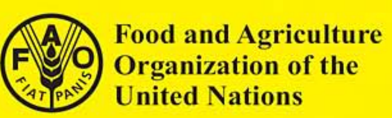
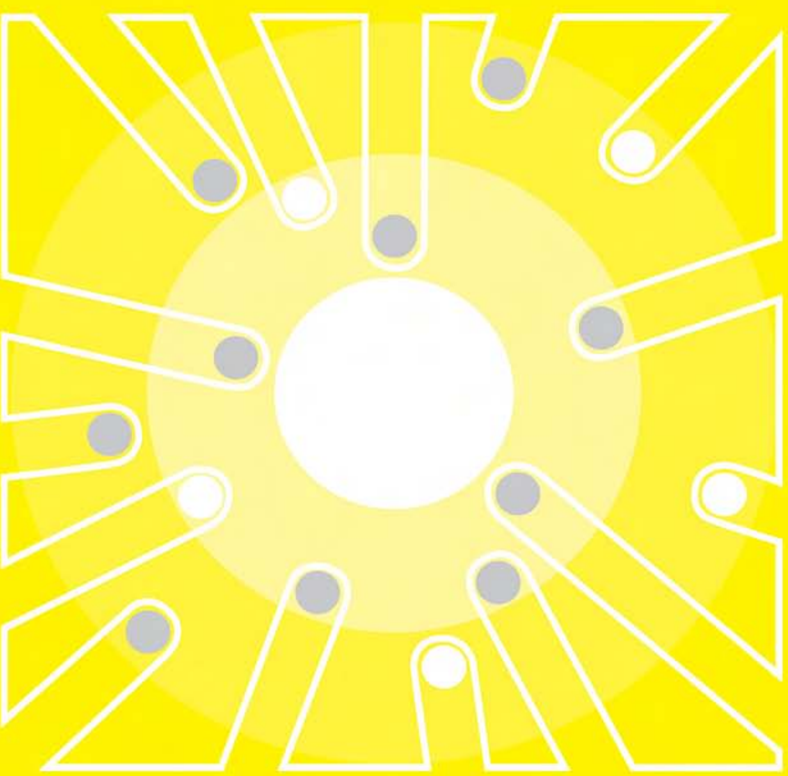
WHO FOOD ADDITIVES SERIES: 64

Safety evaluation of certain food additives and contaminants

Prepared by the
Seventy-third meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)





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PREFACE

The monographs contained in this volume were prepared at the seventy-third meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at WHO headquarters in Geneva, Switzerland, on 8–17 June 2010. These monographs summarize the data on selected food additives (flavouring agents) and contaminants reviewed by the Committee.

The seventy-third report of JECFA has been published by the World Health Organization as WHO Technical Report No. 960. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by temporary advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by M. Sheffer, Ottawa, Canada.

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 organizations participating in WHO concerning the legal status of any country, territory, city or area or 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 organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

SAFETY EVALUATIONS OF GROUPS OF RELATED FLAVOURING AGENTS

INTRODUCTION

Assignment to structural class

Twelve groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

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

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference *122*):

- *Innocuous metabolic products* are defined as products that are known or readily predicted to be harmless to humans at the estimated dietary exposure to the flavouring agent.
- *Endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated dietary exposure to a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

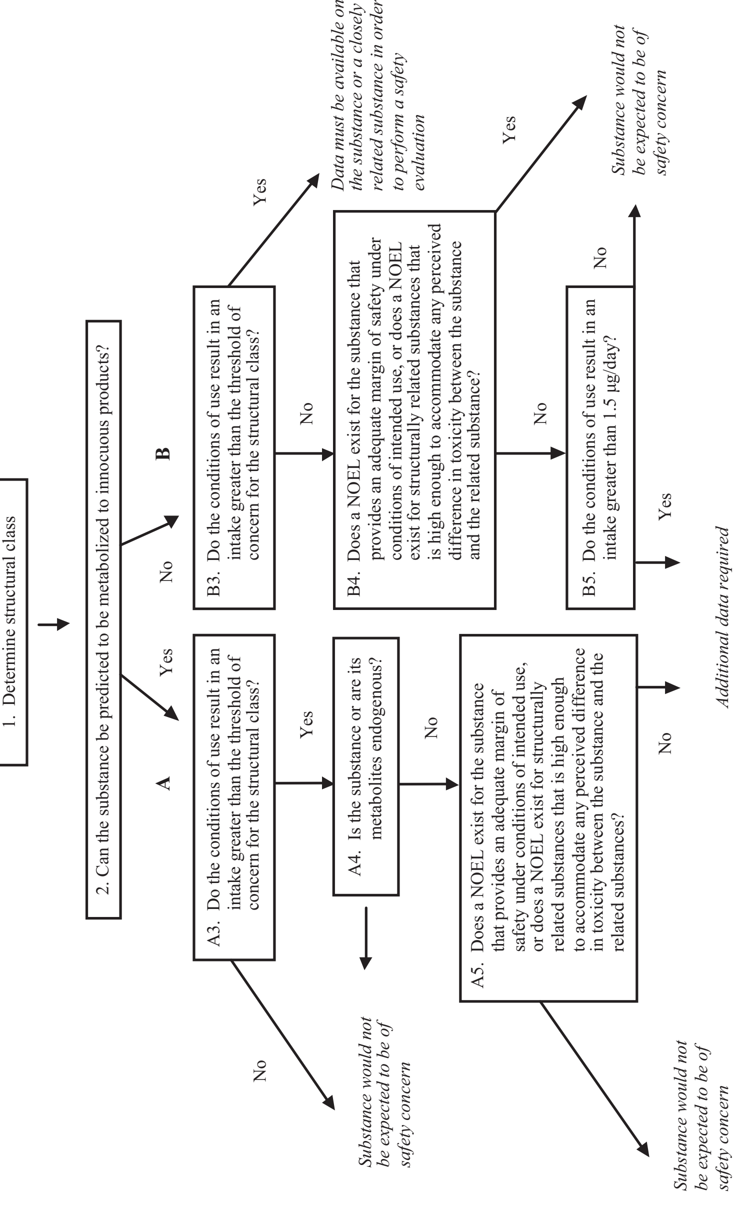
Assessment of dietary exposure

MSDI (maximized survey-derived intake)

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

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

Figure 1. Procedure for the Safety Evaluation of Flavouring Agents



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

SPET (single portion exposure technique)

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

The SPET provides an estimate of dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day. The SPET combines an average (or usual) added use level provided by the flavour industry with a standard portion size from 75 predefined food categories as described by the Committee at its sixty-seventh meeting. The standard portion is taken to represent the mean food consumption for consumers of these food categories. Among all the food categories with a reported use level, the calculated dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.

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

The highest result is used in the evaluation.

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

ALICYCLIC KETONES, SECONDARY ALCOHOLS AND RELATED ESTERS (addendum)

First draft prepared by

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1. EVALUATION

1.1 Introduction

The Committee evaluated 12 additional flavouring agents that are members of a group entitled alicyclic ketones, secondary alcohols and related esters. The additional flavouring agents included one saturated alicyclic ketone (No. 2050), two unsaturated alicyclic ketones (Nos 2049 and 2052), one alicyclic diether (No. 2051), one alicyclic secondary ester (No. 2053), one alicyclic α-hydroxy ketone (No. 2054), two unsaturated alicyclic keto-esters (Nos 2055 and 2056), one tri-unsaturated alicyclic ketone (No. 2057), one di-unsaturated alicyclic keto-hydroxy-diol (No. 2058) and two di-unsaturated bicyclic keto-ethers (Nos 2059 and 2060). The evaluations were conducted according to the Procedure for the Safety Evaluation

of Flavouring Agents (Figure 1, Introduction) (Annex 1, reference *131*). None of these flavouring agents has been evaluated previously.

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

Four of the 12 flavouring agents (Nos 2052, 2054, 2057 and 2058) in this group have been reported to occur naturally and can be found in honey, black teas, green and roasted mate, tomatoes and tomato juice, starfruit, clams, coffee, hazelnuts and grapefruit juice (Nijssen, van Ingen-Visscher & Donders, 2009).

1.2 Assessment of dietary exposure

The total annual volumes of production of the 12 alicyclic ketones, secondary alcohols and related esters are approximately 0.4 kg in the USA and 18 kg in Japan (Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009). Approximately 55% of the total annual volume of production in Japan is accounted for by one substance in this group—namely, cyclotene butyrate (No. 2056).

The estimated dietary exposures for each flavouring agent, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The estimated daily dietary exposure is greatest for (–)-8,9-dehydrotheaspirone (No. 2059) (4000 μg, the SPET value obtained from milk [dairy] and other fermented milk products). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 600 μg, with the SPET yielding the highest estimates. Annual volumes of production of this group of flavouring agents as well as the daily dietary exposures calculated as the MSDI or using the SPET are summarized in Table 2.

1.3 Absorption, distribution, metabolism and elimination

The esters in this group (Nos 2053 and 2055–2056) and the ketal (No. 2051) are predicted to be hydrolysed to their corresponding alcohols and carboxylic acids by carboxylesterases found in abundance in hepatocytes (Heymann, 1980; Anders, 1989; White et al., 1990; Graffner-Nordberg et al., 1998). The resulting alicyclic secondary alcohols can be interconverted enzymatically with the corresponding ketone in vivo. The principal detoxication pathway involves reduction of the ketone to yield the corresponding secondary alcohol, which is conjugated with glucuronic acid and excreted mainly in the urine. Side-chain oxidation, glutathione conjugation of α,β-unsaturated ketones and hydrogenation of endocyclic or exocyclic double bonds are other elimination pathways involved. Polar oxygenated metabolites are excreted primarily in the urine, either unchanged or as conjugates.

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

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II						
2-(*trans*-2-Pentenyl) cyclopentanone	2049	51608-18-5	A3. No, SPET: 450	NR	Note 2	No safety concern
2-Cyclopentylcyclopentanone	2050	4884-24-6	A3. No, SPET: 400	NR	Note 2	No safety concern
2-Cyclohexenone	2052	930-68-7	A3. No, SPET: 200	NR	Note 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
2,6,6-Trimethyl-2-hydroxycyclohexanone	2054	7500-42-7	A3. No, SPET: 300	NR	Note 4	No safety concern
Cyclotene propionate	2055	87-55-8	A3. No, SPET: 300	NR	Note 1	No safety concern
Cyclotene butyrate	2056	68227-51-0	A3. No, SPET: 200	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (mixture of isomers)	2057	13215-88-8	A3. No, SPET: 300	NR	Notes 2 and 3	No safety concern
4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (mixture of isomers)	2058	24427-77-8	A3. No, SPET: 300	NR	Notes 2 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	Are additional data available for substances with an estimated intake exceeding the threshold of concern?[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class III						
(−)-8,9-Dehydrotheaspirone	2059	85248-56-2	B3. Yes, SPET: 4000	The NOAEL of 60 mg/kg bw per day in a 28-day oral study in rats for the structural analogue No. 2060 (Imatanaka, 2003b) is 900 (based on the SPET) and >1 million (based on the MSDI) times the estimated daily dietary exposure to No. 2059 when used as a flavouring agent.	Notes 2 and 3	No safety concern
(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	2060	80722-28-7	B3. Yes, SPET: 600	The NOAEL of 60 mg/kg bw per day in a 28-day oral study in rats for No. 2060 (Imatanaka, 2003b) is at least 6000 times its estimated daily dietary exposure when used as a flavouring agent.	Notes 2 and 3	No safety concern

Table 1 (contd)

bw, body weight; CAS, Chemical Abstracts Service; NOAEL, no-observed-adverse-effect level; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure.

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

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

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

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

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

Notes:

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

Table 2. Annual volumes of production and dietary exposures for alicyclic ketones, secondary alcohols and related esters used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Exposure				Annual volume of natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
2-(*trans*-2-Pentenyl) cyclopentanone (2049)				450	7.5	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
2-Cyclopentylcyclopentanone (2050)				400	7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.1	0.001			
Cyclohexanone diethyl ketal (2051)				400	7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	7	1.9	0.03			
2-Cyclohexenone (2052)				200	3	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
3,3,5-Trimethylcyclohexyl acetate (2053)				150	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.2	0.003			
2,6,6-Trimethyl-2-hydroxycyclohexanone (2054)				300	5	+

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Exposure				Annual volume of natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.3	0.1	0.001			
Cyclotene propionate (2055)				300	5	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.2	0.003			
Cyclotene butyrate (2056)				200	3	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	10	3	0.05			
4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (mixture of isomers) (2057)				300	5	+
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (2058)				300	5	+
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
(−)-8,9-Dehydrotheaspirone (2059)				4000	67	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.02	0.0003			
(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (2060)				600	10	−

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Exposure				Annual volume of natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Total						
Europe	ND					
USA	0.4					
Japan	18					

bw, body weight; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From Gavin, Williams & Hallagan (2008), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

The alicyclic ketones in this group (Nos 2049–2050, 2052 and 2054–2060) are likely to be reduced to the corresponding secondary alcohol and excreted primarily as the glucuronic acid conjugate (Elliott, Parke & Williams, 1959). If a double bond is present, it may be reduced to the corresponding dihydro- derivative (Krasavage, O'Donoghue & Divincenzo, 1982). For metabolites excreted into the

bile, reduction of the double bond may occur, mediated by the gut microflora. Endocyclic double bonds (Nos 2052 and 2055–2060) are more prone to reduction compared with exocyclic double bonds (Nos 2049 and 2057–2058). In addition to reductive pathways, alicyclic ketones containing an alkyl or alicyclic side-chain (Nos 2049, 2050 and 2054–2060) may undergo oxidation of the side-chain to form polyoxygenated metabolites, which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned two flavouring agents (Nos 2051 and 2053) to structural class I, eight flavouring agents (Nos 2049, 2050, 2052 and 2054–2058) to structural class II and two flavouring agents (Nos 2059 and 2060) to structural class III (Cramer, Ford & Hall, 1978).

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

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

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

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

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

A no-observed-adverse-effect level (NOAEL) of 60 mg/kg body weight (bw) per day for (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) was identified in a 28-day oral study (Imatanaka, 2003b). In this study, doses of 12, 60 or 300 mg/kg bw per day were administered by gavage to rats (10 of each sex per dose). No changes attributable to No. 2060 were reported for body weight, food or water consumption, haematological examination or urinalyses. Some behavioural/motor effects were observed at 300 mg/kg bw per day. Changes in

serum enzyme activities and cholesterol and triglyceride levels were reported at the end of the study in those rats treated with the 300 mg/kg bw per day dose. An increase in liver weight was reported for females only at 60 mg/kg bw per day. This change was considered non-adverse and led to the designation of 60 mg/kg bw per day as the NOAEL. This NOAEL provides a margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2060 (SPET = 600 μg/day) when used as a flavouring agent.

(−)-8,9-Dehydrotheaspirone (No. 2059) is a close structural analogue of (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060), and toxicological studies on that compound can be used for the evaluation of No. 2059. The NOAEL of 60 mg/kg bw per day provides a margin of safety of 900 in relation to the highest estimated dietary exposure to No. 2059 (SPET = 4000 μg/day) when used as a flavouring agent. The Committee noted that the margin of safety of 900 between the SPET estimate for No. 2059 and the NOAEL for No. 2060 is lower than the value of 1000, which was proposed as an adequate margin of safety for flavouring agents on the B-side of the decision-tree at the forty-fourth meeting of the Committee (Annex 1, reference *116*). The value of 1000 was based on the comparison of the NOAEL with the MSDI. The Committee noted that the margin of safety for No. 2059 based on the MSDI of 0.02 μg/day and the NOAEL of 60 mg/kg bw per day for No. 2060 exceeds 1 million and concluded that the values of 900 (based on the SPET) and greater than 1 million (based on the MSDI) provided an adequate margin of safety.

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

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

1.5 Consideration of combined intakes from use as flavouring agents

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

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

Flavouring agents in this group with the highest intakes that have the common metabolite cyclohexanol or a cyclohexenol derivative are Nos 1099 and 2053 in structural class I, Nos 1098, 1108, 1109, 1111–1113, 2052 and 2054 in structural class II and No. 2059 in structural class III. In the unlikely event that these

were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 22.5, 4.9 and 0.3 μg/day, respectively, which would not exceed any of the thresholds of concern (i.e. 1800 μg/day for class I, 540 μg/day for class II and 90 μg/day for class III).

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

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

1.6 Consideration of secondary components

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

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the additional key data relevant to the safety evaluation of the group of 12 alicyclic ketones, secondary alcohols and related esters, including 1 saturated alicyclic ketone (No. 2050), 2 unsaturated alicyclic ketones (Nos 2049 and 2052), 1 alicyclic diether (No. 2051), 1 alicyclic secondary ester (No. 2053), 1 alicyclic α-hydroxy ketone (No. 2054), 2 unsaturated alicyclic keto-hydroxy-esters (Nos 2055 and 2056), 1 tri-unsaturated alicyclic ketone (No. 2057), 1 di-unsaturated alicyclic keto-diol (No. 2058) and 2 di-unsaturated bicyclic

keto-ethers (Nos 2059 and 2060). These 12 flavouring agents are additions to a group of 25 flavouring agents evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposure estimates were made using the MSDI approach as well as the SPET.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

Relevant data on the absorption, distribution, metabolism and excretion of these flavouring agents have not been reported since the publication of the original monograph (Annex 1, reference *160*).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for 3 of the 12 additional flavouring agents in this group (Table 3). For 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057), an LD_{50} value in rats of 1000 mg/kg bw was reported (Yoshitake, 2001a). For 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058), an LD_{50} value in rats of 2000 mg/kg bw was reported (Uzuka, 2002). For (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060), an LD_{50} in rats was found to be 1000 mg/kg bw (Yoshitake, 2001b).

These studies demonstrate that the acute oral toxicity of alicyclic ketones, secondary alcohols and related esters is low.

Table 3. Results of acute toxicity studies for alicyclic ketones, secondary alcohols and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Rat; M, F	1000	Yoshitake (2001a)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Rat; M, F	2000	Uzuka (2002)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Rat; M, F	1000	Yoshitake (2001b)

F, female; M, male

(b) Short-term and long-term studies of toxicity

Results of short-term studies of toxicity are available for 2-cyclohexenone (No. 2052), 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057), 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058) and (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060). These studies are summarized in Table 4 and described below. No additional studies of short-term or long-term toxicity on any of the related flavouring agents previously evaluated have become available since their previous evaluation (Annex 1, reference *160*).

(i) 2-Cyclohexenone (No. 2052)

In a subchronic toxicity study, groups of B6C3F1 mice (10 of each sex per dose) were exposed to atmospheres containing 2-cyclohexenone at concentrations of 0, 2.5, 5 or 10 parts per million (ppm) via inhalation for 6 h/day, 5 days/week, for 13 weeks (Cunningham et al., 2001; NTP, 2009). These concentrations correspond to systemic exposure levels of approximately 0, 4.2, 8.4 and 16.8 mg/kg bw per day in mice (Fassett, 1978). Body weights were recorded prior to the daily exposure and weekly thereafter. Prior to the last exposure, blood was collected for clinical pathology. At necropsy, tissue weights were obtained for liver, thymus, right kidney, right testicle, heart and lungs. Additional tissues were collected for histopathology. Vaginal smears were prepared for female mice for the last 12 days of exposure. Vaginal cytology slides were analysed, and the estrous cycle stages were determined for each day. At necropsy, the left testis and epididymis from male mice were collected and weighed, and sperm motility and sperm density counts were conducted. Also, additional blood smears were prepared for 10 animals of each sex per dose, and these slides were stained and evaluated for the presence of micronuclei (as in MacGregor et al., 1990).

No compound-related lesions were identified in the trachea, larynx or lung in either sex (Cunningham et al., 2001). In the nasal cavity, thickening of the respiratory epithelium (hyperplasia) occurred in mice exposed to 10, 5 and 2.5 ppm, whereas erosions and squamous metaplasia were present in the high dose group (10 ppm). These lesions were predominately localized to the nasoturbinates and maxilloturbinates in the most anterior portion (level 1) of the nasal cavity, whereas the respiratory and olfactory epithelia of levels 2 and 3 were reported to be normal. Because of the route of exposure employed in this study (i.e. inhalation), these effects on the respiratory epithelium are not likely to be biologically relevant to the oral administration of the test material or its use as a flavouring agent. Body weights were not significantly different from those of controls after 13 weeks of exposure, and no haematological abnormalities were detected. Relative liver weights were increased significantly in female mice at 5 and 10 ppm and in male mice at 10 ppm, although no accompanying histopathological lesions were reported. The results of bone marrow micronucleus assay and all reproductive end-points evaluated indicated that 2-cyclohexenone produced no clastogenic or reproductive toxicity at exposures up to 10 ppm in mice (Cunningham et al., 2001; NTP, 2009). The NOAEL was considered to be 10 ppm (estimated to be equivalent to 16.8 mg/kg bw per day), the highest dose tested.

Table 4. Results of short-term studies of toxicity with alicyclic ketones, secondary alcohols and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/no. per group[b]	Route	Duration (days)	NOAEL (mg/kg bw per day)	Reference
2052	2-Cyclohexenone	Mouse; NR	3/20	Inhalation	90	16.8[c,d]	Cunningham et al. (2001)
2052	2-Cyclohexenone	Rat; M, F	3/20	Inhalation	90	6.2[c,d]	Cunningham et al. (2001)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Rat; M, F	3/8	Oral (gavage)	14	40	Yoshitake (2001d)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Rat; M, F	3/20	Oral (gavage)	28	16[e]	Imatanaka (2003c)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Rat; M, F	3/8	Oral (gavage)	14	40	Imatanaka (2003a)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Rat; M, F	3/20	Oral (gavage)	28	25	Imatanaka (2004)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Rat; M, F	3/8	Oral (gavage)	14	80	Yoshitake (2001c)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Rat; M, F	3/20	Oral (gavage)	28	60	Imatanaka (2003b)

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

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

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

[c] The highest dose tested.

[d] Exposure level converted from inhalation dosage to oral dosage, as in Fassett (1978).

[e] The Committee considered that the NOAEL may be higher because these effects generally are not considered to be adverse and/or are male rat specific (hyaline droplets).

In a similar subchronic toxicity study by the same authors, groups of Fischer 344 rats (10 of each sex per dose) were exposed daily to atmospheres containing 2-cyclohexenone at concentrations of 0, 2.5, 5 or 10 ppm for 13 weeks (Cunningham et al., 2001; NTP, 2009). These concentrations correspond to systemic exposure levels of approximately 0, 1.55, 3.1 and 6.2 mg/kg bw per day in rats (Fassett, 1978). As with the previous study (i.e. with mice), no compound-related lesions were identified in the trachea, larynx or lung in rats of either sex. Similar local effects likely to be resulting from the route of exposure (as were found in mice), such as hyperplasia and inflammation, were reported. Body weights were not significantly different from those of controls after 13 weeks of exposure, and no haematological abnormalities were detected. Increased relative liver weights were observed in female rats, but were not accompanied by any histopathological evidence of lesions. Both control and treated groups of males exposed to 2-cyclohexenone were reported to show cytoplasmic vacuolization of the centrilobular region of the liver. There was no difference in the incidence or severity of the lesions between test and control animals (NTP, 2009). The results of bone marrow micronucleus assay and all reproductive end-points evaluated indicated that 2-cyclohexenone produced no clastogenic or reproductive toxicity at exposures up to 10 ppm in rats (Cunningham et al., 2001; NTP, 2009). For rats, the NOAEL was considered to be 10 ppm (estimated to be equivalent to 6.2 mg/kg bw per day), the highest dose tested by this route of exposure.

(ii) 4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057)

In a range-finding study, groups of Crj:CD(SD) IGS rats (four of each sex per dose) were administered via gavage 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one at doses of 0, 40, 200 and 1000 mg/kg bw per day for 14 days (Yoshitake, 2001d). In the high dose (1000 mg/kg bw per day) group, decreased spontaneous locomotion in males (4/4) and females (3/4), salivation in males (3/4) and females (2/4), staining of hair in males (4/4) and females (4/4) and staining around the nose and mouth in males (4/4) and females (4/4) were observed. One male died on day 14 of dosing in the 1000 mg/kg bw per day group. Body weights were lower in males from day 3 to day 14 and in females from day 10 to day 14 for the 1000 mg/kg bw per day group. Blood chemistry examination revealed an increase in glutamic pyruvic transaminase (i.e. alanine aminotransferase) activities and total cholesterol levels in both sexes of the high dose group and increases in alkaline phosphatase and γ-glutamyl transpeptidase activities and triglyceride and total bilirubin levels in the females of the high dose group. The clinical signs observed in males in the middle dose group (200 mg/kg bw per day) were staining around the nose and mouth (1/4). No significant differences in blood chemistry values between the 40 or 200 mg/kg bw per day groups and the control group were observed.

