveal changes of toxicological concern. At 6 months, serum concentrations of blood urea nitrogen and creatinine were reduced in male groups treated with 5.0, 7.5, and 10 g/kg b.w. and in female groups treated with 7.5 and 10 g/kg b.w. caramel colour. Similar changes were noted at 12 months. Creatinine levels were within the normal range for the F344 rat, whereas blood urea nitrogen levels were slightly outside this range. Decreased levels of serum total protein, albumin, and globulin were also noted at the 6-month sampling, particularly in male rats. These changes, which were less marked at 12 months, were not accompanied by any pathology in the liver or kidneys and were not considered to be of toxicological importance.

The urinalysis studies revealed generally reduced urine volume and increased specific gravity in both sexes.

At necropsy the changes noted were characteristic of the feeding of high levels of caramel colour, which consisted primarily of brown staining of the gastrointestinal tract and mesenteric lymph nodes and caecal enlargement. Increased kidney weights were noted in both sexes in animals fed caramel colour IV; no histologic alterations were present that could be associated with the increased renal weight, which the authors considered to be related to the water imbalance in these animals.

Microscopic examination of the tissues of these animals did not reveal specific toxicological changes. Pigmentation in the gastrointestinal tract and mesenteric lymph nodes was observed. There was no evidence of reactive hyperplasia to the pigment in the mesenteric lymph nodes of the gastrointestinal tract.

In the chronic portion of the toxicity study, although statistically significant changes were noted in some parameters, they were not considered to be toxicologically important. The highest dose fed, 10 g/kg b.w., was considered to be the no-adverse-effect level.

In the carcinogenicity portion of the study, the observations were generally similar to those in the chronic portion. Survival at 24 months ranged from 64-68% for males and 82-92% for females. Random variations in both benign and malignant neoplasms typical of the F344

strain and this age of animal were observed; however, there were no treatment-related differences. The authors concluded that the feeding of caramel colour IV at doses up to 10 g/kg b.w. for 24 months did not induce neoplastic changes or non-neoplastic changes of toxicological

importance (MacKenzie, 1985a).

Special studies on mutagenicity

Caramel colour IV was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. Salmonella typhimurium (strains TA1535, TA1537, and TA1538) and Saccharomyces cerevisiae were used. Caramel colour IV was not genetically active under the test conditions employed in this study (Brusick, 1974).

Caramel colour IV was evaluated for mutagenicity using the Ames Salmonella/microsome plate test and the Saccharomyces/microsome plate test. The samples tested were blends of 3 samples of caramel colour IV (low colour intensity) and 3 samples of caramel colour IV (high colour intensity). The Salmonella test organisms used were TA98, TA100, TA1535, TA1537, and TA1538. Tests were conducted directly or in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. Tests were conducted over a range of concentrations from 1-50 mg/plate. No signs of genetic activity were observed with any of the samples of caramel colour IV tested using either the Salmonella or Saccharomyces test organisms (Jagannath & Brusick, 1978a).

The clastogenicity of caramel colour IV (low colour intensity) was evaluated in an in vitro cytogenetic assay using cultured Chinese hamster ovary cells without metabolic activation. No increase in chromosome aberrations was observed at concentrations of test material between 5 µg/ml and 5 mg/ml. In the same test, sodium ascorbate produced a positive response at 2 mg/ml (Galloway & Brusick, 1981a).

The clastogenicity of caramel colour IV (high colour intensity) was evaluated in an in vitro cytogenetic assay using Chinese hamster ovary cells without metabolic activation. No increase in chromosome aberrations was observed at concentrations of test material between 5 µg/ml and 5 mg/ml. In the same test, sodium ascorbate produced a positive response at 2 mg/ml (Galloway & Brusick, 1981b).

Two samples of caramel colour IV of medium and high colour intensity were assayed for mutagenic activity in the Ames test using Salmonella strains TA98 and TA100 in the absence or presence of rat hepatic S-9 fraction. Neither sample showed mutagenic activity under the conditions of the test (Ashoor & Monte, 1983).

Thirteen commercial caramel colours (not identified) were examined for mutagenicity in the Ames test using Salmonella strains TA98 and TA100, with and without metabolic activation, and for

DNA-damage effects on E. coli (Wild/pol A⁻; Wild/rec A⁻). None of the caramel-colour samples tested showed mutagenic activity in these tests (Kawana et al., 1980).

Five samples of commercial caramel colour were tested for mutagenic activity against Salmonella strains TA98 and TA100 in the Ames test. All samples were reported to show equivocal results with strain TA100, and 2 were equivocal with TA98 (Kawachi et al., 1980).

Five samples of caramel colour were tested in a chromosome aberration test in a cultured cell line of Chinese hamster lung fibroblasts, in the presence or absence of hepatic S-9 fraction. Ames tests were also conducted using Salmonella strains TA98, TA100, and TA1537, with or without metabolic activation and with a 20-minute pre-incubation step. All samples of caramel colour were designated as positive in the Ames assay and in the chromosome aberration test (aberrations were detected in 20% of metaphase cells) (Ishidate & Yoshikawa, 1980).

The same series of 5 caramel-colour samples as used in the above studies were tested for mutagenicity in the Ames test using Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 and Saccharomyces strain D4. Assays were performed both in the presence and absence of hepatic microsomal S-9 fraction from Aroclor-induced rats in the conventional plate assay and after a pre-incubation step in strains TA100 and TA1535. The results of the mutagenicity assays were negative under all the test conditions and over a concentration range of 1-50 mg/plate for strain TA100 and 1-20 mg/plate for the other strains (Jagannath & Brusick, 1978b).

Special studies on reproduction

Fifteen male and female Wistar rats were given 0 or 10% caramel colour IV solution as their sole fluid source until day 100 and were then mated. Animals of the F₁-generation (25 males and 25 females) were weaned and again given 0 or 10% caramel solution until day 100. There were no adverse effects with regard to the number of litters born and the number of pups/litter. No influence on haematology, growth, food consumption, gross pathology, or histopathology of the F₁-generation at 100 days of age was observed (Haldi & Wynn, 1951).

Six different samples of caramel colour IV (3 single-strength (SS) and 3 double-strength (DS) products), each containing a different level of 4-methylimidazole (between 200 and 850 ppm) were tested in a reproduction study in rats. Two control diets were used, an unmodified stock diet and the stock diet supplemented with starch and cellulose. The various test diets are shown in the following diagramme.

Composition of the test diet (in g)

10	Group		1	2	3	4	5	6	7	8	9
	11	12									
4	Caramel										
	in %		5	10	15	10	10	2	4	6	4
	0	0									
100	stock diet		100	100	100	100	100	100	100	100	100
	100	100									
4.1	wheat										
	starch		3.8	1.9	-	1.9	1.9	4.9	4.1	3.4	4.1
	5.8	-									
4.6	cellulose		4.2	2.2	-	2.2	2.2	5.4	4.6	3.8	4.6
	6.2	-									
SS ^a caramel colour											
IV + 202 ^b			5.6	11.5	17.6						
SS caramel colour											
IV + 400						11.5					
SS caramel colour											
IV + 600							11.5				
DS ^c caramel colour											
IV + 350								2.3	4.5	6.8	
DS caramel colour											
IV + 639											4.5
DS caramel colour											
IV + 852											4.5

^a SS=Single strength

^b Quantity of 4-methylimidazole in ppm

^c DS=Double strength

Twelve groups of 10 male and 20 female weanling Wistar rats were allocated to the above dietary groups and, at week 12, the rats were mated in sub-groups of 5 males and 10 females. After a 3-week mating period, the females were caged individually.

At weaning age of the F_1 generation, 40 males and 40 females were selected from as many different litters as possible within each caramel-colour group and continued on the same diet as the parent generation. Ten males and 10 females of each group were sacrificed after one year (see Sinkeldam *et al.*, 1975, under short-term toxicity studies); the remaining 30 males and 30 females of each group were fed the test diets for 2 years (see Sinkeldam *et al.*, 1976, under long-term toxicity studies). After weaning the F_1 litters, the dams were killed and the implantation sites were counted.

No consistent, dose-related effects on growth of the F_0 animals were noted. No adverse effects were seen on female fertility, litter size, average weight and growth of the pups, or number of implantation sites or sex ratio of the young. In one group (10% SS caramel colour IV + 600 ppm 4-methylimidazole) there was a slight increase in mortality at birth. No teratogenic effects were found (Til & Spanjers, 1973).

A range-finding reproduction study was conducted on F344 rats. Five groups of 12 male and 12 female mature rats were given caramel colour IV at concentrations of 0, 10, 15, 20, or 25% in drinking water for 21 days prior to mating and throughout mating, gestation, and lactation. Animals of the F_0 generation were killed when animals from the F_1 generation were weaned. At weaning, 2 pups/sex/litter were randomly selected from litters that had a minimum of 2 males and 2 females at day 21 and treatment of these animals was continued for 13 weeks post-weaning. Haematology and clinical chemistry were performed on days 45/46 and at termination. Complete gross post-mortem examinations of both F_0 and F_1 animals were performed at sacrifice. Selected organs (caecum, spleen, thymus, liver, kidneys, heart, adrenals, and gonads from animals of the F_0 generation were weighed at autopsy.

Although the dose levels of caramel colour IV in this study were very high (ranging from 8-28 g/kg b.w.), no specific toxic effects were observed. All F_0 -generation animals survived the duration of the study, but 3 F_1 -generation animals were killed accidentally while sampling for clinical chemistry studies at day 45. In the 20- and 25%-dose groups of both generations there was a higher incidence of soft stools than in the controls, and all animals of the F_0 generation showed slight to statistically significant, dose-related

decreases in body weight. Dose-related depression of body-weight gain was also noted in animals of the F₁ generation.

Mating, pregnancy, and fertility rates were comparable for all groups, but the number of implantation sites and of pups alive at days 0, 4, and 21 of lactation in the 20%-dose groups was significantly lower than control values. Litter size was decreased at the 15-, 20- and 25%-dose levels. Pups in the 25%-dose group showed a markedly higher incidence of alopecia and arched spine than controls, and a

generalized poor condition during the last 7 days of suckling. No significant haematological changes were observed at 45 or 90 days post-weaning, except that prothrombin time of the F₁ females in the 15 and 25% groups were significantly greater than controls at day 45. Blood urea nitrogen values were lower than controls at 45 and 90 days but other clinical chemistry values were normal.

At necropsy, dose-related increases in absolute and relative weights of the liver, kidneys, and caecum (full and empty) were observed from animals in the 15%- and higher-dose groups. The only gross treatment-related morphological changes reported were brown/black or green colouration of the contents and mucous membrane of the lower gut and mesenteric lymph nodes (Tierney, 1980).

Special studies on teratogenicity

Teratogenicity tests were performed with caramel colour IV on mice, rats, and rabbits. The doses employed were 0, 16, 74.3, 345, and 1600 mg/kg b.w. in all 3 species.

Mice

Caramel colour IV was administered by gavage to groups of 19-22 pregnant albino CD1 mice at the doses above, beginning on day 6 and continuing through day 15 of gestation. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. On day 17 all dams were subjected to Caesarean section under surgical anaesthesia and the numbers of implantation sites, resorption sites, live and dead foetuses, and body weights of live pups were recorded. All foetuses were examined grossly for external congenital abnormalities, one-third of the foetuses from each litter underwent visceral examination (Wilson technique), and the remaining two-thirds were cleared, stained with Alizarin Red, and examined for skeletal defects.

The number of abnormalities seen in either soft or skeletal tissues did not differ from the number occurring spontaneously in

sham-treated controls (Morgareidge, 1974).

Rats

Caramel colour IV was administered by gavage to groups of 21-24 pregnant Wistar rats at the dose levels above, beginning on day 6 and continuing daily through day 15 of gestation. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. On day 20 dams were subjected to Caesarean section under surgical anaesthesia and the number of implantation sites, resorption sites, live and dead foetuses, and body weights of live pups were recorded. All foetuses were examined for gross, visceral, and skeletal abnormalities as in the mouse experiment.

No clearly discernible effects on nidation or on maternal or foetal survival were observed. The number of abnormalities seen in the test groups did not differ from the number occurring spontaneously in sham-treated controls (Morgareidge, 1974).

Rabbits

Caramel colour IV was administered by gavage to groups of 11-12 pregnant Dutch-belted female rabbits at the doses above beginning on day 6 and continuing daily through day 18 of pregnancy. Body weights were recorded and all animals were observed daily for changes in appearance and behaviour. The does were subjected to Caesarian section under surgical anaesthesia on day 29 and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead foetuses were recorded.

Body weights of the live pups were also recorded. All foetuses were examined grossly for the presence of external congenital abnormalities. The live foetuses from each litter were then placed in an incubator for 24 hours for evaluation of neonatal survival. Surviving pups were sacrificed and all pups examined for visceral abnormalities (by dissection). All foetuses were then cleared with potassium hydroxide, stained with Alizarin Red, and examined for skeletal defects.

No clearly discernible effects on nidation or on maternal or foetal survival were observed. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in sham-treated controls (Morgareidge, 1974).

Special study on haematology

Rats

A study was conducted in rats to determine whether haematological changes were associated with the feeding of high dietary concentrations of caramel colour IV. Sixty-six male rats, mean initial body weights approximately 173 g, were fed caramel colour IV in the diet for 28 days. Sixteen rats were assigned to a control group and 10 rats/group were assigned to levels of 16 or 22% caramel colour IV (high colour intensity) and 34 or 47% caramel colour IV (low colour intensity). Caramel colour III was fed at a 4% level to a positive control group. The parameters observed were body weight, food consumption, haematology (total and differential leucocyte counts), and terminal necropsy with organ weights (caecum - full and empty). Rats were bled from the retro-orbital sinus on days 16, 9, and 2 before treatment and again on days 7, 14, and 28 during treatment.

Feeding of these high dietary levels of caramel colour IV led to reduced rates of body-weight gain. The animals fed caramel colour III gained weight at a rate similar to the controls. Animals receiving caramel colour III had progressively lower relative lymphocyte counts commencing 1 week after treatment and increasing in severity with duration of treatment. This pattern of decreased relative lymphocyte counts was not seen with either sample of caramel colour IV and no significant decreases in lymphocyte counts were observed at any dose level of this caramel colour. Caecal weights were increased in all treatment groups (BIBRA, 1978).

Acute toxicity

No information available.

Short-term studies

Mice

Groups of 10 B₆C₃F₁ mice of each sex were given concentrations of 0, 10, 15, 20, or 30% caramel colour IV in drinking water for 4 weeks. The intake of caramel colour IV expressed in g/kg b.w./day was more than twice the percentage concentration in the drinking water. At 28 days the body weights of male animals at the 30%-dose level were significantly decreased compared to the controls, and transient depressions of body weights were noted at the highest-dose level in females. No significant depressions in body-weight gain were noted at the lower-dose levels in either male or female mice. Fluid consumption was depressed throughout the study among all treatment groups. However, these changes were not consistent with time or dosage. There were no statistically significant differences in food consumption between treated animals and controls. At necropsy, the only treatment-

related effect reported was enlargement of the caecum (Tierney, 1979).

Rats

Groups of 5 rats received either 10 or 20% caramel colour IV solution (equivalent to about 10 or 20 g/kg b.w./day) as their sole source of fluid for 127 days. Only dark faeces and very mild diarrhoea were noted. No adverse effects were noted regarding general health, body weight, food and fluid consumption, haematology, gross pathology, or histopathology (Haldi & Wynn, 1951).

Six groups of 5 male and 5 female weanling rats received 0 or 10% caramel colour IV solution as their sole fluid source for 100, 200, or 300 days. No adverse effects were noted regarding growth, food and fluid intake, haematology, gross pathology, or histopathology (Haldi & Wynn, 1951).

Groups of 16 male and 16 female rats received either 0 or 10% caramel colour IV solution for 100 days and groups of 5 rats received 20% caramel solution for 100 days. At the lower test level, there were no observable abnormalities as regards growth, food consumption, haematology, gross pathology, or histopathology. Only growth and haematology were examined at the higher test level, and the results for both parameters were found to be normal (Haldi & Wynn, 1951).

Groups of 5 male and 5 female rats were given 1 ml/kg b.w. of concentrated caramel colour for 21 days. Some diarrhoea was induced in all animals, but no other abnormalities were noted. Gross pathology and histopathology revealed no significant changes due to administration of the test compound (Foote et al., 1958).

Three groups of 20 male and female rats received either 0 or 11-14 g/kg b.w. of caramel colour IV solutions for 100 days. Growth and food intake did not differ significantly between test and control animals. Gross pathology and histopathology showed no abnormal findings related to administration of the test compound (Haldi & Wynn, 1958).

Four groups of 10 male and 10 female Sprague-Dawley rats received 0, 0.1, 1.0 or 10% caramel colour IV in their diet for 12 weeks. No adverse effects were noted on growth, food consumption, urinalysis, haematology, gross pathology, or histopathology that were related to administration of the caramel colour (Prier, 1960).

Groups of 10 male and 10 female rats received 0, 5, or 10 g/kg caramel colour IV in their diet for 3 months. Weight gain was normal in all groups. Food consumption, haematology, and urinalysis were comparable in all groups. Gross pathology and histopathology showed no

test-related adverse findings (Chacharonis, 1960).

Four groups of 10 male and 10 female Sprague-Dawley rats received 0, 5, 10, or 20% caramel colour IV (low colour intensity) or caramel colour IV (high colour intensity) in their diet for 90 days. In addition, a paired-feeding study involving 5 male rats in 2 groups was run for 23 days (one sample was at the 20% level), and there were no differences in the rate of growth. The only effects attributable to treatment were a mild depression in growth of male rats at the 10 and 20% levels due to unpalatability of the test diet. No other adverse findings were noted in growth, behaviour, mortality, haematology, urinalysis, gross pathology, organ weights, or histopathology (Kay and Calandra, 1962a; 1962b).

Four groups of 10 male and 10 female Sprague-Dawley rats received either 0 or 10% of 3 different caramel colours (one of which was a sample of caramel colour IV) in their diet for 90 days. Weight gains showed a slight reduction compared with controls but food consumption was normal for all groups. No abnormalities were noted with regard to haematology, urinalysis, gross pathology, or histopathology (Chacharonis, 1963).

Four groups of 15 male and 15 female rats received 0, 5, 10, or 20% caramel colour IV in their diet for 90 days. No adverse effects were noted on appearance, behaviour, survival, body weights, food intake, haematology, blood chemistry, urinalysis, organ weights, gross pathology, or histopathology (Oser, 1963).

Four groups of 10 male and 10 female rats received 0, 0.015, 0.3, or 3.0% caramel colour IV in their diet for 90 days. No differences between test and control animals were noted regarding body weight, food consumption, haematology, urinalysis, gross pathology, or histopathology (Nees, 1964).

Four groups of rats received 0, 4, 8, or 16% caramel colour IV in their diet for 3 months. No convulsions or other behavioural abnormalities or signs of neurological damage were seen. No macroscopic or microscopic pathological abnormalities were found in the central nervous system (Sharratt, 1971).

Three groups of 20 female Wistar rats received stock diet to which 0, 10, or 20% caramel colour IV containing 202 ppm 4-methylimidazole was added. During the second week of the experiment these levels of caramel colour IV were increased to 15 and 25% and in week 7, the levels were increased to 25 and 30% caramel colour IV. The diets were administered for 16 weeks followed by a 4-week recovery period.

Food consumption and growth of the test animals were comparable with the controls. Leucocyte counts, collected after 4, 8, 12, and 16 weeks, did not show statistically significant differences among the groups. The relative weights of the caecum, both filled and empty, were distinctly increased after 4 weeks of feeding caramel colour IV. After the recovery period of 4 weeks the increases had disappeared. The relative weight of the thymus was not affected.

Gross examination at autopsy after 16 weeks of feeding caramel colour IV revealed a dose-related, brown-greenish discolouration of the mesenteric lymph nodes in all test animals of the highest-dose level. After the recovery period of 4 weeks the colour change was less, but still visible. Microscopically, the lymph nodes of the test rats showed pigment accumulation which was not noticeably diminished after withdrawal of the caramel for 4 weeks. These results failed to confirm the decreased leucocyte counts that were observed in females fed 5 to 15% caramel colour IV in a 1-year feeding study (Sinkeldam & van der Heyden, 1975).

A 10-week feeding study with 18 groups of 10 male and 10 female Wistar rats was carried out with 3 caramel colours, including caramel colour IV (low colour intensity) and caramel colour IV (high colour intensity). The dietary concentrations were 0, 1.25, 2.5, 5, 10, and 15% caramel colour IV (low colour intensity) and 0, 0.5, 1, 2, 4, and 6% caramel colour IV (high colour intensity).

Both caramel colours caused loose stools at the highest-dose levels, although body-weight gains were not affected. Leucocyte (lymphocyte) counts were not affected in the animals fed samples of caramel colour IV at any of the dose levels. Only slight indications of caecal enlargement were observed. Minimal amounts of pigment were observed in the mesenteric lymph nodes of rats fed 2.5% and higher of the low colour intensity sample, and 1% and higher of the high colour intensity sample (Sinkeldam and van der Heyden, 1976).