In males of the 1000 mg/kg bw per day group, decreased absolute and relative spleen weights as well as increased relative liver and kidney weights were reported. In females of the 200 and 1000 mg/kg bw per day groups, increased absolute and relative liver weights and decreased absolute spleen weights were reported. Enlarged livers were reported in all females of the high dose group, but

were presumed to result from metabolic changes due to administration of the test material. Histopathologically, diffuse hypertrophy of hepatocytes (3/3), prominent nucleoli of hepatocytes of the liver (3/3) and vacuolization of tubular epithelium of the kidney (1/3) were reported in males of the 1000 mg/kg bw per day group. No significant changes were reported in the low dose group (40 mg/kg bw per day) (Yoshitake, 2001d), which can be considered the NOAEL for this 14-day study.

In a follow-up 28-day study using the same protocol as above, groups of Crj:CD(SD) IGS rats (10 of each sex per dose) were administered 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one at doses of 0 (control), 16, 80 and 400 mg/kg bw per day via gavage (Imatanaka, 2003c). A recovery study was also included in which dosed animals (six of each sex at 400 mg/kg bw per day) were allowed to recover for 28 days following the last dose. No significant changes were attributable to 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one administration with respect to clinical signs, food consumption or haematological examinations. Lower body weights were reported in males of the 400 mg/kg bw per day group from day 8 to day 28 of administration. Both sexes in the 400 mg/kg bw per day group had increased γ-glutamyl transpeptidase activities and decreased chloride levels. Males of this group had increased glutamic pyruvic transaminase (i.e. alanine aminotransferase) and alkaline phosphatase activities and total bilirubin levels, along with decreased sodium levels. Females of this group had increased total cholesterol, triglyceride and albumin levels.

Increased absolute and relative liver and kidney weights were reported in both males and females of the 400 mg/kg bw per day group. Histopathological examinations revealed centrilobular hypertrophy of hepatocytes in males and females of the 400 mg/kg bw per day group, as well as focal necrosis in males of the 400 mg/kg bw per day group. Histopathology also revealed increased hyaline droplets in the kidneys of males in the 80 and 400 mg/kg bw per day groups. However, these effects were attributed to α-2u-globulin nephropathy of the male rat. Therefore, this mode of action is not relevant to human renal toxicity and is not indicative of a risk to humans (Capen et al., 1999). Statistically significant increased relative liver weights (female) and kidney weights (male) were reported in the 80 mg/kg bw per day groups. In the recovery study, all changes and variations observed during or upon the termination of the administration period disappeared during the recovery period or at the end of the recovery period.

Based on the increased relative liver weights (females) and kidney weights (males) as well as the increased hyaline droplets in male kidneys, and under the conditions of the study, the author considered the NOAEL for No. 2057 to be 16 mg/kg bw per day (Imatanaka, 2003c). The Committee considered that the NOAEL may be higher because these effects generally are not considered to be adverse and/or are male rat specific (hyaline droplets).

(iii) 4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058)

In a range-finding study using the previously reported protocol (Imatanaka, 2003c), groups of Crj:CD(SD) rats (four of each sex per dose) were administered

4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one via gavage at doses of 0, 40, 200 and 1000 mg/kg bw per day for 14 days (Imatanaka, 2003a).

In the 1000 mg/kg bw per day groups, three out of four males and two out of four females presented with decreased spontaneous locomotion, and one male and one female were observed to have incomplete eyelid closure. Measurement of body weights revealed lower values in males and females in the 1000 mg/kg bw per day group on day 3. Blood chemistry examinations at 1000 mg/kg bw per day revealed increased γ-glutamyl transpeptidase activities and total protein levels in males and females; increased glutamic pyruvic transaminase (i.e. alanine aminotransferase) activities and total bilirubin levels as well as decreased triglyceride levels, albumin to globulin ratio and creatinine levels in males; and increased total cholesterol levels and ketone bodies in females. There were no abnormal clinical signs attributable to 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one in male or female groups receiving less than 1000 mg/kg bw per day.

Increased relative kidney weights were also reported in males of the 1000 mg/kg bw per day group. At necropsy, enlarged livers were reported in males at 200 mg/kg bw per day or more and in females at 1000 mg/kg bw per day. Histopathological examinations revealed hypertrophy of hepatocytes in the liver of males at 200 mg/kg bw per day or more and a ground glass–like appearance of the liver of males at 1000 mg/kg bw per day. Increased relative and absolute liver weights were reported in males receiving 40 mg/kg bw per day or more and in females receiving 200 mg/kg bw per day or more. There were no abnormal histopathological findings that corresponded with the enlarged liver in females (Imatanaka, 2003a).

Based on these results, the main changes caused by 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one were revealed as effects on the liver. Increased organ weights were reported in the kidney, but were not accompanied by histopathological changes. Therefore, the NOAEL of 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one was estimated to be 40 mg/kg bw per day for this 14-day study (Imatanaka, 2003a).

In a follow-up 28-day study using the same protocol as above, groups of Crj:CD(SD) IGS rats (10 of each sex per dose) were administered 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one via gavage at doses of 0 (control), 5, 25 and 125 mg/kg bw per day (Imatanaka, 2004). No changes attributable to 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one were reported in general condition, body weight, food consumption, water consumption, urinalysis or haematological examination. In blood chemical examinations, increased γ-glutamyl transpeptidase activity was noted in the females of the 125 mg/kg bw per day group. Increased total protein levels were noted in males of all groups; however, dose dependency was minor, and the effects were considered incidental. Increased relative liver weights were noted in males of the 25 mg/kg bw per day group. Increased absolute and relative liver weights were noted in males and females of the 125 mg/kg bw per day group. Upon necropsy, enlargement of the liver was observed in males and females of the 125 mg/kg bw per day group. Pelvic dilatation was also reported in the kidney of males in

the 5 and 125 mg/kg bw per day groups, but was not attributed to administration of 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one. In histopathological examinations, centrilobular hypertrophy of hepatocytes was reported in the liver of males and females in the 125 mg/kg bw per day group. Mineralization in the corticomedullary junction was also reported in the kidney of females in the 125 mg/kg bw per day group, but was not considered attributable to 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one administration, because the finding was also reported in the vehicle control group.

On the basis of the biochemical and histopathological observations of males and females in the 125 mg/kg bw per day group, the NOAEL under the present study conditions was concluded to be 25 mg/kg bw per day (Imatanaka, 2004).

(iv) (±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060)

In a range-finding study using the previously reported protocol (Imatanaka, 2003c), groups of Crj:CD(SD) rats (four of each sex per dose) were administered 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one via gavage at doses of 0, 16, 80 and 400 mg/kg bw per day for 14 days (Yoshitake, 2001c). In the high dose (400 mg/kg bw per day) group, clinical signs in females included decreased spontaneous locomotion (4/4), decreased respiratory rate (1/4), stupor (1/4) and staggering gait (4/4). Increases in total cholesterol level, γ-glutamyl transpeptidase activity, triglyceride level and blood urea nitrogen were also reported in females of the 400 mg/kg bw per day group. Blood chemistry revealed an increase in glutamic pyruvic transaminase (i.e. alanine aminotransferase) activities in males at 16, 80 and 400 mg/kg bw per day.

In males and females of the high dose group, increases in absolute and relative liver weights were reported. Also, increased relative weights of brain and adrenals were reported in females of the high dose group. Macroscopic examination revealed enlarged livers in one male and one female in the high dose group (400 mg/kg bw per day). In males, this corresponded with histopathological findings, which included periportal fine vacuolization of hepatocytes, periportal prominent nucleoli of hepatocytes and solitary cysts of the renal cortex. There were no macroscopic or microscopic findings related to the enlarged liver in females (Yoshitake, 2001c). Based on the lack of histological changes, the NOAEL was considered to be 80 mg/kg bw per day.

In a follow-up 28-day study using the same protocol as above, groups of Crj:CD(SD) IGS rats (10 of each sex per dose) were administered 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one via gavage at doses of 0 (control), 12, 60 and 300 mg/kg bw per day (Imatanaka, 2003b). No changes attributable to 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one were reported in body weight, food consumption, water consumption, haematological examination or urinalysis. Decreased spontaneous locomotion, staggering gait and stupor were reported as changes considered to be attributable to administration of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one to females in the 300 mg/kg bw per day group. Salivation was reported in males and females in the 300 mg/kg bw per

day group on day 0, with the incidence of salivation increasing in the first 2 weeks after administration. Staining of the lower abdomen in males in the 300 mg/kg bw per day group was a secondary change accompanying moist hair caused by salivation.

Increased γ-glutamyl transpeptidase activities, total cholesterol levels and triglyceride levels in males and females and increased glutamic pyruvic transaminase (i.e. alanine aminotransferase) activities in males were reported at the end of administration in the 300 mg/kg bw per day group. Increased absolute liver weights in females in the 300 mg/kg bw per day group were reported, as well as increased relative liver weights (considered an adaptive change) in females receiving 60 or 300 mg/kg bw per day and in males receiving 300 mg/kg bw per day. Upon necropsy, enlargement of the liver and spleen were also reported in females of the 300 mg/kg bw per day group; these changes corresponded to histopathological changes, which included diffuse hypertrophy of hepatocytes, increased mitoses of hepatocytes, microgranuloma and periportal lipid droplets of hepatocytes and increased extramedullary haematopoiesis in the spleen. In males, increased hyaline droplets were reported in the kidney in the 300 mg/kg bw per day group. However, these findings were also reported in the vehicle control group and are likely attributable to α-2u-globulin nephropathy of the male rat. Therefore, this mode of action is not relevant to human renal toxicity and is not indicative of a risk to humans (Capen et al., 1999).

On the basis of the biochemical and histopathological observations of males and females in the 300 mg/kg bw per day group, the NOAEL under the present study conditions was concluded to be 60 mg/kg bw per day (Imatanaka, 2003b).

(d) Genotoxicity studies

Genotoxicity data (in vitro and in vivo) are available for 5 of the 12 flavouring agents in this group (Nos 2051, 2052, 2057, 2058 and 2060). Additionally, studies of in vitro genotoxicity have been reported on five flavouring agents in this group that were previously evaluated (Annex 1, reference *160*). The results of all of these studies are summarized in Table 5 and described below.

(i) In vitro

No indication of mutagenic potential was reported in the Ames test when *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were incubated in the presence of cyclohexanone diethyl ketal (No. 2051) at 15–5000 μg/plate with or without S9 activation, using both the direct plate incorporation and preincubation methods (Thompson, 2004). In the direct plate incorporation method, an increase in toxicity was reported in strain TA100 at 5000 μg/plate in the presence and absence of liver activation. With the preincubation method, the test material exhibited toxicity to all bacterial strains from 1500 and 500 μg/plate in the presence and absence of S9 activation, respectively. A light, oily precipitate was reported at 5000 μg/plate, but did not prevent scoring of revertant colonies (Thompson, 2004).

Table 5. Studies of genotoxicity with alicyclic ketones, secondary alcohols and related esters used as flavouring agents

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
2051	Cyclohexanone diethyl ketal	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	15–5000 µg/plate with or without S9 activation	Negative[a,b,c]	Thompson (2004)
2051	Cyclohexanone diethyl ketal	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	15–5000 µg/plate with or without S9 activation	Negative[a,d,e]	Thompson (2004)
2052	2-Cyclohexenone	Reverse mutation	*S. typhimurium* TA97, TA98, TA100 and TA1535	10, 33, 100, 333 and 1000 µg/plate	Negative[a,b]	NTP (1994)
2052	2-Cyclohexenone	Reverse mutation	*S. typhimurium* TA100	240, 360, 721 and 1442 µg/ml[f]	Slightly positive[a,g]	Lutz et al. (1982)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	9.38, 18.8, 37.5, 75, 150 and 300 µg/plate	Negative[a,b,h]	Otsuka (2000)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Reverse mutation	*Escherichia coli* strain WP2*uvr*A	18.8, 37.5, 75, 150, 300 and 600 µg/plate	Negative[a,b,f]	Otsuka (2000)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Chromosomal aberration	Chinese hamster lung fibroblast cells	31.3, 62.5 and 125 µg/ml[i]	Negative[i]	Ajimi (2000a)
				50, 100 and 200 µg/ml[j]	Slightly positive[j]	

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	156, 313, 625, 1250, 2500 and 5000 μg/plate	Negative[a,b]	Ozaki (2000a)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Reverse mutation	*E. coli* strain WP2*uvr*A	156, 313, 625, 1250, 2500 and 5000 μg/plate	Negative[a,b]	Ozaki (2000a)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Chromosomal aberration	Chinese hamster lung fibroblast cells	313, 625, 1250, 2500 and 5000 μg/ml	Negative	Ozaki (2000b)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	19.5, 39.1, 78.1, 156, 313, 625 and 1250 μg/plate	Negative[a,b,k]	Otsuka (1999)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Reverse mutation	*E. coli* strain WP2*uvr*A	19.5, 39.1, 78.1, 156, 313, 625 and 1250 μg/plate	Negative[a,b,l]	Otsuka (1999)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Chromosomal aberration	Chinese hamster lung fibroblast cells	100, 200 and 400 μg/ml[i] 150, 300 and 600 μg/ml[j]	Negative[a]	Ajimi (1999a)
1100	Cyclohexanone	Reverse mutation	*S. typhimurium* TA98 and TA100	1.0 and 98 μg/plate[m] (0.01 and 1 mmol/l)	Negative[a,b]	Kubo, Urano & Utsumi (2002)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1109	2-*sec*-Butylcyclohexanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	10, 33, 100, 333, 1000 and 2500 µg/plate	Negative[a,b,n]	Poth (2003b)
1109	2-*sec*-Butylcyclohexanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	33, 100, 333, 1000, 2500 and 5000 µg/plate	Negative[a,d,n]	Poth (2003b)
1112	Isophorone	Reverse mutation	*S. typhimurium* TA98 and TA100	1.4 and 138 µg/plate[o] (0.01 and 1 mmol/l)	Negative[a,b]	Kubo, Urano & Utsumi (2002)
1113	3-Methyl-5-propyl-2-cyclohexen-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	78.13, 156.3, 312.5, 625 and 1250 µg/plate[i] 78.13, 156.3, 312.5, 625 and 1250 µg/plate[j]	Negative[a,d,p]	Haddouk (2004)
1113	3-Methyl-5-propyl-2-cyclohexen-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	14.65, 29.3, 58.59, 117.2 and 234.4 µg/plate[i] 58.59, 117.2, 234.4, 468.8, 937.5 and 1875 µg/plate[j]	Negative[a,b,q]	Haddouk (2004)
1114	3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	3, 10, 33, 100, 333, 1000 and 2500 µg/plate	Negative[a,b,r]	Poth (2003a)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1114	3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	3, 10, 33, 100, 333, 1000 and 2500 µg/plate	Negative[a,c,r]	Poth (2003a)
In vivo						
2052	2-Cyclohexenone	Micronucleus assay	Mice	4.2, 8.4 and 16.8 mg/kg bw[s] (2.5, 5.0 and 10 ppm)	Negative	Cunningham et al. (2001)
2052	2-Cyclohexenone	Micronucleus assay	Rats	1.6, 3.1 and 6.2 mg/kg bw[s] (2.5, 5.0 and 10 ppm)	Negative	Cunningham et al. (2001)
2057	4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	Micronucleus assay	Mice	125, 250 and 500 mg/kg bw[t]	Negative	Ajimi (2000b)
2058	4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Micronucleus assay	Mice	250, 500 and 1000 mg/kg bw[t]	Negative	Toya (2000); Ishida (2008)
2060	(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one	Micronucleus assay	Mice	250, 500 and 1000 mg/kg bw[t]	Negative	Ajimi (1999b)

[a] With and without metabolic activation.
[b] Preincubation method.
[c] Cytotoxicity was observed in all bacterial strains from 1500 and 500 µg/plate in the presence and absence of S9 activation, respectively.

Table 5 (contd)

[d] Standard plate incorporation method.
[e] Cytotoxicity was reported in strain TA100 at 5000 μg/plate in the presence and absence of liver activation.
[f] Calculated with a relative molecular mass of 96.13.
[g] Cytotoxicity was observed at 721 and 1442 μg/ml.
[h] Cytotoxicity was observed in all strains at concentrations greater than 150 μg/plate with and without metabolic activation.
[i] Without metabolic activation.
[j] With metabolic activation.
[k] Cytotoxicity was observed in four *S. typhimurium* strains at concentrations of 625 μg/plate or greater.
[l] Cytotoxicity was observed in *E. coli* at a concentration of 1250 μg/plate.
[m] Calculated with a relative molecular mass of 98.14.
[n] Cytotoxicity was observed in all strains at concentrations greater than 2500 and 1000 μg/plate with and without S9 activation, respectively.
[o] Calculated with a relative molecular mass of 138.21.
[p] Cytotoxicity was observed in strains TA100 and TA102 at a concentration of 1250 μg/plate without S9 activation.
[q] Cytotoxicity was observed in all strains at concentrations greater than or equal to 468.8 and 234.4 μg/plate with and without S9 activation, respectively.
[r] Cytotoxicity was observed in all strains at concentrations greater than 1000 μg/plate with and without metabolic activation.
[s] Exposure via inhalation; conversion calculated by the method described by Fassett (1978).
[t] Administered via intraperitoneal injection.

No evidence of mutagenicity was observed when 2-cyclohexenone (No. 2052) at 10, 33, 100, 333 and 1000 μg/plate was incubated with *S. typhimurium* strains TA97, TA98, TA100 and TA1535 using the preincubation method, with and without S9 liver activation (NTP, 1994).

Evidence of slight mutagenicity was reported in a modified *S. typhimurium* mutagenicity assay in which strain TA100 was incubated with 2-cyclohexenone (No. 2052) at concentrations of 240, 360, 721 and 1442 μg/ml using the preincubation method. Following exposure to the test material (721 and 1442 μg/ml) for 48 h at 37 °C, the mutagenic activity was determined to be 1 and 3 revertants per micromole, with and without S9 liver activation, respectively. However, these reversions occurred at concentrations (721 and 1442 μg/ml) that were cytotoxic to the bacteria, as indicated by a cell survival rate of approximately 50% (Lutz et al., 1982).

No evidence of mutagenicity was observed when 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057) at 9.38, 18.8, 37.5, 75, 150 and 300 μg/plate was incubated with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 using the preincubation method, with and without metabolic activation (Otsuka, 2000). Similar results were obtained when 4-(2-butenylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one at 18.8, 37.5, 75, 150, 300 and 600 μg/plate was incubated with *Escherichia coli* strain WP2*uvrA* in the presence or absence of metabolic activation (Otsuka, 2000).

In a chromosomal aberration assay, Chinese hamster lung fibroblast (CHL/IU) cells were incubated with 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057) at 31.3, 62.5 and 125 μg/ml without metabolic activation or 50, 100 and 200 μg/ml with metabolic activation for 6 h. Chromosomal aberrations were observed without metabolic activation, but no concentration dependence was reported (Ajimi, 2000a). In a continuous treatment study in which 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one at 25, 50 and 100 μg/ml was incubated with CHL/IU cells for 24 h, no chromosomal aberrations were reported. As there was no concentration dependence for the induction of chromosomal aberrations and because most aberrations occurred at dose levels associated with high toxicity, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was considered not to be genotoxic (Ajimi, 2000a).

No evidence of mutagenicity was observed when 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058) at 156, 313, 625, 1250, 2500 and 5000 μg/plate was incubated with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 using the preincubation method, with and without metabolic activation. Similar results were obtained when 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one at 156, 313, 625, 1250, 2500 and 5000 μg/plate was incubated with *Escherichia coli* strain WP2*uvrA* in the presence or absence of metabolic activation (Ozaki, 2000a).

In a chromosomal aberration assay, Chinese hamster lung fibroblast (CHL/IU) cells were incubated with 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058) at 313, 625, 1250, 2500 and 5000 μg/ml with or without metabolic activation for 6 h (Ozaki, 2000b). Chromosomal aberrations

were not observed with or without metabolic activation. In the continuous treatment study in which 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one at 313, 625, 1250, 2500 and 5000 μg/ml was incubated with CHL/IU cells for 24 h, chromosomal aberrations were reported, but there was no dose dependence. As there was no concentration dependence for the induction of chromosomal aberrations, 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one was considered not to be genotoxic (Ozaki, 2000b).

No evidence of mutagenicity was observed when (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) at 19.5, 39.1, 78.1, 156, 313, 625 and 1250 μg/plate was incubated with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 using the preincubation method, with and without metabolic activation (Otsuka, 1999). Similar results were obtained when (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one at 19.5, 39.1, 78.1, 156, 313 and 625 μg/plate was incubated with *Escherichia coli* strain WP2*uvrA* in the presence or absence of metabolic activation (Otsuka, 1999).

In a chromosomal aberration assay, Chinese hamster lung fibroblast (CHL/IU) cells were incubated with (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) at 100, 200 and 400 μg/ml without metabolic activation or 150, 300 and 600 μg/ml with metabolic activation for 6 h. Chromosomal aberrations were not observed with or without metabolic activation. In a continuous treatment study in which (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one at 75, 150 and 300 μg/ml was incubated with CHL/IU cells for 24 h, no chromosomal aberrations were reported (Ajimi, 1999a).

No indication of mutagenic potential was reported in the Ames test when *S. typhimurium* strains TA98 and TA100 were incubated in the presence (0.01 and 1 mmol/l) of either cyclohexanone (No. 1100) or isophorone (No. 1112) with or without S9 activation (Kubo, Urano & Utsumi, 2002).

No indication of mutagenic potential was reported in the Ames test when *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were incubated in the presence of 33–5000 μg (0.2–32.4 μmol) of 2-*sec*-butylcyclohexanone (No. 1109) per plate with S9 activation or 10–2500 μg (0.06–16.2 μmol) per plate without S9 activation. Toxic effects (i.e. reduction in the number of revertants) were observed with metabolic activation at concentrations greater than 2500 μg/plate in nearly all strains. Precipitation of 2-*sec*-butylcyclohexanone was not observed (Poth, 2003b).

No indication of mutagenic potential was reported when *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were incubated according to the direct plate incorporation method in the presence of 3-methyl-5-propyl-2-cyclohexen-1-one (No. 1113) at 78–1250 μg (0.5–8.2 μmol) per plate with and without S9 activation. A moderate toxicity was noted in strains TA100 and TA102 at a dose level of 1250 μg/plate, without S9 activation (Haddouk, 2004). Similarly, no indication of mutagenic potential was reported when the experiment was performed according to the preincubation method, in which *S. typhimurium* was incubated in the presence of 3-methyl-5-propyl-2-cyclohexen-1-one at 14.65–234.4 μg/plate without S9 activation and at 58.59–1875 μg/plate with S9 activation. A

moderate to marked toxicity was noted at 234.4 μg/plate in all bacterial strains used without S9 activation, whereas moderate to marked toxicity was reported in various strains at dose levels greater than 468.8 μg/plate with liver activation. Precipitation of 3-methyl-5-propyl-2-cyclohexen-1-one was not observed (Haddouk, 2004).

No indication of mutagenic potential was reported in the Ames test when *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 and TA1538 were incubated (by both the standard plate incorporation method and the preincubation method) in the presence of 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (No. 1114) at 3–2500 μg (0.02–15.2 μmol) per plate with or without S9 activation. In the presence and absence of S9 mix, the test material was bacteriotoxic towards all strains at concentrations greater than 2500 μg/plate. Precipitation was not observed (Poth, 2003a).

(ii) In vivo

No increase in the incidence of micronucleated polychromatic erythrocytes in blood smears was observed when male and female mice were administered 2.5, 5.0 or 10 ppm 2-cyclohexenone (No. 2052) (corresponding to systemic exposure levels of approximately 4.2, 8.4 and 16.8 mg/kg bw per day; Fassett, 1978) for 6 h/day via inhalation (Cunningham et al., 2001).

In a similar assay, no increase in the incidence of micronucleated polychromatic erythrocytes in blood smears was observed when male and female rats were administered 2.5, 5.0 or 10 ppm 2-cyclohexenone (No. 2052) (corresponding to systemic exposure levels of approximately 1.6, 3.1 and 6.2 mg/kg bw per day; Fassett, 1978) for 6 h/day via inhalation (Cunningham et al., 2001).

No increase in the incidence of micronucleated polychromatic erythrocytes in 24 or 48 h femoral bone marrow was observed when male and female ICR mice were administered 125, 250 or 500 mg/kg bw per day of 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (No. 2057) via intraperitoneal injection (Ajimi, 2000b).

No increase in the incidence of micronucleated polychromatic erythrocytes in 24 or 48 h femoral bone marrow was observed when male and female ICR mice were administered 250, 500 or 1000 mg/kg bw per day of 4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (No. 2058) via intraperitoneal injection (Toya, 2000; Ishida, 2008).