In a 10-week feeding study, 17 groups of 15 male and 15 female Sprague-Dawley rats were given various caramel colours that included caramel colour IV (low colour intensity) and caramel colour IV (high colour intensity). The dose levels fed were 1.25, 2.5, 5, 10, and 15% for caramel colour IV (low colour intensity) and 0.5, 1, 2, 4, and 6% caramel colour IV (high colour intensity). Two control groups were used.

In the animals fed caramel colour IV (low colour intensity) at the 15% level, the faeces became soft within 2 weeks. The water content of the faeces from these animals was higher than that of the controls, as was the water content of faeces from rats fed 6% caramel

colour IV (high colour intensity). Body-weight gains were slightly decreased in male, but not female, rats fed both samples of caramel colour.

There were no significant changes in total white cell or lymphocyte counts in animals fed either sample of caramel colour IV. No macroscopic or microscopic evidence of abnormal pigmentation of the mesenteric lymph nodes was found in any group of either sample of caramel colour IV. Caecal weights were generally increased in all test groups fed both samples of caramel colour IV (Procter et al., 1976).

Groups of weanling Wistar rats were given caramel colour IV (low colour intensity) or caramel colour IV (high colour intensity) at concentrations of 0, 0.5, 1.0, 2.0, 4.0, or 16.0% in the diet for 10 weeks. Each group contained 15 male or 15 female rats except the control group (60 animals of each sex) and the 16%-dose group

(10 animals of each sex). Food intake and growth were recorded and haematological studies were carried out. Lymph nodes, thymus, spleen, and caecum were examined histologically for distribution of pigment.

An additional 3 groups of 10 rats of each sex were given basal diet or diet containing 16% each of the caramel colours for 10 weeks. At the end of this period all the rats received basal diets. Haematological studies were performed on these animals at 3, 7, 14, and 28 days. Five rats of each sex were killed after 7 days and the remainder after 28 days of feeding basal diet (recovery experiment).

Decreased body-weight gains were noted in animals of both sexes fed 16% caramel colour IV (high colour intensity). No decreases were observed in the groups fed caramel colour IV (low colour intensity). Food intake was not consistently altered in any of the groups fed either sample of caramel colour IV. Occasionally, values for total leucocyte counts were significantly higher or lower than controls in the groups fed both samples of caramel colour IV; however, these changes were not consistent in direction and they were not dose-related. There were no consistent or dose-related differences in lymphocyte counts between the groups fed caramel colour IV and the controls. Liver weights were significantly increased in the group fed 16% caramel colour IV (high colour intensity). Increased relative kidney weights were observed in the groups fed 2, 4, and 16% caramel colour IV (high colour intensity) and 16% caramel colour IV (low colour intensity), although no histological changes were observed. Increased caecal weights were seen only at the 16% feeding level for both caramel colours. At necropsy pigmentation of the lymph nodes was seen at the 16% feeding level of both caramel colours. Microscopically, pigmentation was observed in the mesenteric lymph nodes in the males and females in the groups fed 16% caramel colour IV

(high colour intensity). Relative weights of the liver and kidneys and caecal weights returned to normal during the recovery period (BIBRA, 1977).

Five groups of 30 male and 30 female weanling F344 rats were given caramel colour IV in drinking water at concentrations which provided intakes of 0, 15, 20, 25, or 30 g/kg b.w./day for 13 weeks. After 43 days, 10 animals of each sex from each group were randomly selected for collection of data on urinalysis, haematology, and clinical chemistry, followed by sacrifice and necropsy; similar examinations were performed on survivors at termination. At interim and terminal sacrifice, a detailed necropsy was performed on all animals and a complete histopathological examination was performed on controls and animals in the top-dose group.

Throughout the study, rats given caramel colour IV produced dark-coloured, soft or sticky, liquid and/or odorous, faeces which stained and caused alopecia of the perianal area, most noticeably at the higher-dose levels. Dose-related decreases in food intake, water

consumption (after correction for caramel content), and body-weight gains were observed and were attributed to the poor palatability of the drinking solutions.

Caramel colour IV at the dose levels tested did not significantly affect haematological values at interim or terminal examinations. All treatment groups of both sexes had significantly-reduced blood urea nitrogen and alkaline phosphatase levels at both 45 and 90 days. Total serum protein values of both sexes in the treatment groups were lower than controls at 90, but not at 45, days. These effects may be due to reduced food intake and growth retardation.

Treated rats had reduced urine volume and increased urine specific gravity, protein, ketones, and acidity, which were associated with decreased water consumption. Increased kidney weights were observed at necropsy. Treatment-related decreases in thymus and spleen weights and increased caecal size, with staining of the mucosa of the caecum and colon, were noted. Accumulation of yellowish-tan pigment occurred in macrophages of the mesenteric lymph nodes. No treatment-related histopathological changes were observed in any organs at the highest-dose level (Heidt & Rao, 1980).

A 1-year rat toxicity study was conducted as a continuation of the reproduction study described earlier (Til & Spanjers, 1973). It involved the interim sacrifice of one-fourth of the animals utilized in the 2-year toxicity study described below.

Wistar rats (10 males or 10 females in each group) were selected

from the first litter of parents that were given diets containing 6 samples of caramel colour IV, 3 of which were low colour intensity and 3 of which were high colour intensity. The dose levels employed were 5, 10, and 15% caramel colour IV (low colour intensity) and 2, 4, and 6% caramel colour IV (high colour intensity). Two additional samples of caramel colour IV (low colour intensity) were tested at 10% and 2 additional samples of caramel colour IV (high colour intensity) were tested at 4% in the diet. The animals were sacrificed after a feeding period of 1 year.

No adverse effects on behaviour, growth, food intake, mortality, liver and kidney function tests, urine composition, or organ weights were observed. Haematological indices showed a slight dose-related decrease in total leucocyte counts in females fed one sample of caramel colour IV (low colour intensity) which contained 202 mg/kg methylimidazole. In males, no effect was noted and in a subsequent experiment in which the same sample of caramel colour IV was fed to female rats at levels as high as 15 and 30%, no indications of decreased leucocyte counts were observed after 4, 8, 12, or 16 weeks.

The only finding attributed to the feeding of caramel colour IV consisted of increased accumulation of a yellow-brown pigment and pigment-laden macrophages in the mesenteric lymph nodes of males and females in all caramel colour IV groups. Inflammatory or degenerative changes of the lymphoid tissue were not found (Sinkeldam *et al.*, 1975).

Dogs

Four groups of 3 male and 3 female adult beagle dogs received 0, 6, 12.5, or 25% caramel colour IV in their diet 5 days per week for 90 days. No significant adverse effects on growth, behaviour, food consumption, mortality, liver function, kidney function, haematology, urinalysis, gross pathology, or histopathology were noted (Kay and Calandra, 1962c).

Long-term toxicity studies

Rats

Six samples of caramel colour were tested in a long-term toxicity study, 3 of which were caramel colour IV (low colour intensity) and 3 of which were caramel colour IV (high colour intensity). Each group consisted of 40 male or 40 female weanling Wistar rats, except the control group which had double this number of animals. Animals were selected from the first litter of parents fed diets containing the various caramel colours from weaning age (see reproduction studies, Til & Spanjers, 1973). The dose levels tested were 5, 10, or 15%

caramel colour IV (low colour intensity) and 2, 4, or 6% caramel colour IV (high colour intensity). Two additional samples of caramel colour IV (low colour intensity) were tested at 10% and 2 additional samples of caramel colour IV (high colour intensity) were tested at 4% in the stock diet.

Observations were made of general appearance, behaviour, mortality, growth, food intake, haematological factors, and clinical chemistry of blood and urine. After about 14 months mortality attributed to intercurrent disease was observed in both control and treated groups. Approximately three-quarters of the animals died or were killed before the experiment was terminated at week 104. Organs of animals that died or were killed were weighed and extensive histopathological examinations were carried out. However, one-third of the animals could not be examined histopathologically due to autolysis. At week 104 organs were weighed and extensive histopathological examinations were carried out on all surviving rats of all groups.

No clinical changes were observed in this study except for slightly decreased haemoglobin and haematocrit values at weeks 78 and 98 in males fed caramel colour IV (high colour intensity). Leucocyte

counts were decreased in females fed 10 and 15% of one sample of caramel colour IV (low colour intensity) at weeks 13 and 52, but these changes were not consistent and decreases in lymphocyte counts were not reported. At autopsy, an increased incidence of greenish discoloured mesenteric lymph nodes was observed in most groups fed high levels of caramel colour. Microscopically, an increased pigment-phagocytosis in the mesenteric lymph nodes in all test groups (except the group fed 2% caramel colour IV (high colour intensity)) was observed. No evidence of any other adverse structural or cellular alteration was found. Gross and microscopic examination of the other organs did not reveal any pathological changes attributable to the ingestion of caramel colour IV. An increase in the incidence of neoplastic lesions in the different groups was not found (Sinkeldam *et al.*, 1976).

A long-term toxicity and carcinogenicity study of caramel colour IV was conducted using F344 rats (MacKenzie, 1985a). Details are given above (see special studies on carcinogenicity).

Observations in man

Tolerance studies of caramel colour IV (low colour intensity) and caramel colour IV (high colour intensity) were conducted in human volunteers. The subjects, 10 men and 10 women, ingested caramel colour once daily in simulated soft drinks over 3 test periods of 21 days

each separated by 7-day rest intervals. The test doses were 6 g/day during the first test period, 12 g/day during the second period, and 18 g/day during the third test period.

Haematological, clinical chemical, and routine urinary parameters were studied at the beginning of each ingestion period, after 10 days of ingestion, and at the end of each ingestion period. Most individual values for haemoglobin, haematocrit, RBC, corrected sedimentation rate, WBC, and differentials (neutrophils, basophils, eosinophils, monocytes, and lymphocytes) were found to be within normal limits. There were a few instances of values outside the normal range, indicating mild neutropenia and mild lymphocytosis, but these were not consistent and were unrelated to the ingestion of caramel colour IV. On the other hand, caramel ingestion was associated with an increased frequency of bowel movements and softening or increased liquidity of faeces (Marier et al., 1977a; 1977b).

Comments

The pigmentation of mesenteric lymph nodes and caecal enlargement were considered to be non-specific effects that are of no toxicological significance. The carcinogenicity studies required by the twenty-fourth meeting of the Committee (Annex 1, reference 53)

have been conducted in rats and mice, and no treatment-related neoplastic changes were observed. The material used in these studies conformed to recent specifications. The committee based its evaluation on the no-effect level in these long-term/carcinogenicity studies, to which (in view of the ancillary human data in which no adverse effects other than laxation were observed) a safety factor of 50 was applied.

EVALUATION

Level causing no toxicological effect

Mouse: 10 g/kg b.w./day in drinking water

Rat: 10 g/kg b.w./day in drinking water

Man: No adverse effects other than laxation at levels up to 18 g/day.

Estimate of acceptable daily intake for man

0-200 mg/kg b.w. (0-150 mg/kg b.w. on a solids basis).

REFERENCES

- Ashoor, S.H. & Monte, W.C. (1983). Mutagenicity of commercial caramels. Cancer Letters 18, 187-190.
- BIBRA (1977). The short-term (10 week) feeding study with three caramels in rats. Unpublished report No. 177/2/77 from the British Industrial Biological Research Association, Carshalton, Surrey, England.
- BIBRA (1978). The evaluation of the effect of sulfite ammonia caramels on the lymphocytes of the rat. Unpublished report No. 238/2/78 from the British Industrial Biological Research Association, Carshalton, Surrey, England.
- Brusick, D. (1974). Mutagenic evaluation of compound FDA 71-83, caramel. Unpublished report No. 2468 from Litton Bionetics Inc., Kensington, MD, USA.
- Chacharonis, P. (1960). Acute and chronic toxicity studies on caramel colours A and B. Unpublished report No. S.A. 54219 from Scientific Associates Inc., St. Louis, MO, USA.
- Chacharonis, P. (1963). Acute oral toxicity study in rats on caramel colorings 25A-1, 30B-0, and 30F-1. Unpublished report No. S.A. 79105 from Scientific Associates Inc., St. Louis, MO, USA.
- Foote, W.L., Robinson, R.F., & Davidson, R.S. (1958). Toxicity of caramel color products. Unpublished report of Battelle Memorial Institute, Columbus, OH, USA.
- Galloway, S.M. & Brusick, D.J. (1981a). Mutagenicity evaluation of ETA-36-1 in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Galloway, S.M. & Brusick, D.J. (1981b). Mutagenicity evaluation of ETA-63-1S in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Part II. Unpublished report No. 20990 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Haldi, J. & Wynn, W. (1951). A study to determine whether or not caramel has any harmful physiological effect. I. Unpublished report from Emory University, Atlanta, GA, USA.
- Haldi, J. & Wynn, W. (1958). A study to determine whether or not

caramel has any harmful physiological effect. II. Unpublished report from Emory University, Atlanta, GA, USA.

- Heidt, M. & Rao, G.N. (1980). 90-day subacute toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats. Unpublished report No. 79028 from Raltech Scientific Services, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.
- Ishidate, M. Jr. & Yoshikawa, K. (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation - a comparative study on mutagens and carcinogens. Arch. Toxicol. Suppl. 4, 41-44.
- Jagannath, D.R. & Brusick, D. (1978a). Mutagenicity evaluation of ETA 4-10, ETA 4-11, ETA 4-15 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Jagannath, D.R. & Brusick, D. (1978b). Mutagenicity evaluation of caramel colors ETA 4-8, ETA 4-9, ETA 4-12, ETA 4-13, ETA 4-14 in the Ames Salmonella/microsome plate test. Unpublished report No. 20838 from Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by International Technical Caramel Association.
- Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M., & Sugiyama, T. (1980). Cooperative programme on short-term assays for carcinogenicity in Japan. In Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Scientific Publication No. 27, 323-330. International Agency for Research on Cancer, Lyon, France.
- Kawana, K., Akema, R., Nakaoka, T., Ikeda, H., Takimoto, T., & Kawauchi, S. (1980). Studies on mutagenicities of natural food additives. II. Mutagenicities and antibacterial activities of caramels. Eisei Kagaku 26, 259-263.
- Kay, J.H. & Calandra, J.C. (1962a). Subacute oral toxicity of caramel colorings (coded Sample A) - albino rats. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.
- Kay, J.H. & Calandra, J.C. (1962b). Ninety-day subacute oral toxicity of caramel coloring (coded Sample B) - albino rats. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.
- Kay, J.H. & Calandra, J.C. (1962c). Subacute oral toxicity of caramel

coloring (coded Sample A) - dogs. Unpublished report from Industrial Bio-test Laboratories, Inc., Northbrook, IL, USA.

Mackenzie, K.M. (1985a). Carcinogenicity and chronic toxicity study of caramel color (sulfite ammonia process) type SAC2 in rats (Chronic toxicity portion, Vols. I-V; Carcinogenicity portion, Vols. I-VIII). Unpublished study No. 81184 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.

Mackenzie, K.M. (1985b). Carcinogenicity study of caramel color (sulfite ammonia process) type SAC2 in mice. Vols. I-VI. Unpublished study No. 81185 from Hazleton Laboratories America, Inc., Madison, WI, USA. Submitted to WHO by International Technical Caramel Association.

Marier, G., Berry, G., & Orr, J.M. (1977a) Tolerance study of single strength ammonia sulfite caramel in human volunteers. Unpublished project No. 5612, report No. 2, from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

Marier, G., Berry, G., & Orr, J.M. (1977b) Tolerance study of double strength ammonia sulfite caramel in human volunteers. Unpublished project No. 5711, report No. 2, from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

Morgareidge, K. (1974). Teratologic evaluation of FDA 71-83 (caramel, beverage) in mice, rats and rabbits. Unpublished report No. PB 234-867 from Food and Drug Research Laboratories Inc., Waverly, NY, USA.

Nees, P.O. (1964). Toxicological feeding study of caramel E. Unpublished report from Wisconsin Alumni Research Foundation, Madison, WI, USA.

Oser, B.L. (1963). Toxicological feeding study of "acid-proof" caramel. Unpublished report No. 83911 from Food and Drug Research Laboratories, Inc., Waverly, NY, USA.

Prier, R.F. (1960). The toxicity of double strength acid proof caramel in rats - 12 week feeding test. Unpublished report No. 9070599 from Wisconsin Alumni Research Foundation, Madison, WI, USA.

Procter, B.G., Berry, G., & Chappel, C.I. (1976). A toxicological evaluation of various caramels fed to albino rats. Unpublished report No. 4244 from Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

- Selim, S. (1982). Single and repeat oral dose pharmacokinetic and distribution studies of caramel color concentrate in the rat. Unpublished study No. IT-59r from Primate Research Institute, Holloman Air Force Base, NM, USA. Submitted to WHO by International Technical Caramel Association.
- Sharratt, M. (1971). Unpublished report.
- Sinkeldam, E.J. & van der Heyden, C.A. (1975). Short-term feeding study with caramel SS 202 in albino rats. Unpublished report No. R 4777 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam, E.J. & van der Heyden, C.A. (1976). Short-term (10 week) feeding study in rats with three different ammonia caramels. Unpublished report No. R 5120 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam, E.J., van der Heyden, C.A., & Beems, R.B. (1976). Chronic (two year) feeding study in rats with six different ammonia caramels. Unpublished report No. R 4961 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Sinkeldam, E.J., Willems, M.I., & van der Heyden, C.A. (1975). One year feeding study in rats with six different ammonia caramels. Unpublished report No. R 4767 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.
- Tierney, W.J. (1979). A four-week dose range-finding study of caramel colour no. 3, sample 3-1, in mice. Unpublished project No. 79-2380 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by International Technical Caramel Association.
- Tierney, W.J. (1980). An in-utero range-finding study of caramel colour no. 3, sample 3-1, in rats. Unpublished project No. 79-2405 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by International Technical Caramel Association.
- Til, H.P. & Spanjers, M. (1973). Reproduction study in rats with six different ammonia caramels. Unpublished report No. R 4068 from Centraal Instituut voor Voedingsonderzoek (CIVO/TNO), Zeist, The Netherlands.

See Also:

Toxicological Abbreviations

I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 8028-89-5
CAS No.	8028-89-5
EINECS Name	Caramel (color)
EINECS No.	232-435-9
Structural Formula	-
Molecular Formula	-

Dataset created by: EUROPEAN COMMISSION - European Chemicals Bureau

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

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

Creation date: 19-FEB-2000

Number of Pages: 11

Chapters: all

Edition: Year 2000 CD-ROM edition

Flags: non-confidential

(C) 2000 EUROPEAN COMMISSION
European Chemicals Bureau

1.0.1 OECD and Company Information

-

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

Substance type: natural substance
Physical status: liquid

1.1.1 Spectra

-

1.2 Synonyms

-

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

-

1.6.1 Labelling

-

1.6.2 Classification

-

1.7 Use Pattern

-

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit:

Limit value:

Remark: No exposure limits known

Source: CERESTAR UK LIMITED MANCHESTER
ROQUETTE (CORBY) LIMITED TUNBRIDGE WELLS**1.9 Source of Exposure**

Remark: No data available

Source: CERESTAR UK LIMITED MANCHESTER
ROQUETTE (CORBY) LIMITED TUNBRIDGE WELLS**1.10.1 Recommendations/Precautionary Measures**

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

-

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2.1 Melting Point

-

2.2 Boiling Point

-

2.3 Density

-

2.3.1 Granulometry

-

2.4 Vapour Pressure

-

2.5 Partition Coefficient

-

2.6.1 Water Solubility

Remark: Totally miscible with water
Source: CERESTAR UK LIMITED MANCHESTER

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

Result: non flammable
Source: CERESTAR UK LIMITED MANCHESTER

2.10 Explosive Properties

Result: not explosive
Source: CERESTAR UK LIMITED MANCHESTER

2.11 Oxidizing Properties

Result: no oxidizing properties
Source: CERESTAR UK LIMITED MANCHESTER

2.12 Additional Remarks

-

3.1.1 Photodegradation

-

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2 Monitoring Data (Environment)

-

3.3.1 Transport between Environmental Compartments

-

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

-

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

-

3.8 Additional Remarks

-

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

-

4.2 Acute Toxicity to Aquatic Invertebrates

-

4.3 Toxicity to Aquatic Plants e.g. Algae

-

4.4 Toxicity to Microorganisms e.g. Bacteria

-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

-

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

-

4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

-

5.1 Acute Toxicity**5.1.1 Acute Oral Toxicity**

-

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

-

5.2 Corrosiveness and Irritation**5.2.1 Skin Irritation**

-

5.2.2 Eye Irritation

-

5.3 Sensitization

-

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test
System of testing:	Salmonella typhimurium, strains TA 1535, 1537, 1538, 98 & 100
Concentration:	2.5 - 20 mg/plate
Metabolic activation:	with and without
Result:	negative
Method:	
Year:	1992
Test substance:	other TS
Source:	CERESTAR UK LIMITED MANCHESTER
Test substance:	Caramel Colours I, II, III & IV