No increase in the incidence of micronucleated polychromatic erythrocytes in 24 or 48 h femoral bone marrow was observed when male and female ICR mice were administered 250, 500 or 1000 mg/kg bw per day of (±)-2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (No. 2060) via intraperitoneal injection (Ajimi, 1999b).

(iii) Cytotoxicity, genotoxicity and glutathione depletion

In order to evaluate the cytotoxic and genotoxic potentials of 2-cyclohexenone (No. 2052) as compared with structurally related 2-alkenals, Glaab et al. (2001) performed a series of experiments in mammalian cell lines. V79 cells (Chinese hamster lung fibroblasts) were incubated with 2-cyclohexenone for 1 h

and monitored for concentration-dependent cytotoxicity and subsequently for 24 h to evaluate growth inhibition. The cytotoxicity of 2-cyclohexenone expressed as a median lethal concentration (LC_{50}) (based on membrane integrity) was reported to be 4.75 mmol/l. As such, 2-cyclohexenone was slightly less effective as a cytotoxic agent than cinnamaldehyde (No. 656) and (*E*)-hexenal (No. 1353), with LC_{50} values of 4.45 and 3.67 mmol/l, respectively, or significantly less cytotoxic than (2*E*,6*Z*)-nona-2,6-dienal (No. 1186), which had an LC_{50} value of 0.270 mmol/l. The median inhibitory concentration (IC_{50}) for inhibition of cell growth of V79 cells (percentage of control) was 0.015 mmol/l. Thus, 2-cyclohexenone was less cytotoxic than cinnamaldehyde (No. 656), (*E*)-hexenal (No. 1353) and (2*E*,6*Z*)-nona-2,6-dienal (No. 1186), which had IC_{50} values of 0.010, 0.017 and 0.005 mmol/l, respectively (Glaab et al., 2001).

The ability of 2-cyclohexenone to cause deoxyribonucleic acid (DNA) damage was evaluated using both a standard comet assay in V79 cells as well as a modified comet assay, in which a modified (alkaline) single cell gel electrophoresis is screened for oxidized purines following lysis of Caco-2 cells. DNA-damaging potential was expressed with a DC_{50} value, the concentration at which a test material would induce DNA damage in 50% of the cells. In both assays, 2-cyclohexenone was shown to exhibit a comparable level of DNA-damaging potential compared with that of 2-alkenals. In V79 cells, 2-cyclohexenone had a DC_{50} value of 0.27 mmol/l, whereas cinnamaldehyde (No. 656), (*E*)-hexenal (No. 1353) and (2*E*,6*Z*)-nona-2,6-dienal (No. 1186) had DC_{50} values of 0.49, 0.17 and 0.11 mmol/l, respectively. In Caco-2 cells, 2-cyclohexenone had a DC_{50} value of 0.46 mmol/l, whereas cinnamaldehyde (No. 656), (*E*)-hexenal (No. 1353) and (2*E*,6*Z*)-nona-2,6-dienal (No. 1186) had DC_{50} values of 0.54, 0.25 and 0.12 mmol/l, respectively (Glaab et al., 2001).

The tendency of 2-cyclohexenone to cause glutathione depletion was investigated using a kinetic assay, in which total glutathione was determined in V79, Caco-2 and primary human colon cells (Glaab et al., 2001). In V79 cells, 2-cyclohexenone and the majority of the other compounds induced a depletion of glutathione down to approximately 20% of control at concentrations in the range from 10 to 100 μmol/l. In Caco-2 cells and primary human colon cells, about 5-fold higher concentrations of the test materials had to be incubated with the cells in order to induce total glutathione depletions similar to those in the V79 cells (Glaab et al., 2001).

In conclusion, 2-cyclohexenone (No. 2052) was demonstrated to induce cytotoxicity, growth inhibition and DNA damage and to deplete total glutathione in three mammalian cell lines. DNA damage was observed at concentrations that were 10-fold or more lower than those necessary to induce cytotoxicity in each cell line. However, glutathione depletion was observed at lower concentrations of 2-cyclohexenone than those required to induce DNA damage. In all cell lines, this depletion of glutathione resulted in an increased sensitivity towards reactive oxygen species (i.e. hydrogen peroxide), suggesting that the test material may potentially cause an imbalance of intracellular pro- and anti-oxidants at micromole per litre concentrations (Glaab et al., 2001). Therefore, it is likely that the DNA damage demonstrated in these experiments is due to the increase in sensitivity of the cells

towards oxidative stress related to glutathione depletion. The authors noted that a non-genotoxic mechanism best explains these observed results. The effect of glutathione depletion may, in part, be responsible for the report of genotoxicity in an in vitro unscheduled DNA synthesis assay using rat hepatocytes at a 10 μmol/l concentration (Williams, Mori & McQueen, 1989).

The data indicate that the genotoxic potential of 2-cyclohexenone (No. 2052) is low when used as a flavouring agent.

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ALICYCLIC PRIMARY ALCOHOLS, ALDEHYDES, ACIDS AND RELATED ESTERS (addendum)

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1. EVALUATION

1.1 Introduction

The Committee evaluated 11 additional flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters that was evaluated previously. The additional flavouring agents included three saturated and unsaturated primary alcohols (Nos 1903–1905), four aldehydes (Nos 1900, 1902, 1906 and 1908), two acids (Nos 1899 and 1907), one acetal (No. 1901) and one related ester (No. 1898). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, Introduction) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 26 other members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*). The Committee

concluded that all 26 flavouring agents in this group were of no safety concern at estimated dietary exposures.

Three of the 11 flavouring agents in this group are natural components of foods (Nos 1898, 1905 and 1906). Methyl dihydrojasmonate (No. 1898), for example, has been detected in tea, 1,3-*p*-menthadien-7-al (No. 1906) in cumin seed and honey and *p*-menthan-7-ol (No. 1905) in cherries, citrus fruits, berries, dill and grape brandy (Nijssen, van Ingen-Visscher & Donders, 2009).

1.2 Assessment of dietary exposure

The total annual volumes of production of the 11 flavouring agents in this group are approximately 6321 kg in Europe (European Flavour and Fragrance Association, 2004), 15 388 kg in the USA (Gavin, Williams & Hallagan, 2008) and 93 kg in Japan (Japan Flavor and Fragrance Materials Association, 2005). Methyl dihydrojasmonate (No. 1898) contributes the most to the total annual production volumes in Europe, Japan and the USA (100%, 94% and 100%, respectively).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest daily dietary exposure is estimated for *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899) (3000 µg, the SPET value obtained from non-alcoholic beverages), followed by methyl dihydrojasmonate (No. 1898) (1875 µg, the MSDI). For all but one of the other flavouring agents, the estimated daily dietary exposures were higher using the SPET and were in the range of 0.01–240 µg. Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

1.3 Absorption, distribution, metabolism and elimination

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

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 11 flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters, the Committee assigned 7 flavouring agents (Nos 1899, 1900 and 1902–1906) to structural class I, 3 flavouring agents (Nos 1898, 1907 and 1908) to structural class II and 1 flavouring agent (No. 1901) to structural class III (Cramer, Ford & Hall, 1978).

Table 1. Summary of the results of the safety evaluations of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I						
cis-4-(2,2,3-Trimethylcyclopentyl)-butanoic acid	1899	957136-80-0	A3. Yes, SPET: 3000	A5. Yes. The NOEL of 12 mg/kg bw per day from a 90-day study in rats with the structurally related substance 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde (No. 967) (British Industrial Biological Research Association, 1976) is at least 240 times the estimated daily dietary exposure to No. 1899 when used as a flavouring agent.	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	1900	27939-60-2	A3. No, SPET: 150	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	1902	22451-50-9 (*cis*); 22451-49-6 (*trans*)	A3. No, MSDI: Europe ND USA 0.7 Japan ND	NR	Note 1	No safety concern
d-Limonen-10-ol	1903	38142-45-9	A3. No, SPET: 3	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
p-Menthan-7-ol	1904	5502-75-0	A3. No, SPET: 150	NR	Note 1	No safety concern
p-Menth-1-en-9-ol	1905	18479-68-0	A3. No, SPET: 30	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
1,3-*p*-Menthadien-7-al	1906	1197-15-5	B3. No, SPET: 30	B4. Yes. The NOELs of 15, 33.9 and 33 mg/kg bw per day for, respectively, *trans*,*trans*-2,4-hexadienal (No. 1175), 2-*trans*-4-*trans*-decadienal (No. 1190) and 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (No. 1198) from 14-week studies in rats (Nos 1175 and 1190) and a 4-week study in rats (No. 1198) (Edwards, 1973; Damske et al., 1980; NTP, 2001) are at least 30 000–67 800 times the estimated daily dietary exposure to No. 1906 when used as a flavouring agent.	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II						
Methyl dihydrojasmonate	1898	24851-98-7	A3. Yes, MSDI: Europe 676 USA 1875 Japan 23	A5. Yes. The NOEL of 80 mg/kg bw per day for maternal toxicity from a study of prenatal developmental toxicity in rats (Lewis, 2007) is at least 2580 times the estimated daily dietary exposure to No. 1898 when used as a flavouring agent.	Notes 1 and 2	No safety concern
cis- and *trans*-2-Heptylcyclopropanecarboxylic acid	1907	697290-76-9 (*cis*); 697290-77-0 (*trans*)	A3. No, SPET: 1	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/B4*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarbaldehyde	1908	130932-16-0 (*cis*); 97231-35-1 (*trans*)	A3. No, SPET: 240	NR	Note 1	No safety concern
Structural class III						
Perillaldehyde propyleneglycol acetal	1901	121199-28-8	A3. No, SPET: 3	NR	Note 3	No safety concern

Table 1 (contd)

bw, body weight; CAS, Chemical Abstracts Service; ND, no data reported; NOEL, no-observed-effect level; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

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

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

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

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

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

Notes:

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

Table 2. Annual volumes of production and daily dietary exposures for alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
Methyl dihydrojasmonate (1898)				25	0.4	+
Europe	6 321	676	11			
USA	15 331	1875	31			
Japan	87	23	0.4			
cis-4-(2,2,3-Trimethylcyclopentyl)-butanoic acid (1899)				3000	50	−
Europe	ND	ND	ND			
USA	50	6	0.1			
Japan	ND	ND	ND			
Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde (1900)				150	2.5	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.6	0.2	0.003			
Perillaldehyde propyleneglycol acetal (1901)				3	0.05	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	3.7	1.0	0.02			
(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde (1902)				0.6	0.01	−
Europe	ND	ND	ND			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
USA	6.0	0.7	0.01			
Japan	ND	ND	ND			
d-Limonen-10-ol (1903)				3	0.05	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.3	0.3	0.006			
p-Menthan-7-ol (1904)				150	2.5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
p-Menth-1-en-9-ol (1905)				30	0.5	43
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
1,3-*p*-Menthadien-7-al (1906)				30	0.5	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
cis- and *trans*-2-Heptylcyclopropanecarboxylic acid (1907)				1	0.02	–
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	ND	ND	ND			
(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarbaldehyde (1908)				240	4.0	–

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d,e]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Total						
Europe	6 321					
USA	15 388					
Japan	93					

bw, body weight; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, “eaters only”) = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Qualitative data reported by Nijssen, van Ingen-Visscher & Donders (2009).

[e] Quantitative data for the USA reported by Stofberg & Grundschober (1987). The consumption ratio (annual consumption via food, kg)/(most recent reported production volume as a flavouring agent, kg) was not determined, as no production volume as a flavouring agent was available for the USA.

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

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

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

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

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

Step A5. For methyl dihydrojasmonate (No. 1898), the no-observed-effect level (NOEL) of 80 mg/kg body weight (bw) per day for maternal toxicity from a study of prenatal developmental toxicity in rats (Lewis, 2007) is 2580 times the estimated dietary exposure from its use as a flavouring agent (1875 µg/day as the MSDI).

For *cis*-4-(2,2,3-trimethylcyclopentyl)butanoic acid (No. 1899), the NOEL of 12 mg/kg bw per day for the structurally related substance 2,2,3-trimethyl-cyclopent-3-en-1-yl acetaldehyde (No. 967) from a 90-day study of toxicity in rats (British Industrial Biological Research Association, 1976) is 240 times the estimated dietary exposure to No. 1899 from its use as a flavouring agent (3000 µg/day using the SPET).

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

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

Step B4. The NOELs of 15, 33.9 and 33 mg/kg bw per day for, respectively, the structurally related substances *trans*,*trans*-2,4-hexadienal (No. 1175), 2-*trans*-4-*trans*-decadienal (No. 1190) and 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (No. 1198) from 14-week studies in rats (Nos 1175 and 1190) and a 4-week study in rats (No. 1998) (Edwards, 1973; Damske et al., 1980; NTP, 2001) are 30 000–67 800 times higher than the highest estimated dietary exposure to 1,3-*p*-menthadien-7-al (No. 1906) from its use as a flavouring agent (30 µg/day using the SPET). Although these three structurally related compounds are linear compounds, they contain the same toxicologically relevant groups as No. 1906 (i.e. an α,β-unsaturated aldehyde with two or more double bonds) and are therefore considered suitable for the evaluation of No. 1906. The Committee therefore concluded that 1,3-*p*-menthadien-7-al (No. 1906) would not pose a safety concern at current estimated dietary exposures.

Table 1 summarizes the evaluations of the 11 alicyclic primary alcohols, aldehydes, acids and related esters (Nos 1898–1908) in this group.

1.5 Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*). No common metabolites or homologous series could be identified for the additional flavouring agents in this group. When also considering the flavouring agents in this group evaluated at the fifty-ninth meeting (Annex 1, reference *160*), the different flavouring agents were not members of homologous series, despite having some common structural characteristics. However, two common metabolites were identified: *p*-menth-1-en-9-ol (No. 1905) and perillic alcohol (No. 974), both of which are in structural class I. In the unlikely event that the flavouring agents with the common metabolite *p*-menth-1-en-9-ol (i.e. Nos 971 and 972) and *p*-menth-1-en-9-ol itself were to be consumed concurrently on a daily basis, the estimated combined intakes for Europe, the USA and Japan would not exceed the threshold of concern (i.e. 1800 µg/person per day for class I). In the unlikely event that the flavouring agents with the common metabolite perillic alcohol (i.e. Nos 973 and 975), perillic alcohol itself and No. 1901, which would be metabolized to a structural isomer of perillic acid, were to be consumed concurrently on a daily basis, the estimated combined intakes for Europe, the USA and Japan would not exceed the threshold of concern (i.e. 1800 µg/person per day for class I).

1.6 Consideration of secondary components

Five members of this group of flavouring agents (Nos 1898, 1901, 1902, 1906 and 1908) have assay values of less than 95%. The secondary component of methyl dihydrojasmonate (No. 1898), methyl epi-dihydrojasmonate, is expected to

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

Information on the safety of the secondary components of these flavouring agents is summarized in Annex 5.

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes key aspects relevant to the safety evaluation of 11 alicyclic primary alcohols, aldehydes, acids and related esters, which are additions to a group of 26 flavouring agents evaluated previously by the Committee at its fifty-ninth meeting (Annex 1, reference *160*).

2.2 Additional considerations on intake

Production volumes and dietary exposures estimated both as the MSDI and using the SPET for each flavouring agent are reported in Table 2.

Three of the 11 flavouring agents in the group have been reported to occur naturally in traditional foods (Nijssen, van Ingen-Visscher & Donders, 2009; Table 2). Quantitative data on natural occurrence have been calculated for one of them (*p*-menth-1-en-9-ol, No. 1905) (as described by Stofberg & Grundschober [1987], based on quantitative data reported in Nijssen, van Ingen-Visscher & Donders [2009]).

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters has previously been described in the report of the fifty-ninth meeting (Annex 1, reference *160*). Some relevant additional data have been submitted on one compound evaluated previously (perillyl alcohol or *p*-mentha-1,8-dien-7-ol, No. 974) (O'Brien, 2004). In the previous evaluation, it was concluded that perillyl alcohol is rapidly metabolized and excreted, mainly as urinary metabolites. The absorption and metabolism of perillyl alcohol have been investigated recently in both rats and humans. The oral bioavailability of perillyl alcohol in rats was only about 4.5%. The bioavailability of perillyl alcohol in humans was estimated to be 2%. CaCO-2 permeability studies suggested that the gastrointestinal tract was highly permeable to perillyl alcohol. Studies with CaCO-2 cells, human and liver S9 fractions and cryopreserved human and rat hepatocytes showed that the low oral bioavailability could be attributed to presystemic metabolism in both the gastrointestinal tract and the liver. The phase II metabolites of perillyl alcohol included conjugates of glucuronic acid, cysteine, acetylcysteine, glycine and glutathione (O'Brien, 2004).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for 3 of the 11 flavouring agents in this group. Two flavouring agents (Nos 1898 and 1908) were tested in rats only, and one flavouring agent (No. 1900) was tested in rats and in male mice (Table 3). In rats, the oral LD_{50} values ranged from >2000 to >5000 mg/kg bw (Moreno, 1976, 1977, 1978; Gabriel, 1979; Myers, 1986; Gardner, 1987; Szakonyi, 2006). In male mice, the reported oral LD_{50} value was 1300 mg/kg bw (Gloxhuber & Potokar, 1981). These results support the findings in the previous evaluation (Annex 1, reference *161*) that the oral acute toxicity of alicyclic primary alcohols, aldehydes, acids and related esters is low.

Table 3. Results of studies of acute oral toxicity for alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1898	Methyl dihydrojasmonate	Rats; NR	>5000	Moreno (1976)
1898	Methyl dihydrojasmonate	Rats; M, F	>5000	Gardner (1987)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Rats; M, F	2650 (F)[a] 3040 (M)[a]	Myers (1986)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Rats; NR	3900[b]	Moreno (1978)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Rats; M	2330[b]	Gabriel (1979)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Rats; NR	3600	Moreno (1977)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Mice; M	1300[c]	Gloxhuber & Potokar (1981)
1908	(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarbaldehyde	Rats; F	>2000	Szakonyi (2006)

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

[a] The LD_{50} was calculated using a specific gravity of 0.936 g/cm^3 for 2,4-, 3,5- and 3,6-dimethyl-3-cyclohexenylcarbaldehyde.

[b] The test material was the isomer 2,4-dimethyl-3-cyclohexenylcarbaldehyde.

[c] The test material was the isomer 3,6-dimethyl-3-cyclohexenylcarbaldehyde.

(b) Short-term and long-term studies of toxicity

Only for 1 of the 11 flavouring agents in this group have studies on short-term toxicity been reported. The results of these studies with methyl dihydrojasmonate (No. 1898) are described below. For none of the flavouring agents in this group have studies on long-term toxicity been reported. No additional studies of short-term or long-term toxicity on any of the related substances have become available since their previous evaluation.

In a 14-day range-finding study, groups of five male and five female albino Sprague-Dawley rats were given methyl dihydrojasmonate (No. 1898) at dietary levels aiming at mean daily intakes of 0, 10, 40, 100 and 400 mg/kg bw. The actual intakes were 11, 54, 196 and 424 mg/kg bw per day for males in the four treatment groups and 10, 53 and 102 mg/kg bw per day for females in the three lowest dose

groups. Females in the two highest dose groups displayed significant spillage of the feed, so the mean daily intake in the second highest dose group was based on data for three animals only and the mean daily intake in the highest dose group could not be determined. Animals were examined twice daily for mortality and daily for clinical signs of toxicity. Physical and behavioural examinations were performed twice pretest and weekly during the study. Body weights were measured twice pretest, weekly during treatment and at termination, and food consumption was measured weekly over a 6-day period beginning 1 week prior to treatment. Haematological examinations and clinical chemistry measurements were performed. Macroscopic postmortem examinations were performed on all animals. Weights of the adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes and thymus were determined. Microscopic examinations were performed on the liver and tissues with any lesions detected in the gross examination of the controls and the highest dose group.

All animals survived to termination without any signs of toxicity. The only treatment-related effect observed was significant spillage of the feed by females in the two highest dose groups, accompanied by an up to 8% decrease in body weight gain when compared with controls (Hushka, 2000).

In the subsequent 90-day study of toxicity, groups of 10 male and 10 female Sprague-Dawley rats were given methyl dihydrojasmonate (No. 1898) at dietary levels equal to mean daily intakes of 0, 10, 50 and 100 mg/kg bw for males and females. The highest dose was set based on the results of the 14-day range-finding study. The study was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents; 1998) and was certified for compliance with good laboratory practice (GLP; except for the serology analysis) and quality assurance (QA). Diet and drinking-water were available ad libitum. Observations included mortality, clinical signs, behaviour, body weight, food intake, water consumption, a functional observational battery, motor activity, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, and macroscopic and microscopic pathology.

One control female was sacrificed due to an injury during month 2 of the study. There were no test substance–related deaths or clinical signs. Body weight, body weight gain and food consumption did not differ between the groups. Ophthalmoscopic examinations and functional observational and motor activity tests did not reveal treatment-related effects. There was a slight, but statistically significant, decrease (not dose related) in the mean haematocrit values for males receiving the test material. Similarly, there was a statistically significant increase in the blood potassium level in all male treatment groups and a statistically significant decrease in the mean globulin value for the males in the highest dose group. The males in the highest dose group showed increases of 11–12% in absolute kidney weight (not statistically significant) and relative kidney weight (statistically significant); however, these were not accompanied by any gross abnormalities or histological changes. There were no test substance–related macroscopic and microscopic findings. As all observed differences were small, occurred in one sex only and were not accompanied by macroscopic or microscopic findings, they were

considered not to be of toxicological relevance. It can therefore be concluded that the NOEL is 100 mg/kg bw per day, the highest dose tested in this study (Kelly, 2000).

(c) Genotoxicity

Only for 2 of the 11 flavouring agents in this group have studies of genotoxicity in vitro (Nos 1898 and 1900) and in vivo (No. 1898) been reported. The results of these studies are listed in Table 4 and described below.

(i) In vitro

No genotoxic potential was observed in Ames assays when methyl dihydrojasmonate (No. 1898) and mixture of 2,4-, 3,5- and 3,6-dimethyl-3-cyclohexenylcarbaldehyde (No. 1900) were incubated with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 and TA1538 or *Escherichia coli* WP2 *uvrA* with and without S9 activation in concentrations up to 5000 μg/plate (Gilroy et al., 1978; Gloxhuber & Bellinger, 1978; Willems, 1979; Glover, 1987; Vergnes & Morabit, 1995; Thompson, 2000; Wagner & Klug, 2000; Bowles, 2007; Wakamatsu, 2007).

Methyl dihydrojasmonate (No. 1898) gave positive results in a forward mutation assay conducted in L5178 $tk^{+/-}$ mouse lymphoma cells when tested at concentrations ranging from 58 to 300 μg/ml in the presence and absence of metabolic activation (Ross & Harris, 1979), whereas it gave negative results in another mouse lymphoma forward mutation assay at concentrations ranging from 25 to 325 μg/ml, with and without activation (Cifone, 2001).

(ii) In vivo

In a micronucleus assay, groups of male and female ICR mice were administered a single dose of methyl dihydrojasmonate (No. 1898) at 280 mg/kg bw (5 animals of each sex), 560 mg/kg bw (5 animals of each sex) or 1120 mg/kg bw (15 animals of each sex) by intraperitoneal injection (Gudi & Krsmanovic, 1998). Bone marrow isolated after 24 or 48 h from the femur of these animals revealed no increase in micronucleated polychromatic erythrocytes compared with controls.

In male Sprague-Dawley rats given a single dose of methyl dihydro-jasmonate (No. 1898) at 333 or 1000 mg/kg bw by intraperitoneal injection, there was no evidence of unscheduled deoxyribonucleic acid (DNA) synthesis after liver perfusion 2 or 16 h post-injection (Durward, 2001).

(iii) Conclusion

The results from the genotoxicity studies on methyl dihydrojasmonate (No. 1898) and mixture of 2,4-, 3,5- and 3,6-dimethyl-3-cyclohexenylcarbaldehyde (No. 1900) support the findings in the previous evaluation (Annex 1, reference *161*) that the members of this group of alicyclic primary alcohols, aldehydes, acids and related esters are not expected to exhibit genotoxic potential in vivo.

Table 4. Studies of genotoxicity in vitro and in vivo with alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents

No.	Agent	End-point	Test object	Concentration	Results	Reference
In vitro						
1898	Methyl dihydrojasmonate	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	10–3300 μg/plate, ±S9[a]	Negative[b]	Gilroy et al. (1978)
1898	Methyl dihydrojasmonate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	1.5–1500 μg/plate, ±S9[c]	Negative[b]	Glover (1987)
1898	Methyl dihydrojasmonate	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	15–5000 μg/plate, ±S9[d]	Negative[b]	Thompson (2000)
1898	Methyl dihydrojasmonate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	100–5000 μg/plate, ±S9	Negative[b]	Wagner & Klug (2000)
1898	Methyl dihydrojasmonate	Reverse mutation	*Escherichia coli* WP2 *uvr*A	100–5000 μg/plate, ±S9	Negative[b]	Wagner & Klug (2000)
1898	Methyl dihydrojasmonate	Forward mutation	L5178 $tk^{+/-}$ mouse lymphoma cells	25–275 μg/ml, −S9[e] 25–325 μg/ml, +S9[f]	Negative[b,g]	Cifone (2001)
1898	Methyl dihydrojasmonate	Forward mutation	L5178 $tk^{+/-}$ mouse lymphoma cells	58–300 μg/ml, ±S9	Positive[b,h]	Ross & Harris (1979)

Table 4 (contd)

No.	Agent	End-point	Test object	Concentration	Results	Reference
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	5–5000 µg/plate, ±S9[i,j]	Negative[b]	Bowles (2007)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*E. coli* WP2 *uvr*A	15–5000 µg/plate, ±S9[i,k]	Negative[b]	Bowles (2007)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	30–3000 µg/plate, ±S9[i,l]	Negative[b]	Vergnes & Morabit (1995)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0.0015–0.936 µg/plate, ±S9[i,m,n]	Negative[b]	Willems (1979)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	9.77–313 µg/plate, ±S9[o,p]	Negative[b]	Wakamatsu (2007)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*E. coli* WP2 *uvrA*	9.77–313 µg/plate, ±S9[o,p]	Negative[b]	Wakamatsu (2007)
1900	Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	1–10 000 ppm/plate, ±S9[q,r]	Negative[b]	Gloxhuber & Bellinger (1978)

Table 4 (contd)

No.	Agent	End-point	Test object	Concentration	Results	Reference
In vivo						
1898	Methyl dihydrojasmonate	Micronuclei induction	Mouse; bone marrow	1 × 280, 560 or 1120 mg/kg bw, intraperitoneally[s]	Negative	Gudi & Krsmanovic (1998)
1898	Methyl dihydrojasmonate	Unscheduled DNA synthesis	Male rat hepatocytes	1 × 333 or 1000 mg/kg bw, intraperitoneally[t]	Negative	Durward (2001)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant from rat liver

[a] Precipitation was noted in all strains with and without metabolic activation from 1000 µg/plate onwards, and sparse bacterial lawns were noted in most strains with and without metabolic activation at 3300 µg/plate.

[b] With and without metabolic activation

[c] Test concentrations for all strains without metabolic activation and for TA100 and TA1535 with metabolic activation 1.5–150 µg/plate in both assays. Test concentrations for strains TA98 and TA1537 with metabolic activation 15–1500 µg/plate in first assay and 5–500 µg/plate in second assay. At 1500 µg/plate, toxicity was observed in strain TA1537, and thinning of the background lawn was observed in strain TA98.

[d] Precipitation and/or sparse bacterial lawns were noted in all strains with and without metabolic activation at 5000 µg/plate.

[e] Severe toxicity (less than 20% relative growth) was noted at highest concentrations tested (275 µg/ml in first assay and 200 µg/ml in second assay).

[f] Severe toxicity (less than 20% relative growth) was noted at highest concentrations tested (300 µg/ml in first assay and 325 µg/ml in second assay).

[g] In the first assay, a doubling of the mutant frequency was observed with metabolic activation at the highest concentration tested (300 µg/ml, which was highly cytotoxic [<10% relative growth]). The result was not repeated in the second assay when concentrations up to 325 µg/ml were tested.

[h] A doubling of the mutant frequency was observed at 200 and 300 µg/ml (without metabolic activation) and at 300 µg/ml (with metabolic activation).

[i] Test substance was 2,4-dimethyl-3-cyclohexenylcarbaldehyde.

[j] Sparse bacterial lawns were noted in all strains with and without metabolic activation at 5000 µg/plate, in all strains without metabolic activation at 1500 µg/plate and in some strains with metabolic activation at 1500 µg/plate.

[k] Sparse bacterial lawns were noted with and without metabolic activation at 5000 µg/plate.

Table 4 (contd)

[l] Bacterial growth inhibition was observed in several strains with and without metabolic activation from 1000 μg/plate onwards. Toxicity was observed at 3000 μg/plate in all strains without metabolic activation and in some strains with metabolic activation.

[m] Bacterial growth inhibition and/or toxicity were observed at 0.936 μg/plate, with and without metabolic activation.

[n] Calculated using a density of 0.936 g/cm^3 for the test compound.

[o] Test substance was a mixture of 4,6-dimethyl-3-cyclohexenylcarboxaldehyde and 3,6-dimethyl-3-cyclohexenylcarboxaldehyde.

[p] Bacterial growth inhibition was observed in all strains from 156 μg/plate onwards without metabolic activation and at 313 μg/plate with metabolic activation.

[q] Test substance was 3,6-dimethyl-3-cyclohexenylcarboxaldehyde.

[r] Inhibition of colony growth was observed in all strains with and without metabolic activation from 1000 ppm/plate [concentration as provided in reference] onwards.

[s] Harvest of femur bone marrow after 24 h (all doses) and 48 h (highest dose only). Mortality was observed at 1120 mg/kg bw, and clinical signs were seen from 560 mg/kg bw.

[t] The highest dose is the maximum tolerated dose, based on a range-finding study.

(d) Reproductive toxicity

A study on prenatal developmental toxicity has been reported for 1 of the 11 flavouring agents in this group (methyl dihydrojasmonate, No. 1898). The results of this study are described below.

In a 14-day prenatal developmental toxicity study, groups of 25 presumed pregnant Crl:CD(SD) rats were administered methyl dihydrojasmonate via oral gavage at doses of 0 (corn oil, vehicle), 40, 80 or 120 mg/kg bw per day on days 7 through 20 of the presumed gestation. The doses were based on a range-finding study in which rats were given 0–1000 mg/kg bw per day according to the same dosing schedule and maternal toxicity was observed at doses of 250 mg/kg bw per day and higher. All rats were examined twice daily for signs of severe toxicity and deaths, abortions and premature deliveries. Body weights were recorded on gestational day (GD) 0 and throughout the dosage period. Food consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, 20 and 21. All rats were sacrificed on GD 21, and caesarean sections and a gross necropsy of the thoracic, abdominal and pelvic viscera were performed on all animals. The number and distribution of corpora lutea and implantation sites and the numbers of live and dead fetuses and early and late resorptions were determined. Placentas were examined for size, colour and shape. All fetuses were weighed and examined for sex and gross external alterations. Approximately half of the fetuses in each litter were examined for soft tissue alterations (by dissection), and the remaining fetuses were examined for skeletal alterations.

Rats in both the 80 and 120 mg/kg bw per day groups lost weight after the initial dosage was given. Absolute maternal food consumption was reduced during the entire dosage period in the group given 120 mg/kg bw per day, with statistically significant reductions on GDs 10–15. Relative maternal food consumption was significantly reduced over the entire period in this group. When compared with the vehicle control group, only in the 120 mg/kg bw per day group was the mean maternal body weight reduced throughout the dosing period (GDs 7–21), with statistically significant reductions on GDs 11–15. One dam in the 80 mg/kg bw per day group prematurely delivered its litter on GD 21, but this was not considered related to treatment. In the highest dose group, an increase in sparse hair coat and a significant increase in ungroomed coat occurred. In addition, two rats in the highest dose group had tan areas in the liver lobes and a pale spleen, whereas these effects were not observed in the lower dose groups and controls. All other clinical and necropsy observations were considered unrelated to treatment with the test material. No caesarean section or litter parameters were affected by the treatment with methyl dihydrojasmonate. No gross external, soft tissue or skeletal alterations or differences in ossification sites were attributable to the test material at doses as high as 120 mg/kg bw per day.

On the basis of these data, the no-observed-adverse-effect level (NOAEL) for maternal toxicity is 80 mg/kg bw per day, based on reductions in food consumption and body weight. The NOEL for developmental toxicity was 120 mg/kg bw per day, the highest dose tested (Lewis, 2007; Politano et al., 2008).

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ALIPHATIC ACYCLIC AND ALICYCLIC α-DIKETONES AND RELATED α-HYDROXYKETONES (addendum)

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1. EVALUATION

1.1 Introduction

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

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

Five of the eight additional flavouring agents (Nos 2032 and 2035–2038) in this group have been reported to occur naturally and have been found in black tea, green tea, sherry, beef fat, mutton, lamb, fish, turkey, chicken, guinea hen, coffee, roasted peanuts, soya bean, mushroom, prickly pear, lovage leaf, cocoa, black currants, peppermint oil and buchu oil (Nijssen, van Ingen-Visscher & Donders, 2009). Quantitative intake data from natural occurrence were available for two substances, 3-methyl-2,4-nonedione (No. 2032) and octan-2,3-dione (No. 2036). The consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were calculated to be 177 and 125, respectively.

1.2 Assessment of dietary exposure

The total annual volumes of production of the eight aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones are 2 kg in Europe, 6 kg in the USA and 39 kg in Japan (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009). In Europe, 65% of the annual volume of production is accounted for by 3-methyl-2,4-nonedione (No. 2032) and octan-2,3-dione (No. 2036), and in the USA, 83% of the annual volume of production is accounted for by octan-2,3-dione (No. 2036). Over 84% of the annual volume of production in Japan is accounted for by acetoin propyleneglycol acetal (No. 2033).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest estimates are for acetoin propyleneglycol acetal (No. 2033) and the mixture of 3-hydroxy-5-methyl-2-hexanone and 2-hydroxy-5-methyl-3-hexanone (No. 2034) (450 μg for both, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in the group, the daily dietary exposures range from 0.01 to 400 μg, with the SPET yielding the highest estimates for all, except for 4,5-octanedione (No. 2037). Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

Table 1. Summary of the results of the safety evaluations of aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II							
3-Methyl-2,4-nonedione	2032	113486-29-6	No, SPET: 20	NR	NR	Note 1	No safety concern
Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	2034	163038-04-8 246511-74-0	No, SPET: 450	NR	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
3-Hydroxy-2-octanone	2035	37160-77-3	No, SPET: 400	NR	NR	Note 1	No safety concern
2,3-Octanedione	2036	585-25-1	No, SPET: 3.6	NR	NR	Note 1	No safety concern
4,5-Octanedione	2037	5455-24-3	No, MSDI: Europe 0.01 USA ND Japan 0.9	NR	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
(±)-2-Hydroxypiperitone	2038	490-03-9	No, SPET: 400	NR	NR	Note 2	No safety concern
Structural class III							
Acetoin propyleneglycol ketal	2033	94089-23-3	Yes, SPET: 450	No	Yes. The NOAEL of 330 mg/kg bw per day for the metabolite acetoin (No. 405) in a 90-day study in rats (Gaunt et al., 1972) is at least 41 200 times the estimated daily dietary exposure to No. 2033 when used as a flavouring agent.	Note 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	2039	18114-49-3	Yes, SPET: 400	No	Yes. The NOAEL of 90 mg/kg bw per day for the metabolite 2,3-butanedione (No. 408) in a 90-day study in rats (Colley et al., 1969) is at least 12 800 times the estimated daily dietary exposure to No. 2039 when used as a flavouring agent.	Note 3	No safety concern

bw, body weight; CAS, Chemical Abstracts Service; ND, no intake data reported; NOAEL, no-observed-adverse-effect level; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

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

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

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

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

Notes:

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

Table 2. Annual volumes of production and dietary exposures for aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]	Consumption ratio[e]
		MSDI[b]		SPET[c]			
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day		
3-Methyl-2,4-nonedione (2032)				20	0.3	18	177
Europe	0.8	0.09	0.001				
USA	0.1	0.01	0.0002				
Japan	2	0.6	0.01				
Acetoin propyleneglycol acetal (2033)				450	8	–	NA
Europe	ND	ND	ND				
USA	ND	ND	ND				
Japan	33	10	0.2				
Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone (2034)				450	8	–	NA
Europe	0.1	0.01	0.0002				
USA	0.5	0.06	0.001				
Japan	0.1	0.03	0.0005				
3-Hydroxy-2-octanone (2035)				400	7	+	NA
Europe	0.1	0.01	0.0002				

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]	Consumption ratio[e]
		MSDI[b]		SPET[c]			
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day		
USA	ND	ND	ND				
Japan	ND	ND	ND				
Octan-2,3-dione (2036)				3.6	0.06	627	125
Europe	0.5	0.05	0.001				
USA	5	0.6	0.01				
Japan	0.1	0.03	0.0005				
4,5-Octanedione (2037)				0.8	0.01	+	NA
Europe	0.1	0.01	0.0002				
USA	ND	ND	ND				
Japan	3	0.9	0.015				
(±)-2-Hydroxypiperitone (2038)				400	7	+	NA
Europe	0.1	0.02	0.0003				
USA	ND	ND	ND				
Japan	0.1	0.03	0.0005				

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]	Consumption ratio[e]
		MSDI[b]		SPET[c]			
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day		
1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (2039)				400	7	–	NA
Europe	ND	ND	ND				
USA	0.5	0.06	0.001				
Japan	ND	ND	ND				
Total							
Europe	2						
USA	6						
Japan	39						

bw, body weight; NA, not available; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:

Table 2 (contd)

(annual volume, kg) × (1×10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009).

MSDI (μg/kg bw per day) calculated as follows:

(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (μg/person per day) calculated as follows:

(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.

SPET (μg/kg bw per day) calculated as follows:

(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Quantitative data for the USA reported by Stofberg & Grundschober (1987).

[e] The consumption ratio is calculated as follows:

(annual consumption via food, kg)/(most recent reported volume as a flavouring agent, kg).

1.3 Absorption, distribution, metabolism and elimination

In the report of the fifty-first meeting, biodisposition of flavouring agents in this group was extensively discussed. In rats and mice, orally administered aliphatic α-diketones are rapidly absorbed from the gastrointestinal tract (Gabriel, Ilbawi & Al-Khalidi, 1972). It is anticipated that at low levels of exposure, humans will metabolize aliphatic acyclic α-diketones principally by α-hydroxylation and subsequent oxidation of the terminal methyl group to yield the corresponding ketocarboxylic acid. The acid may undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which could be completely metabolized in the fatty acid pathway and citric acid cycle. At higher concentrations, another detoxication pathway is used, which involves reduction to the diol and subsequent conjugation with glucuronic acid (Westerfeld & Berg, 1943; Williams, 1959; Gabriel, Jabara & Al Khalidi, 1971; Gabriel, Ilbawi & Al-Khalidi, 1972; Otsuka et al., 1996). Aliphatic α-diketones and alicyclic α-hydroxyketones, diketones and hydroxyketones are mainly metabolized by reduction to the corresponding diol, followed by glucuronic acid conjugation and excretion (Mills & Walker, 1990; Ong et al., 1991). Ketals (dioxolanes) are predicted to undergo hydrolysis to yield the corresponding alcohol and ketone (Nos 405, 408 and 2033).

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned six flavouring agents (Nos 2032 and 2034–2038) to structural class II and the remaining two flavouring agents (Nos 2033 and 2039) to structural class III (Cramer, Ford & Hall, 1978).

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

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

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

Step A5. For acetoin propyleneglycol ketal (No. 2033), the no-observed-adverse-effect level (NOAEL) of 330 mg/kg body weight (bw) per day for the metabolite acetoin (No. 405) in a 90-day study in rats (Gaunt et al., 1972) provides a margin of safety of over 40 000 in relation to the highest estimated dietary

exposure to No. 2033 (SPET = 450 μg/person per day) when used as a flavouring agent.

For 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (No. 2039), the NOAEL of 90 mg/kg bw per day for the metabolite 2,3-butanedione (No. 408) in a 90-day study in rats (Colley et al., 1969) provides a margin of safety of approximately 13 000 in relation to the highest estimated dietary exposure to No. 2039 (SPET = 400 μg/person per day) when used as a flavouring agent.

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

Table 1 summarizes the evaluations of the eight aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents (Nos 2032–2039) in this group.

1.5 Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was undertaken based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*). In addition, at this meeting, the Committee also considered combined intakes for structurally closely related series of flavouring agents.

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

The flavouring agent No. 2038 is a member of a structurally closely related series of alicyclic α-diketones or related α-hydroxyketones, which are in structural class II, or predicted to be metabolized to such compounds. The five related flavouring agents with the highest intakes in Europe and in the USA are Nos 418–421 and 425; the flavouring agent with the highest intake in Japan is No. 2033. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be approximately 1000 μg/person per day in Europe, 6 μg/person per day in Japan and 800 μg/person per day

in the USA. These would exceed the threshold of concern (i.e. 540 μg/person per day for class II). However, all of these flavouring agents are expected to be efficiently metabolized and would not saturate available detoxication pathways. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of these substances would not raise concern about safety.

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

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

1.6 Consideration of secondary components

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

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This addendum summarizes the additional key data relevant to the safety evaluation of a group of eight aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones, which included two aliphatic α-diketones, two aliphatic α-hydroxyketones, one aliphatic β-diketone, one alicyclic α,β-unsaturated α-hydroxyketone and one α-hydroxyketal. These flavouring agents are additions to the group of 22 aliphatic acyclic and alicyclic α-diketones and α-hydroxyketones evaluated previously by the Committee at its fifty-first meeting (Annex 1, reference *138*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposure estimates were made using the MSDI approach as well as the SPET.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

Additional information related to the absorption, distribution, metabolism and excretion of the flavouring agents in this group and of structurally related agents has been reported since the preparation of the original monograph (Annex 1, reference *138*) and is described below.

(a) 1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (No. 2039)

In vitro studies conducted on 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone (No. 2039) in gastric juice show 100% hydrolysis to butane-2,3-dione (No. 408) within 12 h (Flavor and Extract Manufacturers Association, 2004). Similar studies conducted in artificial saliva showed 75% hydrolysis of 1,1′-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone at pH 9 to butane-2,3-dione (No. 408) and 100% hydrolysis to butane-2,3-dione at pH 1.5 and 2, within 45 min (Flavor and Extract Manufacturers Association, 2004). These hydrolysis reactions are predictable.

(b) Butane-2,3-diol,[1] a structurally related diol

Butane-2,3-diol is a known metabolic reduction product of acetoin, which is a reduction product of diacetyl (Otsuka et al., 1999). Butane-2,3-diol may also be an intermediate in the in vitro mammalian metabolism of acetaldehyde. Butane-2,3-diol and its oxidation metabolite, acetoin, have been reported as intermediates in the mammalian metabolism of pyruvate in vitro (Montgomery et al., 1993).

Butane-2,3-diol, 2-butanol and 3-hydroxy-2-butanone were identified as metabolites in the serum of guinea-pigs administered methyl ethyl ketone by intraperitoneal injection. Methyl ethyl ketone undergoes α-hydroxylation to form 3-hydroxy-2-butanone, which is subsequently reduced to 2,3-butanediol. The serum half-life of methyl ethyl ketone was reported to be 270 min, and the clearance time of butane-2,3-diol was 6 h (DiVincenzo, Kaplan & Dedinas, 1976). Subsequently, butane-2,3-diol is eliminated as 2,3-butanediol β-glucuronide (Otsuka et al., 1996).

---

[1] OH

OH

(c) 4-Hydroxy-4-methylpentan-2-one,[2] a structurally related β-hydroxyketone

4-Hydroxy-4-methylpentan-2-one, a structurally related β-hydroxyketone, and 4-methyl-2-pentanol were identified in the serum of guinea-pigs administered 4-methyl-2-pentanone by intraperitoneal injection. The principal metabolite, 4-hydroxy-4-methylpentan-2-one, had a clearance time of 16 h. The amount of 4-methyl-2-pentanol in the serum was too low for quantification. These results indicate that 4-methyl-2-pentanone is metabolized by reduction of the carbonyl group to form 4-methyl-2-pentanol and by oxidation at the ω-1 carbon to form 4-hydroxy-4-methylpentan-2-one (DiVincenzo, Kaplan & Dedinas, 1976).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for two of the eight flavouring agents in this group and are summarized in Table 3. For both the mixture of 3-hydroxy-5-methyl-2-hexanone and 2-hydroxy-5-methyl-3-hexanone (No. 2034) and 3-methyl-2,4-nonedione (No. 2032), LD_{50} values in rats were demonstrated to be greater than 2000 mg/kg bw (Strobel, 1998; Pooles, 2003).

Table 3. Results of acute oral toxicity studies for aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
2032	3-Methyl-2,4-nonadione	Rat; F	>2000[a]	Pooles (2003)
2034	Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	Rat; M, F	>2000[a]	Strobel (1998)

F, female; M, male
[a] Highest dose tested. Actual LD_{50} may be higher.

These studies support the Committee's conclusion in the original monograph that the acute oral toxicity of aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones is low.

(b) Short- and long-term studies of toxicity

No studies of short-term or long-term toxicity have been reported for any of the eight flavouring agents in this group. One short-term toxicity study on a structurally related substance not in this group is described below.

[2] O OH

(i) 4-Hydroxy-4-methylpentan-2-one, a structurally related β-hydroxyketone

Groups of 10 albino rats (sex not specified) were administered diacetone alcohol (i.e. 4-hydroxy-4-methylpentan-2-one) (commercial grade), which had been determined to be free of acetone, in their drinking-water for 30 days at concentrations resulting in doses of 0, 10, 40 or 130 mg/kg bw per day. Drinking-water consumption, body weight gain, body length, fatness (g/mm body length), liver weight, kidney weight, macroscopic pathology and histopathology were measured or evaluated. No deaths occurred. In one of the rats at the 40 mg/kg bw per day dose level, cloudy swelling and degeneration of renal tubular epithelium were reported. No adverse effects were reported in any rats at the 10 mg/kg bw per day dose level (Smyth, 1946).

(c) Genotoxicity studies

Genotoxicity testing has been performed on three flavouring agents in the present group (Annex 1, reference *138*). The results of these tests are summarized in Table 4 and described below. Additional studies of genotoxicity on a previously evaluated member of this group, on a metabolite of a previously evaluated member of this group and on a structurally related substance not in this group are also described below.

(i) In vitro

3-Methyl-2,4-nonanedione (No. 2032) was tested for mutagenicity to bacteria by incubation with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537, as well as *Escherichia coli* strain WP2*uvrA*, either alone or with an exogenous induced male rat liver bioactivation system, using a preincubation assay. The test substance alone and with bioactivation was toxic at 1250 and 5000 μg/plate, respectively. At six concentrations up to 1250 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Sasaki, 2006).

The mixture of 3-hydroxy-5-methyl-2-hexanone and 2-hydroxy-5-methyl-3-hexanone (No. 2034) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA97, TA98, TA100, TA102 and TA1535, either alone or with an exogenous bioactivation system, using either plate incorporation or preincubation assays. At concentrations up to 5000 μg/plate, no toxic effects were observed in the plate assay, but variable degrees of toxicity were observed in the preincubation assay. The test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Gocke, 1997).

Table 4. Studies of genotoxicity with aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents

No.	Flavouring agent	End-point	Test object	Concentration (µg/plate)	Results	Reference
In vitro						
2032	3-Methyl-2,4-nonadione	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	39.1, 78.1, 156, 313, 625 and 1250	Negative[a,b,c,d]	Sasaki (2006)
2032	3-Methyl-2,4-nonadione	Reverse mutation	*Escherichia coli* WP2*uvrA*	39.1, 78.1, 156, 313, 625 and 1250	Negative[a,c,d]	Sasaki (2006)
2034	Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102 and TA1535	50–5000	Negative[d,e,f]	Gocke (1997)
2034	Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102 and TA1535	50–5000	Negative[a,d]	Gocke (1997)
2039	1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	100, 316, 1000, 3160 and 5000	Negative[a,d,g]	Stien (2005)

Table 4 (contd)

No.	Flavouring agent	End-point	Test object	Concentration (μg/plate)	Results	Reference
2039	1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	10, 31.6, 100, 316 and 1000	Negative[d,e,h]	Stien (2005)
408	2,3-Butanedione	Forward mutation	Mouse lymphoma L5178Y $TK^{+/-}$ cells	100, 150, 180, 200 and 250	Positive	Whittaker et al. (2008)

[a] Standard plate incorporation method.
[b] Cytotoxicity was observed at greater than 313 μg/plate in all test strains without S9 activation.
[c] Cytotoxicity was observed at 1250 μg/plate in all test strains with S9 activation.
[d] With and without metabolic activation.
[e] Preincubation method.
[f] Cytotoxicity was observed at the highest concentration (i.e. 5000 μg/plate) in all test strains.
[g] Cytotoxicity was observed at the highest concentration (i.e. 5000 μg/plate) in all test strains except TA98 in the plate incorporation assay.
[h] Cytotoxicity was observed at the highest concentration (i.e. 1000 μg/plate) in all test strains in the preincubation assay.