GLP: no data

(1)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: drinking water
Exposure period: 2 yr
Frequency of treatment: continuous
Post. obs. period:
Doses: 0, 2.5, 5.0, 10.0g/kg body wt/day
Result:
Control Group: yes
Method:
Year: 1992 **GLP:** no data
Test substance: other TS
Result: No carcinogenic effect found
Source: CERESTAR UK LIMITED MANCHESTER
Test substance: Caramel Colour IV (Sulphite ammonia)

(2)

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

-

5.11 Experience with Human Exposure

Remark: No decrease found in the number of blood lymphocytes in marginally vitamin B6-deficient humans
Source: CERESTAR UK LIMITED MANCHESTER
Test substance: Caramel Colour III (Ammonia Caramel)

(3)

- (1) Allen et al., Fd. Chem. Toxic. Vol. 30, No. 5, pp.389-395, 1992
- (2) MacKenzie K.M. et al, Fd. Chem. Toxic. Vol. 30, No. 5, pp. 431-443, 1992
- (3) Houben et al Fd. Chem. Toxic. Vol. 30, No. 5, pp.427-430, 1992

7.1 Risk Assessment

-

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

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
ISSN 0512-3054

(NLM classification: WA 712)

The World Health Organization welcomes requests for permission to reproduce or translate its publications, in part or in full. Applications and enquiries should be addressed to the Office of Publications, World Health Organization, Geneva, Switzerland, which will be glad to provide the latest information on any changes made to the text, plans for new editions, and reprints and translations already available.

© World Health Organization 2001

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. All rights reserved.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

**Typeset in Hong Kong
Printed in Switzerland**

2000/13521 — Best-set/Schuler — 6000

Contents

1. Introduction	1
2. General considerations	1
2.1 Modification of the agenda	1
2.2 Principles governing the toxicological evaluation of compounds on the agenda	2
2.2.1 Principles governing the safety evaluation of flavouring agents	2
2.2.2 Need for data	4
2.2.3 Recommendations of the World Health Assembly	4
2.3 Principles governing the establishment and revision of specifications	5
2.3.1 General specifications for enzyme preparations	5
2.3.2 Determination of low concentrations of metals	5
3. Specific food additives	6
3.1 Flavouring agents	6
3.1.1 Furfural	6
3.1.2 Paprika oleoresin	8
3.2 Food colours	8
3.2.1 Caramel colour II	8
3.2.2 Cochineal extract and carmines	10
3.3 Sweetening agents	12
3.3.1 Aspartame–acesulfame salt	12
3.3.2 D-Tagatose	13
3.4 Miscellaneous substances	15
3.4.1 Benzoyl peroxide	15
3.4.2 Nitrous oxide	17
3.4.3 Stearyl tartrate	17
3.4.4 Trehalose	18
4. Flavouring agents evaluated using the Procedure for the Safety Evaluation of Flavouring Agents	20
4.1 Cinnamyl alcohol and related flavouring agents	22
4.1.1 Estimated daily per capita intake	34
4.1.2 Absorption, metabolism and elimination	34
4.1.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents	35
4.1.4 Consideration of combined intakes	37
4.1.5 Conclusions	37
4.2 Furfuryl alcohol and related flavouring agents	37
4.2.1 Estimated daily per capita intake	42
4.2.2 Absorption, metabolism and elimination	42
4.2.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents	42
4.2.4 Consideration of combined intakes	44
4.2.5 Conclusions	44

4.3	Phenol and phenol derivatives	44
4.3.1	Estimated daily per capita intake	53
4.3.2	Absorption, metabolism and elimination	53
4.3.3	Application of the Procedure for the Safety Evaluation of Flavouring Agents	54
4.3.4	Consideration of combined intakes	55
4.3.5	Conclusions	55
4.4	Pulegone and related flavouring agents	56
4.4.1	Estimated daily per capita intake	56
4.4.2	Absorption, metabolism and elimination	57
4.4.3	Application of the Procedure for the Safety Evaluation of Flavouring Agents	57
4.4.4	Consideration of combined intakes	61
4.4.5	Conclusions	61
5.	Contaminants	61
5.1	Cadmium	61
5.1.1	Bioavailability	62
5.1.2	Health effects	63
5.1.3	Dietary intake	66
5.1.4	Estimates of the relationship between dietary intake and renal tubule dysfunction	66
5.1.5	Conclusion	67
5.2	Tin	69
6.	Intake assessments of specific food additives	71
6.1	Calcium from calcium salts of food additives	71
7.	Revision of certain specifications	72
7.1	Food additives	72
7.1.1	Food additives for which previous specifications were designated as "tentative"	72
7.1.2	Food additives considered for revision of specifications	73
7.1.3	Food additives that are also flavouring agents	75
7.2	Flavouring agents	75
7.2.1	Procedure for evaluating proposed specifications for flavouring agents	75
7.2.2	Specifications established up to and including the fifty-third meeting	76
7.2.3	Proposed specifications considered for the first time at the present meeting	76
7.2.4	Comments made at the Thirty-second Session of the Codex Committee on Food Additives and Contaminants	77
7.3	Limits for metals in food additives	78
7.3.1	Emulsifiers	78
7.3.2	Food additives other than emulsifiers	80
8.	Future work	80
9.	Recommendation	81
	Acknowledgement	82

References	82
Annex 1	
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives	83
Annex 2	
Acceptable Daily Intakes, other toxicological information and information on specifications	92
Annex 3	
Further information required or desired	106

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 $\times 10$ ”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 $\times 10$ ” is appropriate currently. However, it recognized the potential usefulness of the other methods and noted that use of the “per capita $\times 10$ ” 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 $\times 10$ ” 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 2mg/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 2.2mg/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 0.28mg/kg of body weight per day. For consumers of distilled spirits at the 95th percentile of consumption, the estimated dietary intake is 0.91mg/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 4–16g/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 16g/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 12g/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 500mg/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:

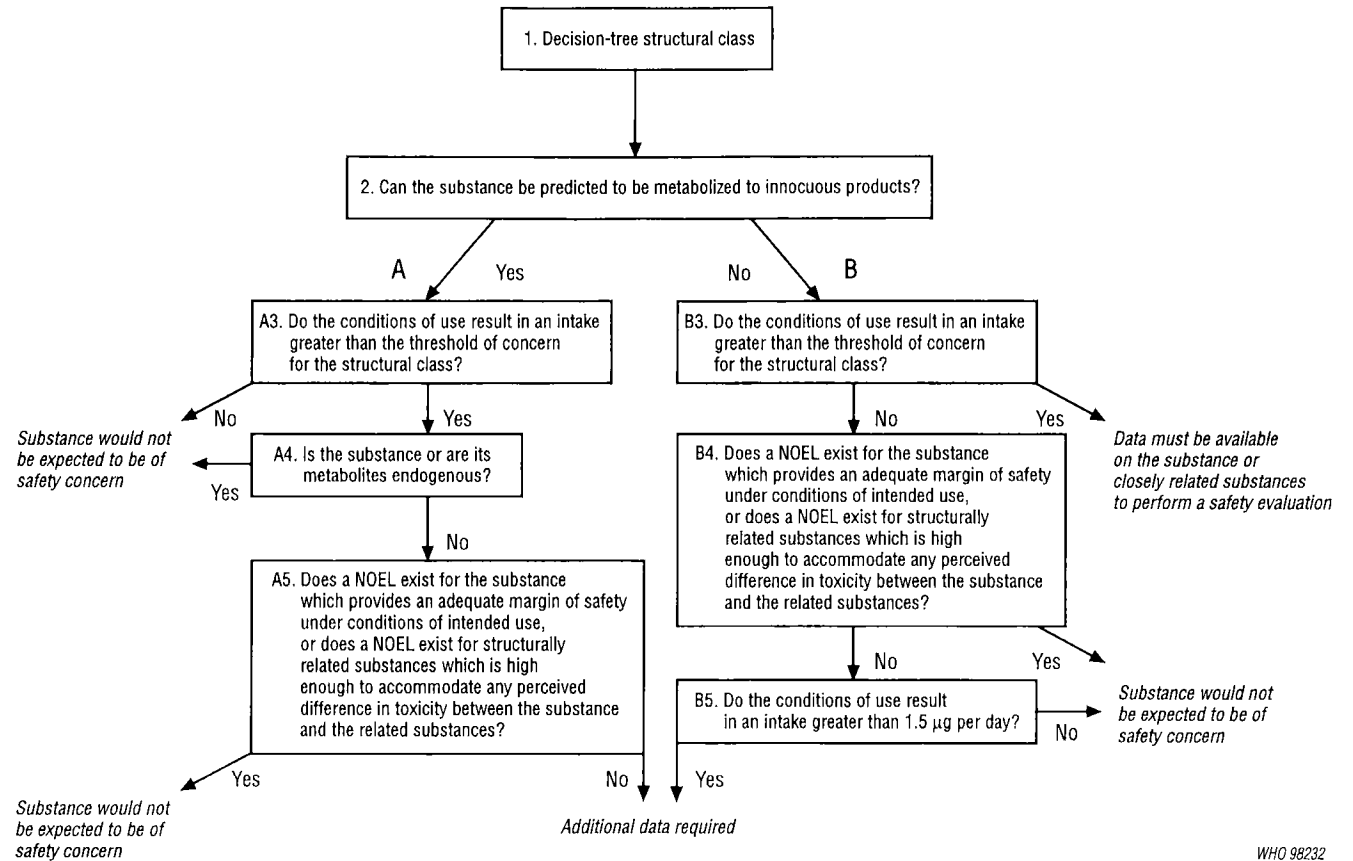
Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

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.