1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)-bis-ethanone (No. 2039) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, either alone or with an exogenous induced rat liver bioactivation system, using either the plate incorporation or preincubation assays. In the plate assay, either alone or with bioactivation, toxicity was observed at 5000 μg/plate, and in the preincubation assay, it was observed at 1000 μg/plate. At five concentrations up to the maximum concentration of 5000 μg/plate in the plate assay, both alone and with bioactivation, and up to the maximum concentration of 1000 μg/plate in the preincubation assay, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Stien, 2005).

2,3-Butanedione (No. 408) (also referred to as diacetyl) was tested for mammalian cell gene mutagenicity in an assay using L5178Y $TK^{+/-}$ mouse lymphoma cells, with an exogenous human liver bioactivation system pooled from 10 individuals of both sexes. Cell death was observed at 500 μg/ml. In the mutagenicity assay, mouse lymphoma cells were exposed to the test material at concentrations up to 500 μg/ml, and the concentrations chosen for cloning were 100, 150, 180, 200 and 250 μg/ml. For the three lower doses, the relative growth of the exposed colonies was approximately 80%, but for the two higher doses, it was reduced to 38% and 31% of the control, respectively. 2,3-Butanedione induced a dose-related increase in the number of mutant colonies over the range of 150–250 μg/ml. The two highest concentrations (i.e. 200 and 250 μg/ml) yielded increases of 90 mutants above the solvent control, which is the criterion for a positive response in this assay. The authors suggested that the increase in the frequency of small colonies is consistent with changes to multiple loci on chromosome 11 as well as functional loss of the thymidine kinase locus (Whittaker et al., 2008).

Butane-2,3-diol, a metabolite of acetoin (No. 405), was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2*uvrA* tester strains. At up to 5000 μg/plate, no toxicity was observed. At eight concentrations up to 5000 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Iwata et al., 1984).

4-Hydroxy-4-methylpentan-2-one, referred to as diacetone alcohol, which is a structurally related β-hydroxyketone, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538, either alone or with exogenous induced male rat and hamster liver bioactivation systems, using the plate incorporation assay. No toxicity was observed at up to 10 000 μg/plate. At five concentrations up to 10 000 μg/plate, the test substance produced no increase in revertant mutants in any of the tester strains alone or with bioactivation, whereas appropriate control substances established the responsiveness of the tester strains (San & Klug, 1993).

4-Hydroxy-4-methylpentan-2-one was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and

TA1538 and *E. coli* WP2 and WP2*uvrA* tester strains, alone or with an exogenous induced rat liver bioactivation system, using the plate incorporation assay. A range of concentrations at 2-fold intervals up to 4000 μg/plate was tested. In two replicate tests, the test substance produced no increase in revertant mutants in any of the strains, whereas appropriate control substances established the responsiveness of the tester strains (Brooks, Meyer & Hutson, 1988).

4-Hydroxy-4-methylpentan-2-one, referred to as diacetone alcohol, was tested for mutagenicity to yeast in the *Saccharomyces cerevisiae* mitotic gene conversion assay, either alone or with an exogenous induced rat liver bioactivation system. At five concentrations up to 5000 μg/ml, the test substance was negative alone and with a bioactivation system (Brooks, Meyer & Hutson, 1988). 4-Hydroxy-4-methylpentan-2-one was tested for induction of chromosomal aberrations in the cultured RL_4 rat liver epithelial-type cell line. An exogenous bioactivation system was not used owing to the fact that RL_4 cells are metabolically competent. Exposure to 4-hydroxy-4-methylpentan-2-one at 4000 μg/ml caused significant toxicity, with greater than 60% growth inhibition of the cells. A marginal increase in chromatid gaps was observed within the concentration range of 2000–4000 μg/ml; however, the effect was not concentration related. In the first experiment, 4-hydroxy-4-methylpentan-2-one concentrations of 750, 1500 and 3000 μg/ml were used, and a small number of chromatid exchanges, breaks and acentric fragments were seen in cultures exposed to 3000 μg/ml. In the second experiment, 4-hydroxy-4-methylpentan-2-one concentrations of 2000, 3000 and 4000 μg/ml were used; however, although some chromatid exchanges, breaks and acentric fragments were seen in cultures exposed to 2000 μg/ml, similar effects were not seen at 3000 μg/ml, and only a single chromatid break and two fragments were seen at 4000 μg/ml. The authors concluded that the observed effect of 4-hydroxy-4-methylpentan-2-one exposure may be related to the length of treatment or osmolality effects (Brooks, Meyer & Hutson, 1988). Brooks, Meyer & Hutson (1988) mentioned that osmolality is believed to have a significant influence on cellular metabolism, leading to chromosomal aberrations, and Galloway et al. (1987) postulated that the solvent and detergent-like properties of a bifunctional (ketone and secondary alcohol) chemical such as 4-hydroxy-4-methylpentan-2-one could lead to a physical effect on the chromosomes of rat liver epithelial-type cells.

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ALIPHATIC ACYCLIC AND ALICYCLIC TERPENOID TERTIARY ALCOHOLS AND STRUCTURALLY RELATED SUBSTANCES (addendum)

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1. EVALUATION

1.1 Introduction

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

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

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

Five of the seven additional flavouring agents (Nos 2027–2031) in this group have been reported to occur naturally and have been found in camomile, figs, lemon juice, black and green teas, calamus, soya bean, pepper and strawberry guava (Nijssen, van Ingen-Visscher & Donders, 2009).

1.2 Assessment of dietary exposure

The total annual volumes of production of the seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances are approximately 18 kg in Europe and 5 kg in Japan (Japanese Flavor and Fragrance Materials Association, 2002, 2005; European Flavour and Fragrance Association, 2004; International Organization of the Flavor Industry, 2009). More than 94% of the total annual volume of production in Europe is accounted for by (+)-cedrol (No. 2030).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest estimates are for (−)-sclareol (No. 2029) and (+)-cedrol (No. 2030) (1500 μg for both, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in this group, the daily dietary exposures range from 0.01 to 900 μg, with the SPET yielding the highest estimates for all. Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

1.3 Absorption, distribution, metabolism and elimination

In the report of the fifty-first meeting, biodisposition of substances in this group was extensively discussed. The esters in this group (Nos 2025 and 2026) can be readily hydrolysed to their component tertiary alcohols and carboxylic acids (Heymann, 1980; Anders, 1989). The hydrolysis products would be readily detoxified primarily by conjugation with glucuronic acid and then excreted primarily in the urine (Williams, 1959; Parke, Rahman & Walker, 1974; Horning et al., 1976; Ventura et al., 1985). The alicyclic tertiary alcohols and alcohols with unsaturation (Nos 2027–2031) undergo ω-oxidation at the allylic position to yield polar metabolites, which can be conjugated and excreted. Metabolites of acyclic alcohols can be further oxidized to eventually yield carbon dioxide.

Table 1. Summary of the results of the safety evaluations of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavouring agents[a,b,c]

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Cubebol	2028	23445-02-5	No, SPET: 3	Note 2	No safety concern
(−)-Sclareol	2029	515-03-7	No, SPET: 1500	Notes 2 and 3	No safety concern
(+)-Cedrol	2030	77-53-2	No, SPET: 1500	Note 2	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
α-Bisabolol	2031	23089-26-1	No, SPET: 150	Note 3	No safety concern

CAS, Chemical Abstracts Service

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

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

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

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

Notes:

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

Table 2. Annual volumes of production and dietary exposures for aliphatic acyclic and alicyclic terpenoid tertiary alcohols ad structurally related substances used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
Dimethylbenzyl carbinyl crotonate (2025)				300	5	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.3	0.005			
Dimethylbenzyl carbinyl hexanoate (2026)				900	15	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.04	0.001			
Caryophyllene alcohol (2027)				80	1	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	2	0.5	0.008			
Cubebol (2028)				3.3	0.06	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1	0.3	0.004			
(−)-Sclareol (2029)				1500	25	+
Europe	0.7	0.07	0.001			
USA	ND	ND	ND			
Japan	ND	ND	ND			
(+)-Cedrol (2030)				1500	25	+
Europe	17	2	0.03			
USA	ND	ND	ND			
Japan	0.9	0.2	0.004			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
α-Bisabolol (2031)				150	3	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
Total						
Europe	18					
USA	0					
Japan	5					

ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2002, 2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2002, 2005; International Organization of the Flavor Industry, 2009). MSDI (µg/kg bw per day) calculated as follows: (µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned all seven of the flavouring agents (Nos 2025–2031) to structural class I (Cramer, Ford & Hall, 1978).

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

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

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

Table 1 summarizes the evaluations of the seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances (Nos 2025–2031) in this group.

1.5 Consideration of combined intakes from use as flavouring agents

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

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

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

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

1.6 Consideration of secondary components

Two flavouring agents in this group (Nos 2027 and 2031) have minimum assay values of less than 95%. The secondary component of caryophyllene alcohol (No. 2027), dihydrocloven-9-ol, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as caryophyllene alcohol, and is considered not to present a safety concern at current estimated dietary exposures. The secondary component of α-bisabolol (No. 2031), β-bisabolol, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as caryophyllene alcohol, and is considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 5.

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the additional key data relevant to the safety evaluation of a group of seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances (see Table 1), which included one aliphatic terpene tertiary alcohol, four alicyclic tertiary alcohols and two esters of phenyl-substituted aliphatic tertiary alcohols. These flavouring agents are additions to the group of 38 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances evaluated previously by the Committee at its fifty-first and sixty-eighth meetings (Annex 1, references *137* and *187*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposure estimates were made using the SPET in addition to the MSDI approach.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

As described in the reports of the fifty-first and sixty-eighth meetings, aliphatic acyclic and alicyclic terpenoids and related esters undergo efficient metabolism. Based on the results of studies in aqueous buffered conditions, simulated gastric juice, simulated human intestinal fluid, blood plasma, whole hepatocytes and liver microsome preparations, terpene esters formed from tertiary alcohols (e.g. linalool) and simple aliphatic carboxylic acids are expected to undergo hydrolysis. Although differences in the rates of hydrolysis occur under in vitro conditions in gastric juice and intestinal fluids, ready hydrolysis is observed in tissue preparations rich in carboxylesterase, including blood and liver. Esters that survive intestinal fluids and blood intact are subject to rapid hydrolysis in the liver. It is expected that the tertiary aromatic alcohols will undergo direct conjugation of the hydroxyl group with glucuronic acid (Williams, 1959).

Additional studies on the absorption, distribution, metabolism and excretion of these flavouring agents have been reported since the preparation of the original monograph (Annex 1, reference *137*).

Plasma pharmacokinetics were investigated following intravenous administration of (−)-sclareol (No. 2029) at 100 mg/kg body weight (bw) to two male Wistar rats. Plasma samples were collected at 5, 15, 30, 60, 180, 360, 720 and 1440 min after administration, and (−)-sclareol concentrations were quantified by gas–liquid chromatography using an internal standard. At 5 min post-injection, the plasma level of (−)-sclareol was 84.9 μg/ml. The (−)-sclareol plasma concentration dropped to 42.9 μg/ml after 180 min and was not detectable at 720 min. This study indicated a rapid biphasic disappearance of (−)-sclareol from plasma following intravenous dosing. The authors suggested that (−)-sclareol may be distributed in fatty tissue owing to its high lipophilicity (Kouzi, McChesney & Walker, 1993).

In a second study by the above authors, (−)-sclareol (No. 2029) was administered to two male Wistar rats by intravenous injection at 100 mg/kg bw and two male Wistar rats by intragastric instillation at 1000 mg/kg bw in 3:1 propylene glycol–ethanol. Urine and faecal samples were collected from all rats at periodic intervals over 144 h or 72 h, respectively. Bile samples were collected only from rats given intravenous injections at periodic intervals over 30 h. No (−)-sclareol was detected in urine, either untreated or treated with β-glucuronidase to liberate conjugates, at any time. No (−)-sclareol was detected in faecal samples from rats treated via intravenous injection, but 9% of the initial dose was found in faecal samples of rats orally administered (−)-sclareol. The bile samples from rats receiving (−)-sclareol by intravenous injection showed very low levels (0.02%) of unchanged (−)-sclareol over a 3 h period. Very low levels (0.04%) of oxidized metabolites were found in bile after 3 h, including 3-α-hydroxysclareol (0.24%), 3-β-hydroxysclareol (0.075%), 18-hydroxysclareol (0.056%) and 3-ketosclareol (0.03%). (−)-Sclareol or its oxidized metabolites were not observed in bile samples collected at any other time during the 30 h study. The authors hypothesized that the low sensitivity of the assay technique may have prevented detection of low levels

of (−)-sclareol and its metabolites over the course of the study. Although only a very small percentage of intravenously injected (−)-sclareol (<0.05%) could be accounted for in these experiments, the authors suggested that other mammalian metabolites were formed that were not detected in the assays used (Kouzi, McChesney & Walker, 1993).

In order to determine the phase I metabolism of (+)-cedrol (No. 2030), Bang & Ourisson (1975) and Trifilieff, Bang & Ourisson (1975) administered (+)-cedrol to rabbits and dogs, respectively. In rabbits, hydroxylation occurred exclusively at C3, yielding the two epimers α-epiisobiotol and α-isobiotol subsequent to dehydration at C7/C8. The metabolic fate in dogs, however, was far less regioselective, with oxidation occurring at multiple ring locations as well as methyl groups. Initial glucuronidation at the hydroxyl function of cedrol is made difficult by steric hindrance. Therefore, elimination involves additional functionalization prior to conjugation. In the case of (+)-cedrol, the hydroxylation of a non-activated saturated carbon atom is the most likely pathway (Bang & Ourisson, 1975; Trifilieff, Bang & Ourisson, 1975; Ishida, 2005).

Terpenoids have been known to affect the activity of various drug-metabolizing hepatic enzymes (Parke & Rahman, 1969). The inhibitory effect of chamomile essential oil and its major constituents (e.g. α-bisabolol, No. 2031) on four selected human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2D6 and CYP3A4) was studied. Increasing concentrations of the test compounds were incubated with individual, recombinant CYP isoforms, and their effect on the conversion of surrogate substances was measured fluorometrically; enzyme inhibition was expressed as median inhibitory concentration (IC_{50}) and inhibition constant (K_i value) in relation to positive controls. α-Bisabolol (IC_{50} = 2.18 mol/l) produced a significant inhibition of CYP2C9 and CYP2D6. As indicated by these in vitro data, chamomile preparations contain constituents that can inhibit the activities of major human drug-metabolizing enzymes; interactions with drugs whose route of elimination is mainly through CYP oxidation (especially CYP1A2) are therefore possible (Ganzera, Schneider & Stuppner, 2006).

Studies in humans, dogs, rabbits and rats have shown that aliphatic acyclic and alicyclic terpenoid tertiary alcohols are absorbed to a small or large extent depending upon lipophilicity and are distributed primarily to adipose tissue. Oxidation to polar metabolites and/or conjugation with glucuronic acid followed by excretion in the urine are expected for all seven of the flavouring agents in this group. Small amounts may be expired in exhaled air. The two esters within this group (Nos 2025 and 2026) are expected to be hydrolysed in humans to their component tertiary alcohols and butenoic acid and hexanoic acid, respectively.

The available data demonstrate that the seven aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances are rapidly absorbed, distributed, metabolized and excreted.

2.3.2 Toxicological studies

(a) Acute toxicity

No studies of acute toxicity have been reported for these flavouring agents since the preparation of the original monograph (Annex 1, reference *137*).

(b) Short-term studies of toxicity

Short-term studies of toxicity have been reported for only three of the seven flavouring agents in this group. The results of these studies with (−)-sclareol (No. 2029), (+)-cedrol (No. 2030) and α-bisabolol (No. 2031) are summarized in Table 3 and described below. Additional short-term (up to 90 days) studies of toxicity for three structurally related substances not in this group have been reported. The results of these studies are also described below.

Table 3. Results of short-term studies of toxicity with aliphatic acyclic and alicyclic terpenoid tertiary alcohols used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOEL[c]/ NOAEL[d] (mg/kg bw per day)	Reference
2029	(−)-Sclareol	Rat; M, F	1/20	Oral (gavage)	28	8.8[c,e]	Merkel (2006)
2030	(+)-Cedrol	Rat; M, F	1/20	Oral (gavage)	28	8.4[c,e]	Merkel (2006)
2031	α-Bisabolol	Rat; M, F	2/40	Diet	28	1860[d,f]	Habersang et al. (1979)
2031	α-Bisabolol	Dog; M, F	2/6	Diet	28	1860[d,f]	Habersang et al. (1979)

F, female; M, male

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

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

[c] No-observed-effect level.

[d] No-observed-adverse-effect level.

[e] Study performed with a single dose that had no observed effect; the value is therefore the highest dose tested.

[f] Adverse effects seen in the lowest dose group; therefore, this is not a true NOAEL.

(i) (−)-Sclareol (No. 2029)

In a 28-day oral toxicity study, Sprague-Dawley rats (10 of each sex per dose) approximately 1 month of age were administered (−)-sclareol (98.3% pure) at a dose of 0 or 10 mg/kg bw per day by intragastric instillation in carboxymethylcellulose. It was determined that the actual (−)-sclareol dose achieved was

8.8 mg/kg bw per day. The rats were observed twice daily for mortality, once daily for signs of toxicity and behavioural changes and weekly for more detailed clinical observations. Body weights were recorded twice during the acclimation period, at the initiation of the testing period, weekly during the exposure period and immediately prior to termination. Individual food consumption was recorded weekly, and water consumption was recorded daily. All rats were fasted overnight prior to each blood collection, which was done during the fourth week of the study and immediately prior to termination. Blood samples were used for haematology, clinical chemistry and serology assessments. All rats were terminated on day 31 of the study. Necropsies were performed on all animals, and selected organs and tissues were evaluated histologically. Group means and standard deviations were calculated for body weight, weekly body weight gain, weekly food consumption, weekly food conversion efficiency, water consumption, organ weights and ratios of organ weights to body weight or brain weight.

There were no test substance–related mortalities in the study. There were no clinical observations, effects on body weight, food consumption, food conversion efficiency, water consumption or clinical chemistry, macroscopic findings or histopathological alterations that were considered attributable to administration of (−)-sclareol. In males that were dosed with (−)-sclareol, increases in mean absolute and mean relative liver weights (organ weight to body weight and organ weight to brain weight ratios) were recorded. The increases were not associated with any evidence of macroscopic or microscopic alterations or increases in serum liver enzymes. Evaluation of individual animal data revealed that the combination of low relative liver weight for one male control animal and high relative liver weight for one male test animal accounted for the majority of the difference in mean liver weights between test and control groups. An increase in kidney weight to brain weight ratio was also observed for male rats administered (−)-sclareol. However, considering the absence of any additional pathological or histopathological findings, these changes were judged by the authors to have limited toxicological significance. In female rats, increases in absolute heart and spleen weights and heart to brain, spleen to brain, liver to brain and heart to body weight ratios were statistically significant compared with those of controls. Also, in female rats, there was a decrease in the brain to body weight ratio. In general, these changes were not found to be consistent between the sexes, and there were no associated clinical pathological or histopathological changes reported in females. The authors concluded that these findings were non-adverse and considered them to be of limited toxicological importance.

In conclusion, in rats dosed with (−)-sclareol, statistically significant changes were found not to correlate with evidence of macroscopic or microscopic alterations or associated enzyme activity. The authors concluded that the minor clinical pathology and histopathological alterations were incidental and therefore toxicologically non-adverse. Other statistically significant organ weight changes were isolated and not consistent between the sexes and were therefore considered not to be related to administration of the test substance. Therefore, under the conditions of the study and based on the toxicological end-points evaluated, the no-observed-adverse-effect level (NOAEL) for (−)-sclareol was 8.8 mg/kg bw per day for both male and female rats (Merkel, 2006).

(ii) (+)-Cedrol (No. 2030)

In a 28-day oral toxicity study, Sprague-Dawley rats (10 of each sex per dose) approximately 1 month of age were administered (+)-cedrol (99.7% pure) at a dose of 0 or 10 mg/kg bw per day by intragastric instillation in carboxymethylcellulose. It was determined that the actual (+)-cedrol dose achieved was 8.4 mg/kg bw per day. The rats were observed twice daily for mortality, once daily for signs of gross toxicity and behavioural changes and weekly for more detailed clinical observations. Body weights were recorded twice during the acclimation period, at the initiation of the testing period, weekly during the exposure period and immediately prior to sacrifice. Individual food consumption was recorded weekly, and water consumption was recorded daily. All animals were fasted overnight prior to each blood collection, which occurred during the fourth week of the study and immediately prior to sacrifice. Blood samples were used for haematology, clinical biochemistry and serology assessments. All animals were terminated on day 31 of the study. Necropsies were performed on all animals, and selected organs and tissues were evaluated histologically. Group means and standard deviations were calculated for body weight, weekly body weight gain, weekly food consumption, weekly food conversion efficiency, water consumption, organ weights and organ weight to body weight or brain weight ratios.

There were no test substance–related mortalities in the study. There were no clinical observations, effects on body weight, food consumption, food conversion efficiency, water consumption or clinical chemistry, macroscopic findings or histopathological alterations that were considered attributable to administration of (+)-cedrol. There were no statistically significant changes in absolute or relative organ weights in male rats treated with (+)-cedrol compared with controls. In female rats, decreases in absolute brain weight and brain weight to body weight and ovary weight to body weight ratios in female rats dosed with (+)-cedrol were all statistically significant compared with controls. Considering the absence of any additional pathological or histopathological findings, these changes were judged by the authors to be non-adverse and to have limited toxicological significance.

In conclusion, in rats dosed with (+)-cedrol, there were no statistically significant changes in either absolute or relative organ weights. Minor clinical pathology and histopathological alterations were considered incidental and therefore toxicologically non-adverse, according to the authors. Other statistically significant organ weight changes were isolated and not consistent between the sexes and therefore considered not to be related to administration of the test substance. Therefore, under the conditions of the study and based on the toxicological end-points evaluated, the NOAEL for (+)-cedrol was 8.4 mg/kg bw per day for both male and female rats (Merkel, 2006).

(iii) α-Bisabolol (No. 2031)

Rats

Groups of 40 rats (strain not specified) (20 of each sex per group) weighing 128–146 g were administered α-bisabolol (98% pure) by intragastric instillation at

a dose of 0, 2.0 or 3.0 ml/kg bw per day for 4 weeks. These dose levels correspond to calculated daily intakes of 0, 1860 or 2790 mg/kg bw per day (assuming a specific gravity of 0.931). In the low dose group, slight hyperactivity and decreased body weight gain were noted. In the high dose group, the mortality rate was 20%, and increased hyperactivity and decreased body weight gain were noted. Postmortem findings in rats of both groups included inflammatory changes in the liver, trachea, spleen, thymus and stomach (Habersang et al., 1979).

Dogs

Groups of Beagle dogs (three of each sex per group) were administered α-bisabolol (98% pure) by intragastric instillation at a dose of 0, 2.0 or 3.0 ml/kg bw per day for 4 weeks. These dose levels correspond to calculated intakes of 0, 1860 or 2790 mg/kg bw per day (assuming a specific gravity of 0.931). After 2 weeks, the high dose was increased to 3720 mg/kg bw per day. A loss of appetite, reduced feed intake and vomiting were observed in two of the six dogs receiving 1860 mg/kg bw per day. At necropsy, an increase in the liver weight to body weight ratio was noted. Reactions and observations were more severe in the high dose group. No other changes were noted compared with controls (Habersang et al., 1979).

(iv) Dihydromyrcenol, a mixture of 2,6-dimethyloct-7-en-2-ol[1] and 2,6-dimethyloct-7-en-2-ol formate (50:50), a structurally related aliphatic material

A 90-day oral toxicity study was conducted in Sprague-Dawley rats approximately 6–8 weeks of age to determine the potential toxicity of 2,6-dimethyloct-7-en-2-ol. Eighty rats were allocated to four groups (10 of each sex per group), and the test substance was administered by intragastric instillation in corn oil at dose levels of 10, 50, 500 and 1000 mg/kg bw per day. A vehicle control group of 10 males and 10 females was administered corn oil alone. All animals were examined for overt signs of toxicity, ill-health or behavioural change immediately before and after dosing and 1 and 5 h after dosing during the work week. Prior to the start of dosing and at weekly intervals thereafter, all rats were observed for signs of functional/behavioural toxicity. Each animal was individually assessed for sensory reactivity to auditory, visual and proprioceptive stimuli. Individual body weights were recorded on day 1, at weekly intervals thereafter and at termination. Food consumption was recorded for each cage group at weekly intervals throughout the study, and water consumption was measured and recorded for each cage group from day 15 onwards. The eyes of all control and high-dose animals were examined prior to the start of the study and at week 12. Haematology, blood chemistry, urinalysis and estrous cycle investigations were performed on all animals from each test and control group during week 7 and at the end of the study on day 90. Blood samples were obtained from the lateral tail vein. On completion of the dosing period, all animals were subjected to a full external and internal examination, any macroscopic abnormalities were recorded and selected organs and tissues were evaluated histologically.

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There were no test substance–related mortalities. No clinically observable signs of toxicity were detected in test or control animals throughout the study period. Incidental findings of noisy respiration, increased salivation, hunched posture and tiptoe gait were evident in 1000 mg/kg bw per day animals throughout the dosing period. Incidents of increased salivation were also evident in 10, 50 and 500 mg/kg bw per day animals, together with isolated incidents of noisy respiration and red/brown staining on a cage train liner for males dosed with 10 mg/kg bw per day. Observations of this nature are often reported following oral administration of unpalatable or slightly irritating test material formulation and are not considered to be an indication of systemic toxicity. Isolated instances of generalized fur loss, scab formation or generalized red/brown stained fur were evident in a number of control and test animals throughout the dosing period. Such observations are commonly observed in laboratory-maintained rats and, in view of the sporadic nature of these findings, were considered to be unrelated to dosing. There were no toxicologically relevant changes in any of the behavioural parameters, functional performance tests or sensory reactivity assessments measured.

A statistically significant reduction in body weight gain was evident in males dosed with 1000 mg/kg bw per day during weeks 6, 7 and 11. Females from the high-dose treatment group showed a reduction in body weight gain during weeks 5, 8, 9 and 10. The effect on body weight gain extended to the 500 mg/kg bw per day group during week 6 for males and during week 9 for females. No such effects were detected in animals of either sex dosed with 50 or 10 mg/kg bw per day. Males dosed with 1000 mg/kg bw per day showed a reduced food conversion efficiency during weeks 6, 7, 9, 10, 11 and 12, whereas females from this dose group showed a reduction in food conversion efficiency during weeks 6, 7, 9 and 10. The effect on food conversion efficiency extended to the 500 mg/kg bw per day dose group during week 6 for males and during week 9 for females. No such effects were detected in food consumption or in food conversion efficiency for animals of either sex dosed with 10 or 50 mg/kg bw per day. Measurements of water consumption revealed an increase for animals of either sex dosed with 500 and 1000 mg/kg bw per day from day 15 onwards. No such effects were detected in animals of either sex dosed with 10 or 50 mg/kg bw per day. There were no test substance–related ocular effects.

Haematological investigation showed a statistically significant reduction in platelet count at week 7 in males dosed with 500 and 1000 mg/kg bw per day. Animals of either sex dosed with 1000 mg/kg bw per day and females dosed with 500 mg/kg bw per day showed a statistically significant reduction in activated partial thromboplastin time. Week 13 assessments revealed reductions in haemoglobin, haematocrit and erythrocyte counts for females dosed with 1000 mg/kg bw per day, with the erythrocyte count extending into the 500 mg/kg bw per day dose group females. No such effects were detected in females dosed with 50 mg/kg bw per day or animals of either sex dosed with 10 mg/kg bw per day. Blood chemistry examinations revealed a statistically significant increase in cholesterol levels for animals of either sex dosed with 500 or 1000 mg/kg bw per day. Males dosed with 1000 mg/kg bw per day also showed statistically significant increases in aspartate aminotransferase and alanine aminotransferase activities, whereas females from this dose group showed statistically significant increases in phosphorus and

chloride levels together with a statistically significant reduction in the albumin to globulin ratio. There were no test substance–related effects on female estrous cycles or on the type or proportion of females with anomalous estrous cycles.

Rats of either sex dosed with 500 and 1000 mg/kg bw per day showed a statistically significant increase in (absolute and relative) liver and kidney weights. There were no test substance–related effects on the concentration, motility or morphology of samples of epididymal sperm. At necropsy, no macroscopic abnormalities were detected. Histopathologically, centrilobular hepatocyte enlargement was observed in males and females dosed with 1000 mg/kg bw per day. However, this is commonly observed in rodent liver following the administration of xenobiotics and, in the absence of associated inflammatory or degenerative changes, is generally considered to be adaptive in nature. A high incidence or severity of groups of basophilic tubules or globular accumulations of eosinophilic material was observed in the kidneys of all male test groups. These findings are consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of α-2u-microglobulin. The incidence of adipose infiltration of the bone marrow was increased in males dosed with 1000 mg/kg bw per day, indicative of marrow hypoplasia. However, there was no evidence of a dose–response relationship.

In conclusion, the oral administration of 2,6-dimethyloct-7-en-2-ol to rats for a period of 90 consecutive days resulted in test substance–related effects in males at all dose levels and in females dosed with 500 and 1000 mg/kg bw per day. Therefore, under the conditions of the study and based on the toxicological endpoints evaluated, the NOAEL for females was considered by the authors to be 50 mg/kg bw per day based on haematological effects at higher doses. For males, the kidney changes identified histopathologically were consistent with well-documented changes that are unique to the male rat, as α-2u-microglobulin is found only in the proximal tubular epithelium of adult male rats. Therefore, this effect is not indicative of a hazard to human health, and the NOAEL was considered by the authors to be 10 mg/kg bw per day for males based on haematological effects at higher doses (Dunster, Watson & Brooks, 2007).

(v) 4-Cyclohexyl-2-methyl-2-butanol,[1] a tertiary alcohol

In a 28-day oral toxicity study, Sprague-Dawley rats (five of each sex per dose) approximately 42 days of age were administered 4-cyclohexyl-2-methyl-2-butanol at 500, 750 or 1500 mg/kg bw per day by intragastric instillation in carboxymethylcellulose, and controls received the vehicle. Animals were observed daily for mortality and clinical signs, and body weights and food consumption were recorded weekly throughout the experiment. Ophthalmoscopy was performed on both eyes of all animals before the start of dosing and on control and high dose groups during week 4. Blood samples were obtained from all animals during week 4, and urine samples were collected from all animals at the end of week 3. At

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termination, all animals were subjected to a detailed necropsy, during which the weights of several organs were recorded and a range of tissues was preserved. A microscopic examination of specified tissues from all control and high-dose animals was performed. All macroscopic lesions were examined from all animals, and statistical analyses were conducted.

There were no deaths during the dosing period, and all animals were unremarkable with regards to clinical signs throughout the study period. Body weights, body weight gains and food consumption were unaffected by administration of 4-cyclohexyl-2-methyl-2-butanol. Ophthalmoscopic examination did not reveal any test substance–related findings or abnormalities. Urinary parameters were unaffected by administration of the test substance. Several statistically significant changes were noted in haematology and blood chemistry; for example, mean glucose levels were reduced in all dosed male and female groups. However, all individual glucose values were within the normal ranges found in the test laboratory; although several animals had values towards the lower end of the range, these changes were considered to be minor and not of any toxicological significance. All other statistically significant changes were within the normal ranges found in the test laboratory and were considered to be unrelated to dosing. Absolute and relative (to body weight) liver weights were increased in males dosed at 750 mg/kg bw per day and in both sexes dosed at 1000 mg/kg bw per day, compared with controls. The values for mean absolute liver weight and mean relative (to body weight) liver weight in these groups were at the upper end or higher than the normal range of the test laboratory. Consequently, a possible association with dosing cannot be excluded at these dose levels. Increased absolute and relative (to body weight) adrenal weights were also noted in females administered 750 and 1000 mg/kg bw per day. Statistically significant changes were noted in relative (to body weight) pituitary and heart weights in males. However, these values were also within the normal ranges found in the test laboratory and were considered not to be related to dosing. There were no test substance–related macroscopic or histopathological findings.

In conclusion, administration of 4-cyclohexyl-2-methyl-2-butanol at a dose level of 1000 mg/kg bw per day was associated with increased absolute and relative (to body weight) liver weights in both sexes. However, the toxicological significance of this finding is unclear, as there were no corresponding histopathological changes. At 750 mg/kg bw per day, absolute and relative (to body weight) liver weights were increased in males only. Because of the liver weight changes at these dose levels, the authors concluded that the NOEL for 4-cyclohexyl-2-methyl-2-butanol was 500 mg/kg bw per day under the conditions of this study (Brownlie, 1995).

(vi) 1-Methyl-3-(2-methylpropyl)cyclohexan-1-ol,[1] a structurally related alicyclic tertiary alcohol

In a 28-day oral toxicity study, young adult Wistar rats (five of each sex per dose) with mean body weights of 185.2 g for males and 140.6 g for females were administered the structurally related material 1-methyl-3-(2-methylpropyl)cyclo-

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hexan-1-ol as a mixture of *cis* and *trans* isomers (referred to as Rossitol QRM2688) at 0, 5, 15 and 150 mg/kg bw per day by intragastric instillation in corn oil. Animals were observed twice daily on working days. All abnormalities, signs of ill-health or reactions to dosing were recorded. The body weight of each animal was recorded once during the acclimatization period, at initiation of dosing and twice per week thereafter. Food consumption was measured over successive 3- to 4-day periods. At the end of the dosing period, haematology and clinical chemistry were conducted on all rats, necropsies were performed on all animals and selected organs and tissues were evaluated histologically. Group means and standard deviations were calculated for body weight, weekly body weight gain, weekly food consumption, weekly food conversion efficiency, clinical chemistry, red/white blood cells counts and organ weights.

None of the animals died during the study. The daily observations did not reveal any dosing-related clinical signs. No significant effects of dosing on any of the functional (i.e. neurobehavioural) measures were observed at any time point during the 4-week dosing period. Mean body weights showed no statistically significant intergroup differences throughout the study. No statistically significant changes in red blood cell and coagulation variables or total and differential white blood cell counts were observed. Clinical chemistry values showed the following statistically significant differences between test rats and controls: increased albumin to globulin ratio in males of the high dose group, increased cholesterol levels in males of the low and intermediate dose groups and increased urea levels in females of the high dose group. Male rats of the high dose group showed a statistically significant increase in relative kidney weight (17%) and relative liver weight (15%). Female rats of the high dose group showed a statistically significant increase in the mean relative liver weight (12%). Female rats of the low dose group showed a statistically significant increase in the relative weight of the ovaries. The latter observation, however, was considered by the authors not to be treatment related, because the absolute ovary weights were normal in the two higher dose groups. Macroscopic examination at autopsy did not reveal treatment-related abnormalities. Microscopic examination did not reveal any treatment-related histopathological changes.

The absence of obvious signs of toxicity demonstrated that the test substance was well tolerated by the rats. The administration of the test substance at 150 mg/kg bw per day was associated with increases in relative kidney weight (males only), relative liver weight, plasma albumin to globulin ratio (males only) and plasma urea level (females only). The lone effects found in the low and intermediate dose groups (increase in plasma cholesterol) were not dose related, and the cholesterol levels in these rats were well within the normal range. Therefore, the increased cholesterol levels in males were not ascribed to exposure to the test substance. The NOAEL of 1-methyl-3-(2-methylpropyl)cyclohexan-1-ol under the conditions of this study was 15 mg/kg bw per day (Appel, 1999).

(c) Long-term studies of toxicity

No additional long-term studies of toxicity of the flavouring agents in this group have been reported since the preparation of the original monograph (Annex 1, reference *137*).

(d) Genotoxicity

Genotoxicity testing has been performed on two substances in the present group and one from the former groups since the previous evaluations (Annex 1, references *137* and *187*). The results of these tests are summarized in Table 4 and described below.

(i) In vitro

(+)-Cedrol (No. 2030) was tested for mutagenicity to bacteria by incubation with *Salmonella typhimurium* tester strains TA97a, TA98, TA100, TA102 and TA1535, either alone or with an exogenous induced rat liver bioactivation system, using a plate incorporation assay. The test substance was toxic at 160 and 500 μg/plate alone and with an exogenous induced rat liver bioactivation system, respectively. At eight concentrations up to 5000 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Scheerbaum, 2001).

α-Bisabolol (No. 2031) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, either alone or with an exogenous induced rat liver bioactivation system, using a plate incorporation assay. The test substance was toxic at 150 and 500 μg/plate alone and with an exogenous induced rat liver bioactivation system, respectively. At seven concentrations up to 500 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (King, 2002).

α-Bisabolol (No. 2031) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA97a, TA98, TA100 and TA1535, either alone or with an exogenous induced rat liver bioactivation system, using a plate incorporation assay. The test substance was toxic at 100 and 200 μg/plate alone and with an exogenous induced rat liver bioactivation system, respectively. At nine concentrations up to 400 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Gomes-Carneiro et al., 2005).

Dihydromyrcenol (2,6-dimethyloct-7-en-2-ol), a structurally related substance, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, either alone or with an exogenous induced rat liver bioactivation system, using a plate incorporation assay. The test substance alone and with bioactivation was toxic at 5000 μg/plate. At seven concentrations up to 5000 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (King, 2000).

Table 4. Studies of genotoxicity with aliphatic acyclic and alicyclic terpenoid tertiary alcohols used as flavouring agents

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
In vitro						
2030	(+)-Cedrol	Reverse mutation	*Salmonella typhimurium* TA97a, TA98, TA100, TA102, TA1535	0, 1.6, 5, 16, 50, 160, 500, 1600 and 5000 μg/plate	Negative[a,b]	Scheerbaum (2001)
2031	α-Bisabolol	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	0, 1.5, 5, 15, 50, 100, 150 and 500 μg/plate	Negative[a,c,d]	King (2002)
2031	α-Bisabolol	Reverse mutation	*S. typhimurium* TA97a, TA98, TA100, TA1535	0, 1, 5, 10, 25, 50, 100, 200, 300 and 400 μg/plate	Negative[a,d,e]	Gomes-Carneiro et al. (2005)
In vivo						
356	Linalool	Micronucleus assay	Male and female mice (Swiss CD-1)	0, 500, 1000 and 1500 mg/kg bw[f]	Negative	Meerts (2001)

[a] With and without metabolic activation.
[b] The test material was bacteriotoxic towards all strains at greater than or equal to 500 and 160 μg/plate with and without S9 activation, respectively.
[c] The test material was bacteriotoxic towards all strains at doses greater than or equal to 500 and 150 μg/plate with and without S9 activation, respectively.
[d] Standard plate incorporation method.
[e] The test material was bacteriotoxic towards all strains at greater than 200 and 100 μg/plate with and without S9 activation, respectively.
[f] Administered via gavage.

Octahydro-2,5,5-trimethyl-2-naphthol,[1] a structurally related substance, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537, as well as *Escherichia coli* strain WP2*uvrA*, either alone or with an exogenous induced rat liver bioactivation system, using a preincubation assay. At eight concentrations up to 500 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Watanabe, 2002).

1,2-Dihydrolinalool,[2] a structurally related substance, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA97, TA98, TA100, TA102 and TA1535, either alone or with an exogenous induced rat liver bioactivation system, using both the plate incorporation and preincubation assays. At six concentrations up to 1000 μg/plate, the test substance produced no increase in revertant mutants in any strain in either assay, whereas appropriate control substances established the responsiveness of the tester strains (Gocke, 1999).

1-Methyl-3-(2-methylpropyl)cyclohexan-1-ol, a structurally related substance, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102 and TA1535, either alone or with an exogenous induced rat liver bioactivation system, using a plate incorporation assay. The test substance alone and with bioactivation was toxic at 250 μg/plate. At five concentrations up to 500 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (van Ommen, 1999).

1-Methyl-3-(2-methylpropyl)cyclohexan-1-ol, a structurally related substance, was tested for induction of chromosomal aberrations in cultured Chinese hamster ovary cells. An exogenous rat liver bioactivation system was used. Test substance concentrations of 200 μg/ml or greater caused significant toxicity, with cell death and precipitation. No biologically relevant or statistically significant increase in the number of cells with structural aberrations was observed (de Vogel, 1999).

(ii) In vivo

Linalool (No. 356) was tested for in vivo mutagenicity in the bone marrow micronucleus assay in mice. Four groups (five of each sex per group) received a single dose of the test substance by intragastric instillation in corn oil; two of these groups were administered linalool at a dose of 1500 mg/kg bw, and one group each was administered 500 or 1000 mg/kg bw. Vehicle controls received corn oil, and

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[1] OH

[2] HO

positive controls received cyclophosphamide. Systemic toxic signs were recorded at least once a day. The animals were terminated 24 or 48 h after dosing, both femurs were removed and bone marrow smears were made and analysed for micronuclei.

The animals of the groups dosed with linalool showed no decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a lack of toxic effects of this compound on erythropoiesis. No increase in the frequency of micronucleated polychromatic erythrocytes was found in the linalool-dosed animals compared with the vehicle controls, whereas cyclophosphamide produced an increase. Therefore, linalool was not mutagenic in the micronucleus test under the experimental conditions used (Meerts, 2001).

(e) Developmental and reproductive studies of toxicity

(i) α-Bisabolol (No. 2031)

A study was conducted in an unspecified strain of rats to determine the potential developmental toxicity of α-bisabolol. In two experiments, groups of unspecified numbers of presumed pregnant rats were administered α-bisabolol (98% pure) at a dose of 0, 0.25, 0.50 or 1.0 ml/kg bw per day in the first experiment and 3.0 ml/kg bw per day in the second experiment by intragastric instillation on days 6–15 of pregnancy. These dose levels correspond to calculated daily intakes of 0, 233, 466, 931 and 2793 mg/kg bw per day (assuming a specific gravity of 0.931). Upon sacrifice, the uteri of all animals were examined for numbers of live or dead fetuses, resorptions and deformities. At the highest dose of the test substance administered, there were reduced numbers of live fetuses in addition to reduced fetal weights and increased numbers of resorptions. No adverse effects were observed in any other dose group. The NOAEL for reproductive effects in an unspecified strain of rats is 931 mg/kg bw per day (Habersang et al., 1979).

In a study to assess the developmental toxicity of α-bisabolol, an unspecified number of female New Zealand White rabbits 8–9 months of age were administered the test material (98% pure) at a dose of 0, 0.3, 1 or 3 ml/kg bw per day by intragastric instillation on days 6–18 of pregnancy. These dose levels correspond to calculated daily intakes of 0, 279, 931 and 2793 mg/kg bw (assuming a specific gravity of 0.931). Caesarean sections were performed at day 30, and fetuses were examined for visceral and skeletal malformations. The highest dose of the test substance reduced the numbers of live fetuses, in addition to reducing fetal weights and increasing numbers of resorptions. No adverse effects were observed in the middle and low dose groups. The NOAEL for reproductive effects in the New Zealand White rabbit is 931 mg/kg bw per day (Habersang et al., 1979).

(ii) Linalool (No. 356)

Linalool was studied for potential developmental toxicity in rats. Four groups of presumed pregnant female Sprague-Dawley rats (25 per group) were administered linalool (99.5% pure) at a dose of 0, 250, 500 or 1000 mg/kg bw per day by intragastric instillation in corn oil on gestational days 7–17. Observations for

viability, adverse clinical signs, abortion and premature delivery were conducted before and approximately 1 h following dosing and once thereafter. Body weights were recorded prior to the start of the study and daily during the dosage and post-dosage periods. Food consumption was recorded on days 0, 7, 10, 12, 15, 18 and 21. Caesarean section and necropsy were conducted on all animals on gestational day 21. The uteri of apparently non-pregnant rats were examined while pressed between glass plates, to confirm the absence of implantation sites. The number and distribution of corpora lutea were recorded. The uterus of each rat was removed and examined for pregnancy, number and distribution of implantations, fetal mortality and early and late resorptions. Fetuses were removed from the uterus, weighed, euthanized and examined microscopically for sex and gross external alterations. Approximately one half of each litter was examined for soft tissue alterations, using a variation of Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared, stained and examined for skeletal alterations.

No deaths related to the administration of linalool occurred. One dam in the low dose group delivered early on the day of scheduled sacrifice. This delivery was considered unrelated to administration of linalool because the observation was not dose dependent. There were no macroscopic lesions at necropsy in this dam, and her litter consisted of 12 live-delivered pups and one early in utero resorption. All pups appeared normal for this dam, and no external, soft tissue or skeletal alterations occurred in these pups.

There were no clinical signs of toxicity. All clinical observations were considered to be unrelated to administration of linalool because they were not dose dependent or the observations were transient. All necropsy observations were considered to be unrelated to administration of linalool because they were not dose dependent or they occurred in a single rat per dosage group. Body weight gains were reduced by 11% in the 1000 mg/kg bw per day dose group during the dosing period (in comparison with control). Body weights and body weight gains were unaffected by dosages of linalool as high as 500 mg/kg bw per day. In comparison with the control group, absolute and relative food consumption values were reduced and significantly reduced (by 7%), respectively, in the 1000 mg/kg bw per day dose group for the entire dosage period. Absolute and relative food consumption values were unaffected by dosages of linalool as high as 500 mg/kg bw per day.

No caesarean section or litter parameters were affected by dosages of linalool as high as 1000 mg/kg bw per day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, per cent resorbed conceptuses, per cent live male fetuses and fetal body weights were similar among the three dosage groups and vehicle control group. No dam had a litter that consisted of all resorbed conceptuses. All placentas appeared normal. No macroscopic external, soft tissue or skeletal alterations appeared to be caused by dosages of linalool as high as 1000 mg/kg bw per day. There were no dose-dependent or significant differences in the litter or fetal incidences of any macroscopic external, soft tissue or skeletal alterations. All ossification averages were comparable to vehicle control group values and did not significantly differ among the groups.

Based on these data, the maternal NOAEL of linalool is 500 mg/kg bw per day. The 1000 mg/kg bw per day dose caused non-significant reductions in body weight gain and also reduced absolute and relative food consumption values during the dosage period. However, following the completion of the dosing period, these effects were reversed. The developmental NOEL is 1000 mg/kg bw per day, the highest dose tested. No embryo-fetal effects were observed at the highest dose tested (Lewis, 2006).

(iii) 2,6-Dimethyloct-7-en-2-ol, a structurally related aliphatic terpenoid tertiary alcohol

A study was conducted in Sprague-Dawley rats to determine the potential developmental toxicity of 2,6-dimethyloct-7-en-2-ol. Four groups of presumed pregnant female rats (25 per group) were administered 2,6-dimethyloct-7-en-2-ol at a dose of 0, 250, 500 or 1000 mg/kg bw per day by intragastric instillation in corn oil on gestational days 7–17. Observations for viability, adverse clinical signs, abortion and premature delivery were conducted before and approximately 1 h following dosing and once thereafter. Body weights were recorded prior to the start of the study and daily during the dosage and post-dosage periods. Food consumption was recorded on days 0, 7, 10, 12, 15, 18 and 21. On gestational day 21, all rats were terminated, and caesarean sections and necropsies were conducted on all animals. The uteri of apparently non-pregnant rats were examined while pressed between glass plates, to confirm the absence of implantation sites. The number and distribution of corpora lutea were recorded. The uterus of each rat was removed and examined for pregnancy, number and distribution of implantations, fetal mortality and early and late resorptions. Fetuses were removed from the uterus, weighed, euthanized and examined microscopically for sex and gross external alterations. Approximately one half of each litter was examined for soft tissue alterations, using a variation of Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared, stained and examined for skeletal alterations.

Body weight gains in the high dose group were reduced by 5% when compared with controls; weight losses were observed after the first two doses. Although these observations were not significant, they were considered to be evidence of a threshold level for maternal toxicity. Both maternal absolute and relative food consumption values were significantly reduced in the 1000 mg/kg bw per day group compared with the vehicle control. Reduced food consumption was most prominent on gestational days 7–10, which correlated with the weight losses and reduced weight gains that occurred during the initial days of the dosing period. 2,6-Dimethyloct-7-en-2-ol doses of 250 or 500 mg/kg bw per day had no adverse effects on maternal food consumption or body weight gains.

Pregnancy occurred in 22–25 rats per dosage group. Body weights for combined male and female fetuses were reduced approximately 3% in the 1000 mg/kg bw per day group compared with vehicle controls (the reduction was statistically significant for females). Although the values were within the historical control range for the testing facility, the reduction in fetal body weight correlated with the reduced maternal body weight gains and food consumption in the dosage period

and for the entire dosage and gestation periods. No other caesarean section or litter parameters were affected by dosages of 2,6-dimethyloct-7-en-2-ol as high as 1000 mg/kg bw per day.

Fetal evaluations were based on 22–25 litters, which corresponded to 295–358 fetuses per dosage group. Fetal alterations were defined as either malformations (irreversible changes that occur at low incidences with this species and strain) or variations (common findings in this species and strain and reversible delays or accelerations in development). Upon inspection, there were no fetal gross external alterations or fetal soft tissue or skeletal malformations observed in the experiment. There were no observable soft tissue variations, and skeletal variations were limited to two reversible minor changes. First, there was evidence of a threshold (but statistically significant) increase in supernumerary ribs, along with associated significant increases and decreases in the respective numbers of thoracic and lumbar vertebrae. Second, there was evidence of a small but statistically significant retardation in ossification of the metatarsal bones in the hind paws, evident as a reduction in the mean number of ossified metatarsal bones.