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

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

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

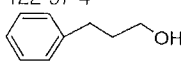
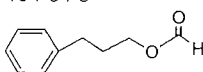
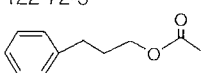
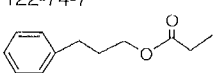
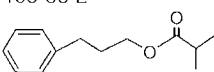
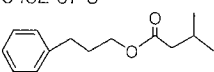
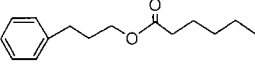
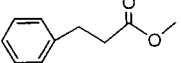
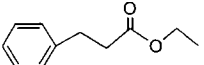
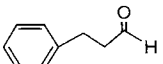
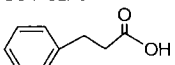
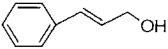
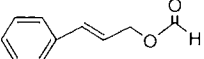
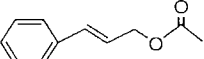
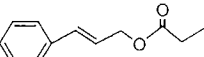
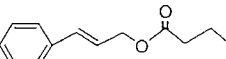
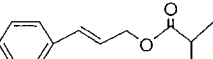
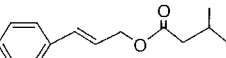
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
3-Phenyl-1-propanol (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	

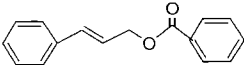
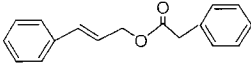
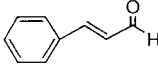
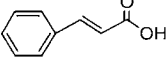
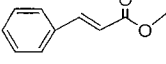
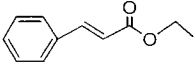
Table 1 (continued)

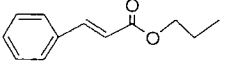
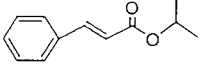
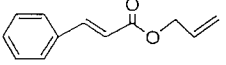
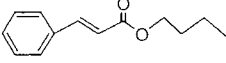
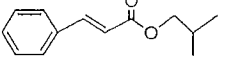
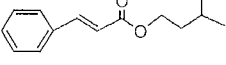
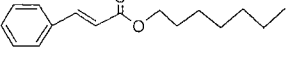
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
3-Phenylpropyl hexanoate	642	6281-40-9 	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
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

No safety
concern

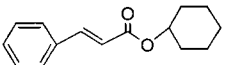
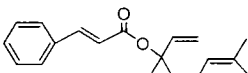
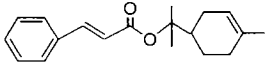
Table 1 (*continued*)

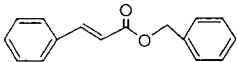
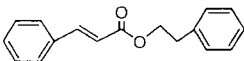
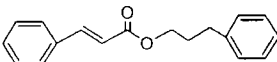
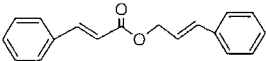
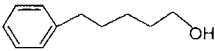
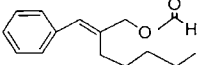
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Cinnamyl benzoate	760	5320-75-2 	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
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

No safety concern

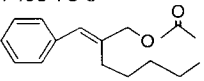
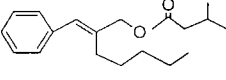
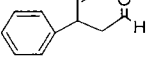
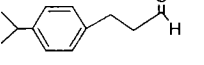
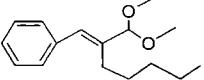
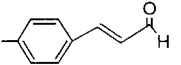
Table 1 (*continued*)

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
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

No safety concern

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-(<i>p</i> -Isopropylphenyl) propionaldehyde (3-(<i>p</i> -cumenyl)propionaldehyde)	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	
<i>p</i> -Methylcinnamaldehyde (3-(4-methylphenyl)-2-propenal)	682	1504-75-2 	No Europe: 0.01 USA: 0.9	NR	NR	See note 4	

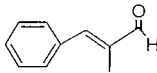
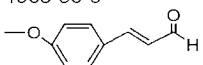
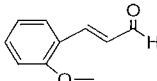
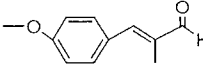
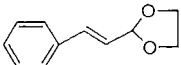
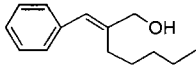
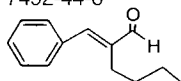
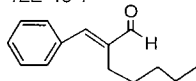
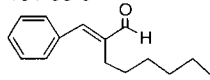
α -Methylcinnamaldehyde	683	101-39-3 	No Europe: 3 USA: 390	NR	NR	See note 4	No safety concern
<i>p</i> -Methoxycinnamaldehyde	687	1963-36-6 	No Europe: 0.04 USA: 0.01	NR	NR	See note 4	
<i>o</i> -Methoxycinnamaldehyde	688	1504-74-1 	No Europe: 0.6 USA: 71	NR	NR	<i>o</i> -Methoxycinnamaldehyde is oxidized 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	
<i>p</i> -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 >10000 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 750g/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 2.5mg of cinnamaldehyde (No. 656), 1.8mg of cinnamyl alcohol (No. 647) and 2.8mg of methyl cinnamate (No. 658). The estimated daily per capita intakes in the USA are 59mg of cinnamaldehyde (No. 656), 1.9mg of cinnamyl alcohol (No. 647) and 0.83mg of methyl cinnamate (No. 658). The estimated daily per capita intakes of all the other flavouring agents in the group are in the range 0.003–690µg, 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 (1800 μ g

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.

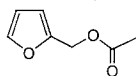
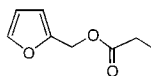
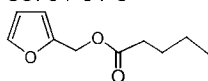
4.2 **Furfuryl alcohol and related flavouring agents**

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 >10000 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 >100000 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 >100000 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 >100000 times the estimated intake of furfuryl pentanoate when used as a flavouring agent	

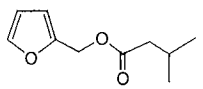
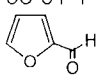
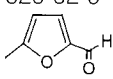
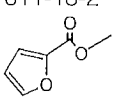
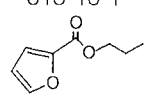
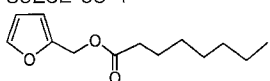
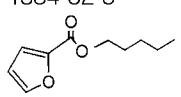
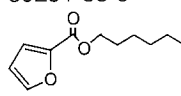
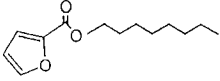
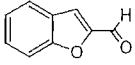
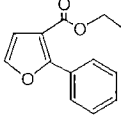
Furfuryl 3-methylbutanoate ^d (2-furanylmethyl-3-methylbutanoate)	743	13678-60-9		No Europe: 0.03 USA: 1	Yes The NOEL of 53mg/kg of body weight per day for related substance No. 450 is >1 million times the estimated intake of furfuryl 3-methylbutanoate when used as a flavouring agent	No safety concern
Furfural ^d (2-furfuraldehyde)	450	98-01-1		No Europe: 520 USA: 460	Yes The NOEL of 53mg/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 53mg/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 53mg/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 53mg/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 53mg/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 53mg/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 53mg/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 53mg/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 25mg/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 13mg/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 540µg per day and 90µg per day, respectively. All intake values are expressed in µg per day.

^d An ADI for furfural of 0–0.5mg/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 (540µg and 90µg, respectively). Accordingly, the evaluation of all 15 substances proceeded to step B4.

Step B4

For furfural (No. 450), the NOEL of 53mg/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 25mg/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

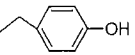
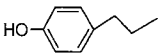
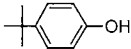
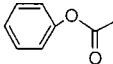
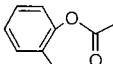
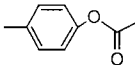
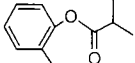
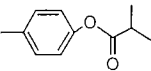
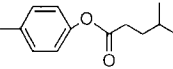
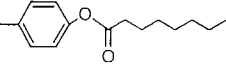
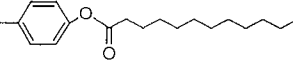
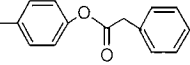
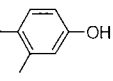
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
Phenol	690	108-95-2 	No Europe: 6 USA: 1	NR	NR	See note 1	No safety concern
<i>o</i> -Cresol	691	95-48-7 	No Europe: 290 USA: 0.1	NR	NR	See note 1	
<i>m</i> -Cresol	692	108-39-4 	No Europe: 0.1 USA: 0.1	NR	NR	See note 1	
<i>p</i> -Cresol	693	106-44-5 	No Europe: 1 USA: 1	NR	NR	See note 1	
<i>p</i> -Ethylphenol (4-ethylphenol)	694	123-07-9 	No Europe: 4 USA: 0.1	NR	NR	See note 1	
<i>o</i> -Propylphenol (2-propylphenol)	695	644-35-9 	No Europe: 0.1 USA: 1	NR	NR	See note 1	

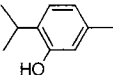
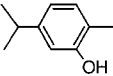
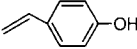
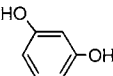
Table 3 (continued)

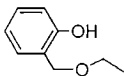
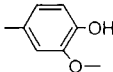
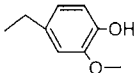
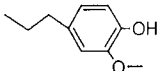
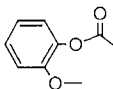
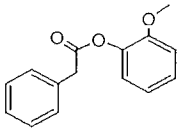
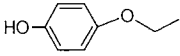
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
<i>p</i> -Propylphenol (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 (<i>p</i> - <i>tert</i> - 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	
<i>o</i> -Tolyl acetate	698	533-18-6 	No Europe: 0.1 USA: 40	NR	NR	See note 2	
<i>p</i> -Tolyl acetate	699	140-39-6 	No Europe: ND USA: 70	NR	NR	See note 2	
<i>o</i> -Tolyl isobutyrate	700	36438-54-7 	No Europe: 0.03 USA: 0.1	NR	NR	See note 2	

<i>p</i> -Tolyl isobutyrate	701	103-93-5 	No Europe: 0.04 USA: 0.01	NR	NR	See note 2
<i>p</i> -Tolyl 3-methylbutyrate (<i>p</i> -tolyl isovalerate)	702	55066-56-3 	No Europe: 0.4 USA: 0.1	NR	NR	See note 2
<i>p</i> -Tolyl octanoate	703	59558-23-5 	No Europe: 0.03 USA: 1	NR	NR	See note 2
<i>p</i> -Tolyl laurate	704	10024-57-4 	No Europe: ND USA: 0.3	NR	NR	See note 2
<i>p</i> -Tolyl phenylacetate	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

No safety concern

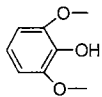
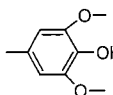
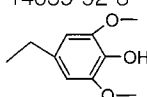
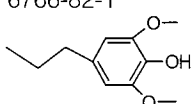
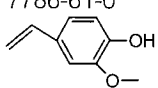
Table 3 (continued)

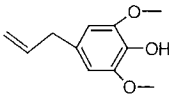
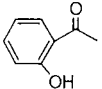
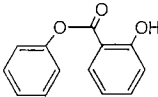
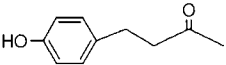
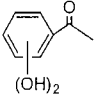
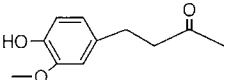
Flavouring agent ^b	No.	CAS no. and structure	Step A3 ^c Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for the substance or related substance?	Comments	Conclusion based on current intake
2,3,6-Trimethylphenol	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	
<i>p</i> -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 (<i>o</i> -methoxyphenol)	713	90-05-1 	No Europe: 51 USA: 16	NR	NR	See note 1	

<i>o</i> -(Ethoxymethyl)-phenol	714	20920-83-6		No Europe: 2 USA: 0.01	NR	NR	See note 1
2-Methoxy-4-methylphenol (2-methoxy- <i>p</i> -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 (<i>p</i> -ethoxyphenol)	720	622-62-8		No Europe: ND USA: 0.4	NR	NR	See note 1

No safety concern

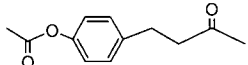
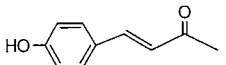
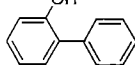
Table 3 (continued)

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- <i>p</i> -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
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-hydroxybenzoate)	736	118-55-8		No Europe: 9 USA: 8	NR	NR	See note 1
4-(<i>p</i> -Hydroxyphenyl)-2-butanone (4-(4-hydroxyphenyl)-butan-2-one)	728	5471-51-2		Yes Europe: 2800 USA: 3800	No	Yes ^d	See note 1
Dihydroxyacetophenone (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

No safety concern

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-(<i>p</i> -Acetoxyphenyl)-2-butanone (4-(<i>p</i> -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 0–0.2mg/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 0–0.4mg/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 2.8mg in Europe and 3.8mg in the USA.