Results from the present study indicate that 2,6-dimethyloct-7-en-2-ol at a dose of 1000 mg/kg bw per day produced threshold levels of maternal and developmental toxicity. The significant reductions in maternal food consumption and the associated reductions in maternal body weight gains suggest that a maternally toxic level was tested. Many of the minor developmental alterations observed in the study are often observed at maternally toxic doses (Khera, 1981) and, because they disappear with continued growth (Wickramaratne et al., 1985; Wickramaratne, 1988), are generally not considered to be adverse effects. Other effects (e.g. delay in ossification of metatarsals) were not considered to be of toxicological importance because the values were within the historical range observed at the testing facility. As such, the maternal and developmental NOAELs for 2,6-dimethyloct-7-en-2-ol are both considered to be 500 mg/kg bw per day, and the test material was not considered to be a selective developmental hazard in rats (Politano et al., 2008).

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ALIPHATIC AND AROMATIC AMINES AND AMIDES (addendum)

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1. EVALUATION

1.1 Introduction

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

The Committee evaluated 49 other members of this group of flavouring agents at its sixty-fifth and sixty-eighth meetings (Annex 1, references *178* and *187*). For 36 of the 37 flavouring agents evaluated at the sixty-fifth meeting, the

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

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

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

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

1.2 Assessment of dietary exposure

The total annual volumes of production of the nine additional flavouring agents in this group are 21 kg in Europe, 1001 kg in the USA and 3 kg in Japan (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009). In Europe and the USA, greater than 99% of the annual volume of production is accounted for by *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) and *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), respectively. In Japan, 100% of the annual volume of production is accounted for by 3-(methylthio)propylamine (No. 2004).

The estimated dietary exposures for each of the flavouring agents, calculated as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest estimate is for choline chloride (No. 2003) (200 000 μg, the SPET value obtained from bread and ordinary bakery ware). For the other flavouring agents in the group, the daily dietary exposures range from 0.02 to 48 000 μg, with the SPET yielding the highest estimate for all. Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

Table 1. Summary of the results of the safety evaluations of aliphatic and aromatic amines and amides used as flavouring agents[a,b,c]

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Are additional data available for substances with an estimated intake exceeding the threshold of concern? (follow-on from step B3)[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	2006	958660-02-1; 958660-04-3	B3: Yes, SPET: 200		The NOAEL of 8 mg/kg bw per day in a 28-day study in rats for the structurally related *N*-ethyl 2-isopropyl-5-methylcyclo-hexanecarboxamide (No. 1601) (Miyata, 1995) is at least 2400 times the estimated daily dietary exposure to No. 2006 when used as a flavouring agent.	Note 3	No safety concern
(±)-*N*-Lactoyl tyramine	2007	781674-18-8	B3: Yes, SPET: 20 000		Additional data are available, but inadequate margins of safety are provided from the NOELs for structurally related substances.	Notes 3 and 4	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Are additional data available for substances with an estimated intake exceeding the threshold of concern? (follow-on from step B3)[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
N-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	2008	847565-09-7	B3: Yes, SPET: 2400		The NOAEL of 10 mg/kg bw per day in a 28-day study in rats (Eapen, 2007) is at least 250 times the estimated daily dietary exposure to No. 2008 when used as a flavouring agent.	Note 3	No safety concern
N-*p*-Benzeneacetonitrile menthanecarboxamide	2009	852379-28-3	B3: Yes, SPET: 3000		The NOEL of 300 mg/kg bw per day in a 90-day study in rats (Eapen, 2006) is at least 6000 times the estimated daily dietary exposure to No. 2009 when used as a flavouring agent.	Note 3	No safety concern
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	883215-02-9	B3: Yes, SPET: 48 000		Additional data are not available.	Notes 3 and 4	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Are additional data available for substances with an estimated intake exceeding the threshold of concern? (follow-on from step B3)[e]	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	51115-77-6	B3: Yes, SPET: 27 000		Additional data are not available.	Notes 3 and 4	Additional data required to complete evaluation

bw, body weight; CAS, Chemical Abstracts Service; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

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

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

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

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

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

Notes:

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

Table 2. Annual volumes of production and daily dietary exposures for aliphatic and aromatic amines and amides used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
Choline chloride (also includes choline) (2003)				125 000	2083	+[d]
Europe	0.5	0.05	0.0008			
USA	ND	ND	ND			
Japan	ND	ND	ND			
3-(Methylthio)propylamine (2004)				200	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	3	1	0.02			
N-Ethyl-2,2-diisopropylbutanamide (2005)				35 000	583	–
Europe	ND	ND	ND			
USA	1000	122	2			
Japan	ND	ND	ND			
Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (2006)				800	13	–
Europe	ND	ND	ND			
USA	1	0.06	0.001			
Japan	ND	ND	ND			
(±)-*N*-Lactoyl tyramine (2007)				20 000	333	–
Europe	0.2	0.02	0.0003			
USA	ND	ND	ND			
Japan	ND	ND	ND			
N-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (2008)				2 400	40	–
Europe	0.1	0.01	0.0001			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
USA	ND	ND	ND			
Japan	ND	ND	ND			
N-*p*-Benzeneacetonitrile menthanecarboxamide (2009)				3 000	50	–
Europe	20	2	0.04			
USA	ND	ND	ND			
Japan	ND	ND	ND			
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide (2010)				63 000	1050	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide (2011)				35 000	583	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Total						
Europe	21					
USA	1001					
Japan	3					

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

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:

Table 2 (contd)

(annual volume, kg) × (1 × 10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009).
MSDI (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (μg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Natural occurrence in food reported in Zeisel et al. (2003).

1.3 Absorption, distribution, metabolism and elimination

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

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

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

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

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

Published studies on choline chloride (No. 2003) show that it is absorbed readily, metabolized to betaine in the liver and kidney and used in the synthesis of endogenous substances, such as acetylcholine.

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the additional flavouring agents, the Committee assigned two flavouring agents (Nos 2003 and 2004) to structural class I. The remaining seven flavouring agents (Nos 2005–2011) were assigned to structural class III (Cramer, Ford & Hall, 1978).

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

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

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

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

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

For cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (No. 2006), available data on the structurally related *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) give a no-observed-adverse-effect level (NOAEL) of 8 mg/kg body weight (bw) per day from a 28-day study in rats (Miyata, 1995). This provides a margin of safety of about 2400 in relation to the

highest estimated dietary exposure to No. 2006 (SPET = 200 µg/day) when used as a flavouring agent.

For *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008), available data give a NOAEL of 10 mg/kg bw per day from a 28-day study in rats (Eapen, 2007). This provides a margin of safety of 250 in relation to the highest estimated dietary exposure to No. 2008 (SPET = 2400 µg/day) when used as a flavouring agent. The Committee noted that the margin of safety of No. 2008 based on the MSDI of 0.01 µg/day exceeds 60 million and concluded that the values of 250 (based on the SPET) and greater than 60 million (based on the MSDI) provide an adequate margin of safety.

For *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009), available data give a no-observed-effect level (NOEL) of 300 mg/kg bw per day from a 90-day study in rats (Eapen, 2006). This provides an adequate margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2009 (SPET = 3000 µg/day) when used as a flavouring agent.

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

For (±)-*N*-lactoyl tyramine (No. 2007), available data on the structurally related nonanoyl 4-hydroxy-3-methoxybenzylamide (No. 1599) give a NOEL of 8.4 mg/kg bw per day from a 90-day study in rats (Posternak, Linder & Vodoz, 1969). This provides a margin of safety of 25 in relation to the highest estimated dietary exposure to No. 2007 (SPET = 20 000 µg/day) when used as a flavouring agent. The NOELs for other structurally related flavouring agents, such as *N*-[2-(3,4-dimethoxy-phenyl)ethyl]-3,4-dimethoxycinnamic acid (No. 1777) or *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide (No. 1776), give similarly low margins of safety. The Committee therefore concluded that additional data on (±)-*N*-lactoyl tyramine (No. 2007) would be necessary to complete the safety evaluation.

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

Table 1 summarizes the evaluations of the nine aliphatic and aromatic amines and amides used as flavouring agents in this group (Nos 2003–2011).

Table 3. Combined dietary exposure for the homologous or closely related series within this group of aliphatic and aromatic amines and amides used as flavouring agents

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

1.5 Consideration of combined intakes from use as flavouring agents

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

This group of flavouring agents contains members of several homologous or closely related series—namely, aliphatic primary amines, aliphatic tertiary amines, amines with an alkyl aromatic side-chain and aliphatic unsaturated amides. In the unlikely event that the flavouring agents in this group in any of these homologous, closely related series were to be consumed concurrently on a daily basis, the estimated combined intakes would be as shown in Table 3.

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

1.6 Consideration of secondary components

Two flavouring agents in this group (Nos 2007 and 2009) have minimum assay values of less than 95%. The secondary components of (±)-*N*-lactoyl tyramine (No. 2007) are lactic acid and ethyl lactate. Lactic acid (No. 930) is endogenous, and ethyl lactate (No. 931) is expected to be hydrolysed to lactic acid. These substances were evaluated at the fifty-seventh meeting of the Committee (Annex 1, reference *154*) and concluded to be of no safety concern at estimated dietary exposures as flavouring agents. The secondary component of *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) is *N*-*p*-benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*). This substance is a stereoisomer of No. 2009, is expected to share the same metabolic fate as the primary substance and is not considered to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 5.

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes key aspects relevant to the safety evaluation of nine aliphatic and aromatic amines and amides, which are additions to a group of 49 flavouring agents evaluated previously by the Committee at its sixty-fifth, sixty-eighth and sixty-ninth meetings (Annex 1, references *178*, *187* and *190*).

2.2 Additional considerations on intake

There is no additional information on estimated dietary exposures. Dietary exposure estimates were made using the SPET in addition to the MSDI approach.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

General information on the metabolism of the aliphatic and aromatic amines and amides was previously provided in the reports of the sixty-fifth and sixty-eighth meetings (Annex 1, references *178* and *187*). Further information relevant to the substances considered in this report is provided below.

(a) Choline chloride (No. 2003)

Choline is endogenous to humans and other mammals. Choline and its derivatives serve as precursors to acetylcholine and phospholipids. Choline also plays important roles in structural integrity of cell membranes, methyl metabolism, cholinergenic neurotransmission, transmembrane signalling and lipid and cholesterol transport and metabolism.

Dietary choline is absorbed primarily in the jejunum and, because it is water soluble, enters the portal circulation (Sanford & Smyth, 1971; Flower et al., 1972; Kuczler, Nahrwold & Rose, 1977). The principal products of choline biodegradation are trimethylamine (Asatoor & Simenhoff, 1965) and betaine. The formation of trimethylamine has been attributed to gut microflora, as markedly reduced trimethylamine formation is observed in germ-free rats and antibiotic-sterilized human guts or when choline is administered via intravenous injection (De La Huerga & Popper, 1951; De La Huerga, Popper & Steigmann, 1951; Prentiss et al., 1961; Zeisel, Wishnok & Blusztajn, 1983).

Choline is readily transformed into betaine by the actions of choline dehydrogenase and betaine aldehyde dehydrogenase, which are present in the liver (Bernheim & Bernheim, 1938) and kidney (Bernheim & Bernheim, 1933). Some choline is biotransformed into acetylcholine, a neurotransmitter, via choline acetyltransferase and acetyl coenzyme A (Nachmansohn & Machado, 1943). Choline kinase, which is widely distributed in mammalian tissues, is responsible for the phosphorylation of choline (Brophy et al., 1977).

A group of eight healthy male volunteers was provided a diet deficient in choline for 3 weeks, while a control group of seven healthy male volunteers was provided a diet with typical levels of choline (700 mg/day). During the first week of the study, the control and test groups were fed the same diet, including choline. In weeks 2–4, the test group was provided the choline-deficient diet, and then at week 5, both control and test groups were fed a diet providing typical levels of choline. For the test group, plasma choline and phosphatidylcholine levels dropped by 30%, while erythrocyte phosphatidylcholine levels dropped by 15%. For the test group during choline deprivation, the serum alanine aminotransferase activity increased steadily during the test period until choline was restored to the diet at week 5. All other parameters were comparable to those of control volunteers (Zeisel et al., 1991).

Figure 1. Metabolism of (±)-*N*-lactoyl tyramine in simulated gastric fluid (SGF)

(+/-)-N-Lactoyl tyramine hydrolysis in SGF

% of product degrade

time (h)

SGF 37C no enzyme

SGF 37C pepsin

(b) *(±)-*N*-Lactoyl tyramine (No. 2007)*

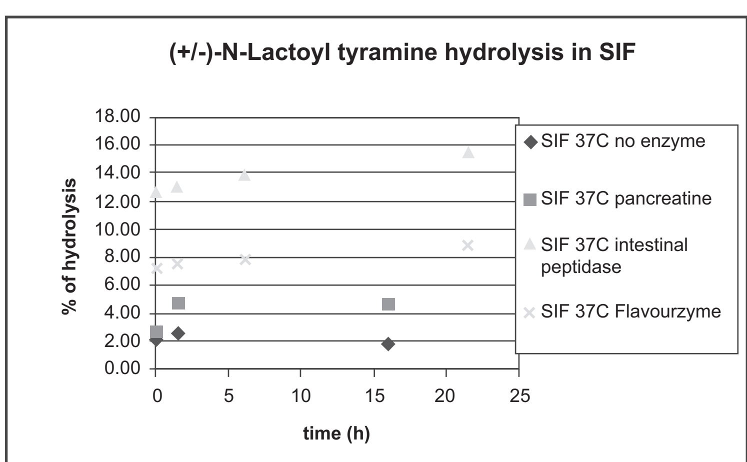
Hydrolysis studies have been performed on (±)-*N*-lactoyl tyramine in vitro under acid conditions and subsequently in both simulated gastric and intestinal conditions. In the initial experiment under acid conditions, a solution of 10 mg of (±)-*N*-lactoyl tyramine in 1 ml of 6 N DCl/D_2O was prepared, and the hydrolytic reaction was carried out at room temperature, followed by measuring ^{1}H-nuclear magnetic resonance after various reaction times. The experiment showed that (±)-*N*-lactoyl tyramine was highly stable under acid conditions, as only a small percentage of lactic acid was observed after 19 h.

In the hydrolysis studies under simulated gastric fluid conditions, experiments were carried out in triplicate at 37 °C, with 1.6 ml of a 0.6 g/ml (±)-*N*-lactoyl tyramine solution in 50 ml of simulated gastric fluid, with or without the addition of 0.6 g of pepsin. Following shaking (100 revolutions per minute [rpm]), 1 ml samples were taken for analysis at 0, 1 and 3 h. Based on the results, presented in Figure 1, it was determined that no significant hydrolysis of (±)-*N*-lactoyl tyramine occurred in gastric conditions, either with or without pepsin.

In the hydrolysis study under simulated intestinal fluid conditions, experiments were carried out in triplicate at 37 °C, with 1.6 ml of a 0.6 g/ml (±)-*N*-lactoyl tyramine solution in 50 ml of simulated intestinal fluid, with or without the addition of 0.5 g of pancreatin. Following shaking (100 rpm), 1 ml samples were taken for analysis at 0, 1 and 15 h. Additional experiments were carried out in 10 ml simulated intestinal fluid with 150 µl of 0.6 g/ml (±)-*N*-lactoyl tyramine and with 0.25 g of peptidase or 10 µl Flavourzyme 1000L (a fungal complex of exopeptidases and endoproteases), after which 1 ml samples were taken at 0, 1.5, 6 and 21 h. Based on the data shown in Figure 2, it was determined that no significant hydrolysis occurred in simulated intestinal fluid or in simulated intestinal fluid with pancreatin.

These in vitro data support the conclusion that no significant hydrolysis of (±)-*N*-lactoyl tyramine occurs in the digestive or intestinal tract environment (Anon., 2006).

Figure 2. Metabolism of (±)-*N*-lactoyl tyramine in simulated intestinal fluid (SIF)



In order to determine the metabolic fate of and identify potential detoxication pathways for (±)-*N*-lactoyl tyramine (No. 2007), a metabolite profile following in vitro incubation with rat hepatocytes was performed. A liquid chromatography–photodiode array–mass spectrometry (LC-PDA-MS) method was developed to analyse incubated samples containing (±)-*N*-lactoyl tyramine and its metabolites. The test material was incubated at a concentration of 10 µmol/l at 37 °C with Sprague-Dawley rat hepatocytes for 1, 60 and 120 min in duplicate, and the metabolic activity of the hepatocytes was verified by measuring CYP-dependent and phase II enzymatic activities in the sample. An insignificant difference (3%) in viability between hepatocytes exposed to (±)-*N*-lactoyl tyramine and solvent control indicated that the test substance was not cytotoxic at 10 µmol/l. The samples were centrifuged for 5 min and subjected to LC-PDA-MS analysis. Two additional peaks were formed during the incubation of (±)-*N*-lactoyl tyramine with Sprague-Dawley rat hepatocytes. One peak, with *m/z* 386, was identified as a glucuronic acid conjugate of (±)-*N*-lactoyl tyramine. The second peak, with *m/z* 301, could not be identified, and it was questioned by the authors whether this compound was truly a metabolite of (±)-*N*-lactoyl tyramine, as essential structural features of the parent compound were not reflected in the fragmentation pattern in this metabolite (Meerts, 2007).

(c) N-p-*benzeneacetonitrile menthanecarboxamide (No. 2009)*

The potential for *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) to metabolize and release cyanide after ingestion to levels above the human background levels of cyanide (in the range of 3.5–106 µg/l) (Maehly & Swensson, 1970; IPCS, 2004) has been examined. An in vitro metabolism experiment was performed in cryopreserved hepatocytes prepared from rat and human livers. Incubation of *N*-*p*-benzeneacetonitrile menthanecarboxamide was carried out with

hepatocytes (1 × 10^6 cells/ml) suspended in 0.25 ml of Waymouth's medium supplemented with fetal bovine serum, insulin, L-glutamine, sodium pyruvate and dexamethasone. Sodium thiosulfate (800 µmol/l) was added to the medium to ensure that cyanide was trapped under conditions of the test system and was enzymatically transformed to thiocyanate (NCS^-). The medium was saturated with a 95:5 mixture of oxygen and carbon dioxide. The incubation temperature was 37 ± 1 °C, and the atmosphere was a 95:5 mixture of air and carbon dioxide at 95% humidity. Cyanide release was evaluated over a range of concentrations of *N*-*p*-benzeneacetonitrile menthanecarboxamide (0, 50, 150 and 250 µmol/l). After 0, 1, 2 and 4 h of incubation, the reactions were stopped by centrifugation. Incubations of *N*-*p*-benzeneacetonitrile menthanecarboxamide with boiled hepatocytes at concentrations of 0, 50, 150 and 250 µmol/l were used to distinguish between actual metabolites and chemical degradation products. Positive control incubations were carried out with hepatocytes in which the test article was replaced with one of two positive controls (sodium cyanide and benzyl cyanide) to determine if the hepatocytes were metabolically competent for this type of compound. Viability of the hepatocytes was determined in the presence and absence of test articles and positive controls at each time point by Trypan Blue exclusion.

Per cent viability patterns were consistent between rat and human hepatocytes, although the human cells showed better viability overall. *N*-*p*-Benzeneacetonitrile menthanecarboxamide did not appear to cause significant cell death in rat or human hepatocytes at 250 µmol/l. Sodium cyanide showed some toxicity over the 4 h time frame of the experiment in rat and human hepatocytes at 150 µmol/l. However, at 250 µmol/l, benzyl cyanide showed dramatic toxicity at both 0 and 4 h in rat and human hepatocytes; at 150 µmol/l in human hepatocytes, survival was improved, but still low at 4 h.

Control samples without any *N*-*p*-benzeneacetonitrile menthanecarboxamide in rat and human hepatocytes showed a small increase in thiocyanate levels with time. As there was no external source of cyanide, this was determined to be attributable to metabolism of endogenous cyanide. *N*-*p*-Benzeneacetonitrile menthanecarboxamide at 250 µmol/l in rat and human hepatocytes also showed a small increase in thiocyanate levels with time. These levels of thiocyanate were not different from background and much lower than levels observed with the positive controls. Sodium cyanide and benzyl cyanide (positive controls) at 50 and 150 µmol/l, respectively, in rat hepatocytes and 150 and 250 µmol/l, respectively, in human hepatocytes showed an increase in thiocyanate levels with time, indicating that cyanide is released in these test systems and metabolically transformed to thiocyanate (Table 4). *N*-*p*-Benzeneacetonitrile menthanecarboxamide at 0 and 250 µmol/l in boiled rat and human hepatocytes showed no increase in thiocyanate levels with time, confirming that measured increases in thiocyanate levels with the other samples were attributable to the metabolism of sodium cyanide and benzyl cyanide.

In conclusion, thiocyanate levels from *N*-*p*-benzeneacetonitrile menthanecarboxamide are low and do not increase during 4 h of incubation at 250 µmol/l with rat or human hepatocytes, indicating that little or no cyanide is released. The maximum test concentration for *N*-*p*-benzeneacetonitrile menthanecarboxamide

Table 4. Mean thiocyanate levels detected upon release from* N-p-*benzeneacetonitrile menthanecarboxamide, sodium cyanide and benzyl cyanide in vitro by rat and human hepatocytes

Incubation time (h)	Mean thiocyanate levels in hepatocytes (µg/dl)							
	N-*p*-Benzeneacetonitrile menthanecarboxamide				Sodium cyanide		Benzyl cyanide	
	0 µmol/l	250 µmol/l	0 µmol/l (boiled)	250 µmol/l (boiled)	50 µmol/l	150 µmol/l	150 µmol/l	250 µmol/l
Rat								
0	20.0	33.3	40.0	35.0	525	575	360	670
1	73.3	170	—	—	—	—	—	—
2	133	230	—	—	—	—	—	—
4	313	303	45.0	30.0	1220	2560	1310	2390
Human								
0	60.0	63.3	10.0	10.0	170	255	125	105
1	80.0	103	—	—	—	—	—	—
2	93.3	76.7	—	—	—	—	—	—
4	90	140	15.0	15.0	565	855	345	330

was limited to 250 µmol/l because of its poor solubility in the incubation medium. Thiocyanate levels for sodium cyanide and benzyl cyanide (positive controls) increased significantly during 4 h of incubation with rat or human hepatocytes, indicating that cyanide is released and transformed to thiocyanate. Comparison of the thiocyanate levels for *N*-*p*-benzeneacetonitrile menthanecarboxamide with those for the positive controls demonstrates clearly that *N*-*p*-benzeneacetonitrile menthanecarboxamide produces background levels of thiocyanate. Even at a sodium cyanide concentration of 50 µmol/l, thiocyanate levels are much higher than background levels. Sodium cyanide at 50 and 150 µmol/l after 4 h of incubation with rat or human hepatocytes showed thiocyanate levels approximately proportional to the initial concentrations. Benzyl cyanide at 150 and 250 µmol/l after 4 h of incubation with rat hepatocytes showed thiocyanate levels approximately proportional to the initial concentrations; however, with human hepatocytes, benzyl cyanide showed similar thiocyanate levels for both concentrations (Wolff & Skibbe, 2007).

(d) N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768)*

Male Sprague-Dawley rats (three per dose) administered *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide at 0.01, 0.1, 1, 10, 30 or 100 mg/kg bw via gavage showed rapid clearance of the majority of the dose from

serum within 4–24 h post-administration. With all dosed groups except for the 100 mg/kg bw group, the serum concentration of *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide was below the limit of detection between 24 and 48 h. The time to maximum serum concentration (T_{max}) across all groups was 0.3 h, demonstrating the rapid clearance. Similarly, when male Sprague-Dawley rats were injected intraperitoneally with *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide at 0.1 mg/kg bw, the serum levels of the test material were cleared within the first 8 h post-injection and below the limit of detection at 24 h (Ikeda, 2008a).

Male Beagle dogs (three per dose) administered *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide at 0, 0.01, 0.1, 1, 10, 30 or 100 mg/kg bw via gavage also showed efficient clearance of the majority of the test material from the serum within the first 24 h. The T_{max} across all of the groups ranged from 1 to 2 h, demonstrating rapid clearance. The group of male Beagle dogs administered *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide at 0.1 mg/kg bw by intraperitoneal injection also showed rapid clearance of the test material from the serum in the first 24 h, dropping below the limit of detection by 48 h (Ikeda, 2008b).

Eight healthy male volunteers consumed gelatine capsules providing *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide doses of 0.01 mg/kg bw or 0.02 mg/kg bw, and blood samples were taken at 5, 15 and 30 min and 1, 2, 3, 4, 6, 8 and 24 h. The *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide was well absorbed following oral administration, and the peak plasma concentration was observed at 4 h post-ingestion. The plasma concentration returned to baseline levels by 24 h post-ingestion (Ikeda, 2008c).

When comparing the three studies, Ikeda (2008c) concluded that clearance of *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide from the serum when administered via gavage followed the order rat > dog > human.