Other flavouring agents for which the estimated daily per capita intakes are in the range 37–300µg include *o*-cresol (No. 691) (290µg in Europe), *o*- and *p*-tolyl acetate (Nos 698 and 699) (40µg and 70µg, respectively, in the USA), 2-methoxy-4-methylphenol (No. 715) (37µg in Europe), thymol (No. 709) (59µg in Europe and 160µg in the USA), guaiacol (No. 713) (51µg in Europe), 2-methoxy-4-propylphenol (No. 717) (210µg in Europe) and zingerone (No. 730) (40µg in Europe and 83µg in the USA). The estimated daily per capita intakes of all other flavouring agents in the group are in the range 0.01–16µg. 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 (>500mg/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 (1800µg and 90µg, 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 100mg/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.

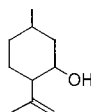
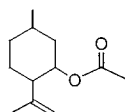
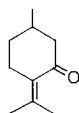
4.4.3 **Application of the Procedure for the Safety Evaluation of Flavouring Agents**

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 (<i>p</i> -menth-8-en-3-ol)	755	89-79-2 	No Europe: 6 USA: 7	Yes The NOEL of 0.44mg/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 0.44mg/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 (<i>p</i> -menth-4(8)-en-3-one)	753	89-82-7 	No Europe: 2 USA: 2	Yes The NOEL of 0.44mg/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 (<i>trans-p</i> -menth-8-en-3-one)	754	29606-79-9	No Europe: 1 USA: 0.01	Yes The NOEL of 0.44mg/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
<i>p</i> -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 0.44mg/kg of body weight per day for related substance No. 753 is >10000 times the estimated intake of <i>p</i> -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 0.44mg/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 1800µg per day and 540µg 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 (1800µg and 540µg, 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 0.44mg/kg of body weight per day for pulegone derived from this study is >10000 times the estimated intake of 0.033µg/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 0.44mg/kg of body weight per day for pulegone in the 90-day study in rats is >1000 times the estimated intake of 0.4µg/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 >10000 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 $\mu\text{g/g}$ 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 $\mu\text{g/g}$, 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 $\mu\text{g}/24\text{h}$ or the estimated concentration in the renal cortex was $>50\mu\text{g/g}$. 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 $>1000\mu\text{g}$ of β_2 -microglobulin in the urine per 24h, 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 1.2g 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 60kg. The PTWI corresponds to a daily intake of 1µg/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 7µg/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 2mg/kg of body weight to a PTWI of 14mg/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 <1mg/day to about 14mg/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 0.5–3h, and recovery occurs within 48h. 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 1400mg/kg (corresponding to a dose of 4.4–6.7mg/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 2mg/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 stearyl-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
Diethyl 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 stearyl-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, Lajarthé, 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 }	
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” ⁱⁱ

^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	No safety concern
3-Phenylpropyl formate (benzenepropanol formate)	637	N	
3-Phenylpropyl acetate (benzenepropanol acetate)	638	N	
3-Phenylpropyl propionate (benzene-propanol 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 (benzene-propanal)	645	N, T	
3-Phenylpropionic acid (benzene-propionic acid)	646	N	
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	No safety concern
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-(phenyl-methylene)heptyl formate)	676	N, T	
α -Amylcinnamyl acetate (2-(phenyl-methylene)heptyl acetate)	677	N, T	
α -Amylcinnamyl isovalerate (2-(phenyl-methylene)heptyl isovalerate)	678	N, T	
3-Phenyl-4-pentenal (3-phenylpent-4-enal)	679	N	
3-(<i>p</i> -Isopropylphenyl)propionaldehyde (3-(<i>p</i> -cumenyl)propionaldehyde)	680	N	
α -Amylcinnamaldehyde dimethyl acetal ((2-(dimethoxymethyl)-1-heptenyl) benzene)	681	N, T	
<i>p</i> -Methylcinnamaldehyde (3-(4-methyl-phenyl)-2-propenal)	682	N	
α -Methylcinnamaldehyde	683	N	
<i>p</i> -Methoxycinnamaldehyde	687	N	
<i>o</i> -Methoxycinnamaldehyde	688	N	
<i>p</i> -Methoxy- α -methylcinnamaldehyde (4'-methoxy-2-methylcinnamaldehyde)	689	N	

Structural class II

Cinnamaldehyde ethylene glycol acetal (2-styryl-1,3-dioxolane)	648	N, T	No safety concern
α -Amylcinnamyl alcohol (2-pentyl-3-phenylprop-2-en-1-ol)	674	N	
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
<i>o</i> -Cresol	691	N	
<i>m</i> -Cresol	692	N	
<i>p</i> -Cresol	693	N	
<i>p</i> -Ethylphenol (4-ethylphenol)	694	N	
<i>o</i> -Propylphenol (2-propylphenol)	695	N	
<i>p</i> -Propylphenol (4-propylphenol)	696	N	
2-Isopropylphenol	697	N	
4-(1,1-Dimethylethyl)phenol (<i>p</i> - <i>tert</i> -butylphenol)	733	N	
Phenyl acetate	734	N	
<i>o</i> -Tolyl acetate	698	N, T	
<i>p</i> -Tolyl acetate	699	N	
<i>o</i> -Tolyl isobutyrate	700	N	
<i>p</i> -Tolyl isobutyrate	701	N	
<i>p</i> -Tolyl 3-methylbutyrate (<i>p</i> -tolyl isovalerate)	702	N	
<i>p</i> -Tolyl octanoate	703	N	

Flavouring agent ^a	No.	Specifications ^b	Conclusion based on current intake
<i>p</i> -Tolyl laurate	704	N, T	No safety concern
<i>p</i> -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	
<i>p</i> -Vinylphenol (4-ethenylphenol)	711	N, T	
Resorcinol	712	N	
Guaiacol (<i>o</i> -methoxyphenol)	713	N	
<i>o</i> -(Ethoxymethyl)phenol	714	N	
2-Methoxy-4-methylphenol (2-methoxy- <i>p</i> -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 (<i>p</i> -ethoxyphenol)	720	N, T	
2,6-Dimethoxyphenol	721	N	
4-Methyl-2,6-dimethoxyphenol (2,6-dimethoxy- <i>p</i> -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'-hydroxyacetophenone)	727	N	
Phenyl salicylate (phenyl 2-hydroxybenzoate)	736	N	
4-(<i>p</i> -Hydroxyphenyl)-2-butanone (4-(4-hydroxyphenyl)butan-2-one)	728	N	
Dihydroxyacetophenone (dihydroxy-1-phenylethanone)	729	N, T	
Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone)	730	N	
4-(<i>p</i> -Acetoxyphenyl)-2-butanone (4-(<i>p</i> -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 ^c (biphenyl-2-ol)	735	N, T	No safety concern

Pulegone and related flavouring agents

Structural class I

Isopulegol (<i>p</i> -menth-8-en-3-ol)	755	N	} No safety concern
Isopulegyl acetate	756	N	

Structural class II

Pulegone (<i>p</i> -menth-4(8)-en-3-one)	753	N	} No safety concern
Isopulegone (<i>trans-p</i> -menth-8-en-3-one)	754	N	
<i>p</i> -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 1.2g 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 <i>Aspergillus awamori</i> , var. ^b	—	W
Carbohydrase from <i>Aspergillus oryzae</i> , var. ^b	—	W
Chlorine dioxide ^b	—	W
Diatomaceous earth	—	R
Diethyl pyrocarbonate ^b	—	W
Isoamyl gallate ^b	—	W
Lipase from <i>Aspergillus oryzae</i> , var. ^b	—	W
Potassium persulfate ^b	—	W
Rennet from <i>Endothia parasitica</i> ^b	—	W
Rennet from <i>Rhizomucor</i> species (<i>Mucor</i> species)	—	R
Food additives considered for revision of specifications		
α -Amylase from <i>Aspergillus oryzae</i> , var.	—	R
α -Amylase and glucoamylase from <i>Aspergillus oryzae</i> , var.	—	R
Amyloglucosidase from <i>Aspergillus niger</i> , var. ^c	—	R, T
Blackcurrant extract ^c	—	R, T
Cellulase from <i>Penicillium funiculosum</i>	—	R
Curcumin	—	R
Diethyl ether	—	R
β -Glucanase from <i>Trichoderma harzianum</i>	—	R
Guaiac resin	—	R
Hemicellulase from <i>Aspergillus niger</i> , var.	—	R
Microcrystalline cellulose	—	R
Microcrystalline wax	—	R
2-Nitropropane ^d	—	W
Oxystearin ^e	—	R, T
Pectinase from <i>Aspergillus niger</i> , var.	—	R
Pentasodium triphosphate	—	R

Substance	No.	Specifications ^a
Protease from <i>Aspergillus oryzae</i> , 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
<i>d</i> - α -Tocopherol, concentrate	—	R
1,1,2-Trichloroethylene ^e	—	W
Flavouring agents^f		
Allyl propionate (2-propenyl propanoate)	1	R
Allyl tiglate (2-propenyl <i>trans</i> -2-methyl 2-butenate)	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 (<i>trans</i> -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
<i>cis</i> -3- and <i>trans</i> -2-Hexenyl propionate (<i>cis</i> -3- and <i>trans</i> -2-hexenyl propanoate)	147	R
Heptyl butyrate (heptyl butanoate)	154	R
Octyl butyrate (octyl <i>n</i> -butyrate)	155	R
Decyl butyrate	156	R
<i>cis</i> -3-Hexenyl hexanoate (<i>cis</i> -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
<i>n</i> -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 <i>H</i>)-furanone	222	R
γ-Heptalactone (5-propyldihydro-2(3 <i>H</i>)-furanone)	225	R
γ-Octalactone (5-butyldihydro-2(3 <i>H</i>)-furanone)	226	R
4,4-Dibutyl-γ-butyrolactone (5,5-dibutyldihydro-2(3 <i>H</i>)-furanone)	227	R
δ-Octalactone (6-propyltetrahydro-2-pyrone)	228	R
γ-Nonalactone (5-pentyldihydro-2(3 <i>H</i>)-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 <i>H</i>)-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
<i>cis</i> -3-Hexenal ((<i>Z</i>)-hex-3-enal)	316	R
3-Hexenoic acid (hex-3-enoic acid)	317	R
<i>cis</i> -4-Hexenal ((<i>Z</i>)-hex-4-enal)	319	R
<i>cis</i> -4-Heptenal ((<i>Z</i>)-hept-4-en-1-al)	320	R
<i>cis</i> -6-Nonen-1-ol ((<i>Z</i>)-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 ((<i>Z</i>)-octadeca-9-enoic acid)	333	R
Methyl 3-hexenoate (methyl hex-3-enoate)	334	R, T
Methyl <i>cis</i> -4-octenoate ((<i>Z</i>)-methyl oct-4-enoate)	337	R, T
Ethyl <i>cis</i> -4-octenoate ((<i>Z</i>)-ethyl oct-4-enoate)	338	R, T
Ethyl <i>cis</i> -4,7-octadienoate (ethyl (<i>Z</i>)-octa-4,7-dienoate)	339	R
Methyl 3-nonenate (methyl non-3-enoate)	340	R
Ethyl <i>trans</i> -4-decenoate (ethyl (<i>E</i>)-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 (<i>p</i> -menth-1-en-8-yl formate)	367	R, T
Terpinyl butyrate (<i>p</i> -menth-1-en-8-yl butyrate)	370	R, T
Terpinyl isobutyrate (<i>p</i> -menth-1-en-8-yl isobutyrate)	371	R
Terpinyl isovalerate (<i>p</i> -menth-1-en-8-yl isovalerate)	372	R, T
<i>p</i> -Menth-3-en-1-ol	373	R
<i>p</i> -Menthan-2-ol	376	R
Dihydrocarvone (<i>p</i> -menth-8-en-2-one)	377	R
<i>l</i> -Carvone (<i>p</i> -mentha-6,8-dien-2-one)	380.2	R
Carvyl propionate (1- <i>p</i> -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
<i>l</i> -Menthyl lactate	433	R
5-Hydroxy-2-dodecenoic acid δ-lactone (6-heptyl-2 <i>H</i> -dihydro-2-pyrone)	438	R
4-Carvomenthenol (<i>p</i> -menth-1-en-4-ol)	439	R
4-Thujanol (2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hexan-2-ol)	441	R
<i>dl</i> -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
<i>S</i> -Methyl thioacetate (<i>S</i> -methyl acetothioate)	482	R
Methyl thiobutyrate (<i>S</i> -methyl butanethioate)	484	R
<i>S</i> -Methyl 2-methylbutanethioate	486	R
<i>S</i> -Methyl 3-methylbutanethioate	487	R
4-(Methylthio)-2-butanone	497	R
4,5-Dihydro-3(2 <i>H</i>)-thiophenone	498	R
2-Methyltetrahydrothiophen-3-one (4,5-dihydro-2-methyl-3(2 <i>H</i>)-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
<i>o</i> -(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- <i>p</i> -Menthene-8-thiol	523	R
Thiogeraniol (3,7-dimethyl-2(<i>trans</i>),6-octadiene-1-thiol)	524	R
<i>o</i> -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
<i>p</i> -Mentha-8-thiol-3-one (8-mercapto-3- <i>p</i> -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(<i>trans</i>)-butenedioic acid)	618	R
<i>L</i> -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

Date issued : 11/16/2017

SDS number : CO38-1

Date revised : 06/28/2023

Revision number : 2

1. Identification

Product code: CO38-1 / M100**Product description:** Caramel Color Additive**Molecular formula:** Trade Secret**Relevant identified uses:** Color Additive**Manufacturer / Supplier**

REFRESCO BEVERAGES USA INC.

1001 10TH AVENUE

COLUMBUS, GA 31901

Customer Service: 1-800-742-4554**Emergency telephone number (24 hour)****Poison Control Center (Medical) :** (877) 800-5553**CANUTEC (Canadian Transportation) :** (613) 996-6666**CHEMTREC (US Transportation) :** (800) 424-9300**CANUTEC (Outside US and Canada) :** (01-703) 527-3887

2. Hazard identification

Label elements

This item is not hazardous

Potential health effects**Eye:** May be mildly irritating to the eyes.**Skin:** Substance does not generally irritate and is only mildly irritating to the skin.**Ingestion:** Not harmful in small quantities. Relatively large quantities may cause gastrointestinal irritation and diarrhea.**Routes of entry:** Inhalation, Ingestion, and Eye/Skin Contact.

3. Composition/information on ingredients

Chemical name	Vol. %	CAS No.
Caramel Color 100%	~ 100	8028-89-5

4. First-aid measures

Eye: Immediately flush eyes with plenty of water. Get medical attention, if irritation persists.**Skin:** Wash with soap and water to remove. Seek medical attention if irritation develops.**Ingestion:** If swallowed, do NOT induce vomiting. Give victim a glass of water or milk. Call a physician or poison control center immediately. Never give anything by mouth to an unconscious person.**Inhalation:** Remove to fresh air if needed.**Most important symptoms and effects, both acute and delayed****Chronic effects:** Refer to Section 11 for Toxicological Information.

5. Fire-fighting measures

Flammable class: Non-Flammable.**Suitable extinguishing media:** Use media suitable to the surrounding fire such as water fog or fine spray, alcohol

foams, carbon dioxide and dry chemical. Do not use a solid water stream as it may scatter and spread fire.

Hazardous decomposition products: None

6. Accidental release measures

Small spill: Contain any spills immediately and stop the leak if you can do it without risk. Absorb the liquid with absorbent pads or cover with an absorbent material (such as dry earth, sand, or other non-combustible material). Collect the cleanup residues and transfer to containers for disposal.

Large spill: Keep unnecessary and unprotected personnel from entering the area of any spill.

Environmental precautions

Water spill: Prevent entry to sewers and water ways.

General procedures: Clean area with soap and water.

Special protective equipment: For personal protection see section 8.

7. Handling and storage

Precautions for safe handling: Follow all MSDS/label precautions even after container is emptied because it may retain product residues.

Conditions for safe storage: Store in dry, cool, well-ventilated area. Do not store under direct sun light. Store at room temperature.

Storage temperature: 7°C (45°F) Minimum to 33°C (91°F) Maximum

Shelf life: 365 Days

8. Exposure controls/personal protection

Appropriate engineering controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower.

Individual protection measures, such as personal protective equipment

Skin protection - other: Wear appropriate protective clothing to prevent skin or eye exposure.

Occupational hygiene practices: 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.

Comments: Use appropriate PPE as necessary. Safety glasses at minimum.

9. Physical and chemical properties

Physical state: Liquid

Appearance: Viscous Liquid

Color: Blackish-Brown to Reddish Tan

Odor: Burnt Sugar aroma

Odor threshold: No data available.

pH: ~ 2.6 to 10.5

Melting point: No data available.

Freezing point: ≤ 0°C (32°F)

Initial boiling point and boiling range: ≥ 100°C (212°F)

Flash point: > 93°C (201°F) ASTM D7094

Evaporation rate (n-butyl acetate = 1): No data available.

Explosion limit / flammability limit notes: No data available.

Vapor pressure: No data available.

Relative vapor density: No data available.

Relative density: ~ 1.24 to 1.40 at 25°C

Solubility: Water: 100%

Partition coefficient n-octanol/water (logarithmic value): No data available.

Auto-ignition temperature: No data available.

Decomposition temperature: No data available.

Dynamic viscosity: ~ 10 to 5000 CPS

Oxidizing properties: No data available.

Percent volatiles: No data available.

10. Stability and reactivity

Reactivity: No dangerous reaction known under conditions of normal use.

Dangerous polymerization: Product will not undergo polymerization.

Chemical stability: Stable under normal temperatures and pressures.

Conditions to avoid: Excess heat

Incompatible materials: None known.

11. Toxicological information

Acute toxicity

Notes: None Classified.

Skin corrosion / irritation: Not Classified.

Serious eye damage / irritation: Not Classified.

Respiratory or skin sensitization: Not Classified.

Germ cell mutagenicity: Not Classified.

Carcinogenicity

Notes: Not Classified.

Reproductive toxicity: Not Classified.

Specific Target Organ Toxicity - single exposure: Not Classified.

Specific Target Organ Toxicity - repeated exposure: Not Classified.

Aspiration hazard: Not Classified.

General comments: For More Information Regarding Potential Health Effects, refer to Section 2.

12. Ecological information

Ecotoxicological information:

May contribute to biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

Comments: Ecological Information is not Reported.

13. Disposal considerations

Disposal methods: This material is not considered a hazardous material and may be disposed of in landfill or by incineration.

General comments: Dispose of in accordance with local, regional, national and international regulations. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner.

14. Transport information

USA Department of Transport Regulations (DOT)

Transport hazard class(es): Non-Hazardous

ADR / RID - road / rail

Hazard class: Non-Hazardous

ICAO - air

Transport hazard class(es): Non-Hazardous

IMDG - sea

Transport hazard class(es): Non-Hazardous

15. Regulatory information

UNITED STATES

SARA Section 311/312 Hazard Categories

Title III notes: Product contains no chemicals subject to the reporting requirements of EPCRA.

16. Other information

Prepared by: DPT **Date revised:** 06/28/2023

Revision summary: This SDS replaces the 09/10/2019 SDS. Revised: **Section 6:** General procedures, Large spill, Small spill. **Section 13:** Disposal methods.

Manufacturer supplemental notes: Department issuing SDS: Regulatory Affairs.

Manufacturer disclaimer: The above information relates only to the product and not to its use in combination with any other material or in any particular process. 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 Refresco'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 process and operations. Appropriate warnings and safe handling procedures should be provided to all handlers and users. The intended use and the specific conditions and factors relating to such use, must be done in accordance with applicable laws and regulations.