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for four of the nine flavouring agents in this group and are summarized in Table 5. In rats, an LD_{50} of 550 mg/kg bw has been reported for *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), whereas LD_{50} values of >2000 mg/kg bw have been reported for cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (No. 2006), *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008) and *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) (Mallory, 2004; Findlay, 2006; Moore & Groom, 2006; Vaeth, 2007).

These data indicate that the acute oral toxicity of these aliphatic and aromatic amides is low.

(b) Short-term studies of toxicity

Results of short-term studies of toxicity are available for *N*-(2-(pyridin-2-yl)-ethyl)-3-*p*-menthanecarboxamide (No. 2008), *N*-*p*-benzeneacetonitrile menthane-

Table 5. Results of oral acute toxicity studies with aliphatic and aromatic amides used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
2005	*N*-Ethyl-2,2-diisopropylbutanamide	Rat; F	550	Findlay (2006)
2006	Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	Rat; F	>2000	Vaeth (2007)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Rat; F	>300	Moore & Groom (2006)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Rat; F	>2000	Moore & Groom (2006)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Rat; M, F	>2000	Mallory (2004)

F, female; M, male

carboxamide (No. 2009) and *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide (No. 1768). They are summarized in Table 6 and described below.

(i) N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008)*

In a 28-day subchronic toxicity study, male and female Crl:CD(SD) rats (five of each sex per group) were fed a diet that provided *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008) at dose levels of 0 (control), 100, 300 or 1000 mg/kg bw per day. Animal survival, clinical condition, detailed physical and arena observations, sensory reactivity, grip strength, motor activity, body weight, food consumption, haematology, blood chemistry, urinalysis, organ weight, macroscopic observations and microscopic histopathological investigations were used to assess potential toxicity. Overall body weight gains (days 1–28) for treated males and females were reduced when compared with those of the controls. This was the result of an initial suppression of weight gain over the first few days of the study. Body weight gains during the recovery (R) period (days R1–R14) for previously treated animals were similar to those of controls. Food consumption for week 1 was reduced for males receiving 300 or 1000 mg/kg bw per day. Food intake for these animals remained slightly reduced for weeks 2–4 of treatment (but not dose related) and for previously treated males in weeks R1 and R2. The appearance and behaviour of the animals were unaffected by treatment. Haematological investigations on day 29 of treatment revealed slightly reduced haemoglobin concentration in males and females receiving 1000 mg/kg bw per day. Platelet counts were increased for males receiving 100, 300 or 1000 mg/kg bw per day. Activated partial thromboplastin time was prolonged for males receiving 1000 mg/kg bw per day. Investigations on day 15 of the recovery period revealed that the mean haemoglobin concentration for previously treated males and the haematocrit, haemoglobin and red blood cell values for previously treated females were still reduced. Biochemical investigations on day 29 of treatment revealed reduced alkaline phosphatase activity for all treated

Table 6. Results of short-term studies of toxicity for aliphatic and aromatic amides used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOAEL (mg/kg bw per day)	Reference
2008	*N*-(2-(Pyridin-2-yl)-ethyl)-3-*p*-menthanecarboxamide	Rat; M, F	3/10	Diet	28	100[c]	Chase (2006)
2008	*N*-(2-(Pyridin-2-yl)-ethyl)-3-*p*-menthanecarboxamide	Rat; M, F	3/16	Diet	28	10	Eapen (2007)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Rat; M, F	2/20[d] 1/30[e]	Diet	91	300	Eapen (2006)
1768	*N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide	Dog; M, F	3/8	Oral	90	30	Hopson (2008)

F, female; M, male

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

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

[c] Lowest dose tested. A true NOAEL could not be established owing to the presence of fatty vacuolation in the liver at all dose levels.

[d] For dose levels of 100 and 300 mg/kg bw per day.

[e] For dose level of 1000 mg/kg bw per day.

female groups and reduced aspartate aminotransferase activity for females receiving 300 or 1000 mg/kg bw per day. Bilirubin levels were reduced for males and females receiving 300 and 1000 mg/kg bw per day. Cholesterol and triglyceride concentrations were increased for all treated male groups and females receiving 300 or 1000 mg/kg bw per day. In addition, females receiving 1000 mg/kg bw per day had increased glucose values. Sodium and phosphorus concentrations were reduced for males receiving 1000 mg/kg bw per day, calcium concentrations were increased for males and females receiving 300 or 1000 mg/kg bw per day and increased potassium levels were recorded for both sexes at 1000 mg/kg bw per day. Total protein concentration, mainly due to globulin, was slightly increased in females receiving 300 or 1000 mg/kg bw per day, resulting in a corresponding decrease in albumin to globulin ratio. Investigations on day 15 of the recovery period revealed that, with the exception of triglyceride values in females (which did show a good degree of resolution), the above-mentioned differences were no longer evident. Urinalysis during week 4 revealed reduced protein concentrations and pH levels for males receiving 1000 mg/kg bw per day. These changes were no longer apparent during week 2 of the recovery period.

Liver weights were increased for males and females that received 100, 300 or 1000 mg/kg bw per day, and spleen weights were slightly increased for females that received 300 or 1000 mg/kg bw per day. At the end of the recovery period, liver weights for previously treated females were still slightly increased. Dark or enlarged livers were observed for males and females that received 300 or 1000 mg/kg bw per day. Pale areas were also seen in the liver of males and females that received 100, 300 or 1000 mg/kg bw per day. There were no liver changes seen on completion of the 2-week recovery period. Centrilobular hepatocyte hypertrophy was seen in males and females that received 100, 300 or 1000 mg/kg bw per day. For males, this was associated with hepatocyte cytoplasmic fat vacuolation, mainly in the midzonal region. Following the 2-week recovery period, centrilobular hepatocyte hypertrophy (minimal) was recorded in one out of five males previously receiving 1000 mg/kg bw per day, and increased hepatocyte cytoplasmic vacuolation (minimal or slight) was recorded in two out of five. For females, there was no evidence of centrilobular hepatocyte hypertrophy following the 2-week recovery period. Follicular cell hypertrophy in the thyroid gland was seen in males and females that received 100, 300 or 1000 mg/kg bw per day. Following the 2-week recovery period, follicular cell hypertrophy (minimal) was noted in two out of five males previously receiving 1000 mg/kg bw per day. There was no evidence of follicular cell hypertrophy in females following the 2-week recovery period.

In summary, dietary administration of *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide to CD rats for 4 weeks at doses of 100, 300 and 1000 mg/kg bw per day was generally well tolerated, although treatment-related changes were detected in the liver and thyroid. The follicular cell hypertrophy in the thyroid at 100, 300 and 1000 mg/kg bw per day is considered secondary to the liver hepatocyte hypertrophy. The majority of changes present at termination had resolved or showed partial recovery after a 2-week period without treatment. However, owing to the presence of fatty vacuolation in the liver at all dose levels, a NOAEL could not be established in this study (Chase, 2006).

In a second study at lower dose levels, three groups of CD rats (eight of each sex per group) received *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide via the diet at doses of 10, 50 or 300 mg/kg bw per day. A control group of eight male and five female rats received basal diet throughout the treatment period. During the study, body weight, food consumption, clinical condition, detailed physical and arena observations, sensory reactivity, grip strength, motor activity, haematology, blood chemistry, special chemistry (triiodothyronine [T_3], thyroxine [T_4], thyroid stimulating hormone [TSH], reverse T_3), urinalysis, organ weight, macroscopic observations and microscopic histopathological investigations were conducted and subsequently used to assess potential toxicity. All rats survived to the scheduled necropsy. There were no clear differences in final body weights between groups. There were no treatment-related effects on haematological parameters. However, some statistically significant ($P < 0.05$ or $P < 0.01$ using Dunnett's test) differences were observed when the control and treated groups were compared. These findings included lower absolute monocyte counts in the 10 and 50 mg/kg bw per day group males; however, these group mean differences were not considered to be treatment related, because the values did not show a dose-related response and were not of

a magnitude that would be considered to be toxicologically significant. Treatment-related changes in serum chemistry parameters found on day 28 included higher total protein, albumin and globulin in the 300 mg/kg bw per day group males; higher cholesterol in the 300 mg/kg bw per day group males and females; lower triglycerides in the 300 mg/kg bw per day group males; higher total T_3 in the 300 mg/kg bw per day group males and females and the 50 mg/kg bw per day group females; and higher TSH in the 50 and 300 mg/kg bw per day group males. There were no treatment-related effects on urinalysis parameters that were considered to be toxicologically significant.

Macroscopic changes that could be attributed to treatment were not observed during the primary necropsy. Upon histopathological examination, one of the eight males in the 300 mg/kg bw per day group had mild centrilobular hepatocellular hypertrophy. Males and females in the 300 mg/kg bw per day group had minimal or mild follicular cell hypertrophy of the thyroid gland. One male in the 50 mg/kg bw per day group had mild follicular cell hypertrophy of the thyroid gland.

In conclusion, dietary administration of *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide to CD rats for 4 weeks at doses of 10, 50 and 300 mg/kg bw per day was generally well tolerated. Treatment-related changes were detected in the liver and thyroid. The follicular cell hypertrophy in the thyroid at 50 and 300 mg/kg bw per day is considered to be associated with the liver hepatocyte hypertrophy. Therefore, the NOAEL was determined to be 10 mg/kg bw per day under the conditions of this study (Eapen, 2007).

(ii) N-p-*Benzeneacetonitrile menthanecarboxamide (No. 2009)*

In a 90-day subchronic toxicity study, male and female Crl:CD(SD) rats were fed a diet providing *N-p*-benzeneacetonitrile menthanecarboxamide at doses of 0 (control), 100, 300 or 1000 mg/kg bw per day for 90 consecutive days. Animal survival, clinical condition, body weight, food consumption, haematology, blood chemistry, urinalysis, organ weight, macroscopic observations and microscopic histopathological investigations were used to assess potential toxicity. All male and female rats survived until the scheduled primary and recovery necropsies. The females administered 1000 mg/kg bw per day exhibited a slightly higher mean methaemoglobin value at study week 13. This minor change was not believed to be adverse owing to the very low magnitude of the difference, its occurrence within the reference range (0.0–1.6%) and the complete recovery by study week 17. There were no other treatment-related effects on haematology data in males or females. However, some statistically significant ($P < 0.05$ or 0.01 using Dunnett's test) differences were observed when the control and treatment groups were compared. These findings included haemoglobin, monocyte percentage, large unstained cell percentage and absolute counts in the males at study week 17; absolute monocyte counts in the 1000 mg/kg bw per day group males; haemoglobin and haematocrit in treated female groups; red cell count in the 100 mg/kg bw per day group females; and mean corpuscular volume, absolute neutrophil count, absolute monocyte count and large unstained cell count in the females at study week 17. These group mean differences were not considered to be treatment related, because the values did not

show a dose-related response and were of a magnitude that was not considered to be toxicologically significant.

Cholesterol and potassium levels were slightly higher in the 300 and 1000 mg/kg bw per day group males at study week 13, but the increases were not considered to be toxicologically significant. Except for aspartate aminotransferase activity in the 300 mg/kg bw per day group females, alanine and aspartate aminotransferase activities were statistically significantly lower in all test article–treated female groups at study week 13. Triglyceride levels were statistically significantly lower at study week 13 in the 300 and 1000 mg/kg bw per day group females. The changes in aspartate aminotransferase and alanine aminotransferase activities and triglyceride levels were not believed to be adverse, as slight lowering of these values is of no known toxicological importance. All of the aforementioned serum chemistry changes recovered by study week 17. There were no other treatment-related effects on serum chemistry end-points. However, some statistically significant ($P < 0.05$ or 0.01 using Dunnett's test) differences were observed when the control and treatment groups were compared. These findings included aspartate aminotransferase activity in the 300 mg/kg bw per day group males; phosphorus and sodium concentrations in the males at study week 17; and albumin to globulin ratio in the females at study week 17. These group mean differences were not considered to be test article related, because the values showed an effect at lower but not higher doses or occurred only at the recovery interval. There were no treatment-related effects or statistically significant findings for urinalysis end-points.

There were no organ weight changes in the spleen of rats from the 90-day study. However, liver weights (relative to body weight) in male and female rats administered 300 or 1000 mg/kg bw per day were marginally increased in a statistically significant manner, probably associated with hepatic enzyme induction. At the primary necropsy, absolute brain weight was lower in female rats in the 100 mg/kg bw per day group only. Following the recovery period, heart weight (relative to body weight) of male rats in the 1000 mg/kg bw per day group was higher, whereas the absolute heart and relative (to body and brain weights) heart weight values were lower in female cohorts. In addition, these females also exhibited lower values for the ovary (absolute) and thymus (absolute and relative to body or brain weight) weights relative to the control group. However, these differences in absolute weights and relative to body or brain weight ratios were discordant, or the dose association was incoherent with no histological correlates; thus, these organ weight changes were considered to be of no toxicological significance. There were no treatment-related histological changes in rats examined after the primary and recovery necropsies. Histological changes encountered were considered to be incidental findings, manifestations of spontaneous diseases or related to some aspect of experimental manipulation other than administration of the test article. There was no treatment-related alteration in the incidence, severity or histological character of those incidental and spontaneous tissue alterations.

In conclusion, administration of *N-p*-benzeneacetonitrile menthanecarboxamide via the diet to Crl:CD(SD) rats at doses of 100, 300 or 1000 mg/kg bw per day for 90 days was well tolerated with no clinical signs of toxicity or mortality. Based on clinical pathology, organ weight and morphological pathology data, the NOAEL was 300 mg/kg bw per day in this study (Eapen, 2006).

(iii) N*1-(2,4-Dimethoxybenzyl)*-N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768)*

In a 90-day oral toxicity study, Beagle dogs (four of each sex per group) were provided a daily oral capsule delivering 0, 10, 30 or 100 mg/kg bw. Parameters recorded included survival, clinical observation, body weight, food consumption, ophthalmic and electrocardiogram examinations, clinical pathology, organ weights and macroscopic and microscopic pathology.

There were no treatment-related changes in clinical observations, body weight, food consumption, electrocardiogram examination, haematology, coagulation or urine chemistry parameters. The 100 mg/kg bw per day female groups showed a decrease in mean body weight relative to controls. One male in the 100 mg/kg bw per day group was found moribund on day 15. He presented ocular opacity, body weight loss and hypoactive behaviour on day 14. On day 15, the condition had deteriorated, with the addition of a jaundiced appearance. The animal was euthanized and at necropsy showed hepatotoxicity, evidenced by hepatocellular degeneration/necrosis, bile stasis and a neutrophilic infiltrate of the liver. Another male in the high intake group showed similar ocular opacity on day 15 and was removed from treatment for 4 days while blood samples were collected and analysed. The clinical pathology data for both animals indicated elevated alanine aminotransferase and alkaline phosphatase activities and total bilirubin levels. The second animal's condition improved, with no recurrence of the clinical signs, and dosing resumed on day 19. Liver weight parameters were increased in the males (significantly) and females given 100 mg/kg bw per day and females given 30 mg/kg bw per day compared with controls. Microscopically, moderate perivascular extramedullary granulopoiesis (males only) or increased vacuolation of hepatocytes may have contributed to the increased liver weight in these dose groups. A treatment relationship is possible for the perivascular extramedullary granulopoiesis but is less certain with regard to the vacuolation.

The NOAEL was 30 mg/kg bw per day based on the adverse liver findings in the 100 mg/kg bw male group and unscheduled death (Hopson, 2008).

(c) Genotoxicity studies

In vitro and in vivo genotoxicity testing has been performed on five flavouring agents in this group. The results of these studies are summarized in Table 7 and described below.

Table 7. Results of genotoxicity studies with the aliphatic and aromatic amines and amides used as flavouring agents

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
In vitro						
2005	*N*-Ethyl-2,2-diisopropylbutanamide	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	25, 50, 100, 250, 500, 1000, 2500 and 5000 μg/plate	Negative[a]	Wells (2006)
2005	N-Ethyl-2,2-diisopropylbutanamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100, 250, 500, 1000, 2500 and 5000 μg/plate	Negative[a,b,c]	Wells (2006)
2005	*N*-Ethyl-2,2-diisopropylbutanamide	Reverse mutation	*Escherichia coli* WP2 *uvr*A pKM101	25, 50, 100, 250, 500, 1000, 2500 and 5000 μg/plate	Negative[a]	Wells (2006)
2005	*N*-Ethyl-2,2-diisopropylbutanamide	Reverse mutation	*E. coli* WP2 *uvr*A pKM101	100, 250, 500, 1000, 2500 and 5000 μg/plate	Negative[a,b,c]	Wells (2006)
2006	Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	10, 31.6, 100, 316 and 1000 μg/plate	Negative[a,d]	August (2007)
2006	Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	10, 31.6, 100, 316 and 1000 μg/plate	Negative[a,b,d]	August (2007)
2007	(±)-*N*-Lactoyl tyramine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100, 333, 1000, 3330 and 5000 μg/plate	Negative[a]	Sikkelerus (2005)

Table 7 (contd)

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
2007	(±)-*N*-Lactoyl tyramine	Reverse mutation	*E. coli* WP2 *uvr*A	3, 10, 33, 100, 333, 1000, 3330 and 5000 μg/plate	Negative[a]	Sikkelerus (2005)
2007	(±)-*N*-Lactoyl tyramine	Forward mutation	L5178Y TK+/− mouse lymphoma cells	1, 3, 10, 33, 100, 333, 1000 and 1952 μg/ml	Negative[a,e]	Buskens (2005)
2007	(±)-*N*-Lactoyl tyramine	Forward mutation	L5178Y TK+/− mouse lymphoma cells	1, 3, 10, 33, 100, 333, 1000 and 1952 μg/ml	Positive[a,f,g]	Buskens (2005)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	5, 15, 50, 150, 500, 1500 and 5000 μg/plate	Negative[a,h]	May (2007)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	50, 150, 500, 1500 and 5000 μg/plate	Negative[a,b,h]	May (2007)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Reverse mutation	*E. coli* WP2 *uvr*A pKM101	50, 150, 500, 1500 and 5000 μg/plate	Negative[a,h]	May (2007)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Reverse mutation	*E. coli* WP2 *uvr*A pKM101	50, 150, 500, 1500 and 5000 μg/plate	Negative[a,b,h]	May (2007)
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Chromosomal aberration	Human lymphocytes	260, 280 and 300 μg/ml[i] 100, 200 and 300 μg/ml[j]	Negative	Mason (2007)

Table 7 (contd)

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
2008	*N*-(2-(Pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide	Chromosomal aberration	Human lymphocytes	25, 50 and 160 µg/ml[k]	Negative	Mason (2007)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate	Negative[a]	Sokolowski (2004)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33, 100, 333, 1000, 2500 and 5000 µg/plate	Negative[a,b]	Sokolowski (2004)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Reverse mutation	*E. coli* WP2 *uvr*A pKM101	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate	Negative[a]	Sokolowski (2004)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Reverse mutation	*E. coli* WP2 *uvr*A pKM101	33, 100, 333, 1000, 2500 and 5000 µg/plate	Negative[a,b]	Sokolowski (2004)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Chromosomal aberration	Human lymphocytes	746.08, 1492.15 and 2984.3 µg/ml[i] 373.04, 1492.15 and 2984.3 µg/ml[j]	Negative	Bowen (2006)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Chromosomal aberration	Human lymphocytes	46.63, 186.52 and 1492.15 µg/ml	Negative[k]	Bowen (2006)

Table 7 (contd)

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
In vivo						
2007	(±)-*N*-Lactoyl tyramine	Micronucleus induction	NMRI BR mouse bone marrow cells	500, 1000 and 2000 mg/kg bw per day[l]	Negative[m]	Sikkelerus (2006)
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	Micronucleus induction	CD-1 mouse bone marrow cells	500, 1000 and 2000 mg/kg bw per day[l]	Negative[n]	Pritchard (2006)

[a] With and without metabolic activation.
[b] Preincubation method.
[c] Reduced background lawns were observed at concentrations of 1000 μg/plate and higher.
[d] Precipitation was observed at 1000 μg/plate.
[e] Three hours of treatment.
[f] Twenty-four hours of treatment.
[g] Cytotoxicity was observed at 88% and 100% at 1000 μg/ml and 1952 μg/ml, respectively.
[h] Cytotoxicity was observed at 5000 μg/plate.
[i] These concentrations were tested without S9 metabolic activation and with 3 h of treatment and 18 h of recovery.
[j] These concentrations were tested with S9 metabolic activation, 3 h of treatment and 18 h of recovery.
[k] These concentrations were tested without S9 metabolic activation and with 21 h of treatment.
[l] Administered via gavage.
[m] Bone marrow was harvested at 24 and 48 h.
[n] Bone marrow was harvested at 24 h.

(i) In vitro

In reverse mutation assays with *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2 *uvrA*, there was no evidence of genotoxicity with *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide (No. 2006), (±)-*N*-lactoyl tyramine (No. 2007), *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008) or *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) at concentrations of up to 5000 μg/plate, with and without S9 activation (Sokolowski, 2004; Sikkelerus, 2005; Wells, 2006; August, 2007; May, 2007).

In two independent forward mutation assays with L5178Y mouse lymphoma cells, there was no evidence of genotoxicity with (±)-*N*-lactoyl tyramine at concentrations of 1, 3, 10, 33, 100, 333, 1000 and 1952 μg/ml, with and without metabolic activation. In the first experiment, the incubation time was 3 h. No toxicity was observed, and (±)-*N*-lactoyl tyramine did not induce a significant increase in the mutation frequency at the thymidine kinase (*TK*) locus, in the presence or absence of metabolic activation. In the second experiment, the incubation times were 24 h and 3 h in the absence and presence of metabolic activation, respectively. With metabolic activation (3 h incubation time), no toxicity was observed in the mouse lymphoma cells, and (±)-*N*-lactoyl tyramine did not induce a significant increase in the mutation frequency at the *TK* locus. After incubation for 24 h in the absence of metabolic activation, (±)-*N*-lactoyl tyramine did induce a dose-related increase in the mutant frequency at the *TK* locus at the highest test conditions in which there were viable cell colonies (1000 μg/ml). However, cytotoxicity was observed to be 88% at 1000 μl/ml and approached 100% when cells were subjected to the highest test level of 1952 μg/ml. It is concluded that under these experimental conditions, (±)-*N*-lactoyl tyramine was non-mutagenic in this in vitro experiment (Buskens, 2005).

In a standard chromosomal aberration assay when human lymphocytes were cultured in the presence of *N*-(2-pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide (No. 2008) at 0, 25, 50, 100, 160, 200, 220, 260, 280 and 300 μg/ml with and without S9 metabolic activation, there was no evidence of genotoxicity (Mason, 2007). No genotoxicity was reported in a similar chromosomal aberration assay when human lymphocytes were cultured in the presence of *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) at 0, 46, 190, 370, 750, 1500 or 3000 μg/ml with and without S9 metabolic activation (Bowen, 2006).

(ii) In vivo

In a standard micronucleus bone marrow assay, groups of five male NMRI BR mice were administered (±)-*N*-lactoyl tyramine (No. 2007) at 0 (negative or positive control), 500, 1000 or 2000 mg/kg bw per day via a single oral (gavage) dose. Bone marrow of the groups treated with (±)-*N*-lactoyl tyramine was sampled 24 h or 48 h (highest dose only) after dosing. Bone marrow of the negative and positive control groups was harvested 24 h and 48 h after dosing, respectively. No increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the polychromatic erythrocytes of the bone marrow of animals treated

with (±)-*N*-lactoyl tyramine. The incidence of micronucleated polychromatic erythrocytes in the bone marrow of all negative control animals was within the historical negative control data range. Cyclophosphamide, the positive control substance, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes. It is concluded that (±)-*N*-lactoyl tyramine is not clastogenic in the micronucleus test under the experimental conditions of this in vivo test procedure (Sikkelerus, 2006).

In a similar micronucleus bone marrow assay, groups of seven male CD-1 mice were administered *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) at 0 (negative or positive control), 500, 1000 or 2000 mg/kg bw per day via gavage on two occasions approximately 24 h apart. Bone marrow smears were obtained from seven male animals in the negative control and in each of the treatment groups 24 h after the second dose, and bone marrow smears were also obtained from five animals in the positive control group 24 h after a single dose (mitomycin C; 12 mg/kg bw). No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes and no substantial decreases in the proportion of polychromatic erythrocytes were observed in mice treated with *N*-*p*-benzeneacetonitrile menthanecarboxamide at any treatment level, compared with vehicle control values. The positive control compound produced significant increases in the frequency of micronucleated polychromatic erythrocytes. It is concluded that *N*-*p*-benzeneacetonitrile menthanecarboxamide did not show evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes or bone marrow cell toxicity in mice, when administered orally via gavage in this in vivo test procedure (Pritchard, 2006).

(iii) Conclusions for genotoxicity

The five flavouring agents of this group gave consistently negative results in in vitro mutation assays conducted in *S. typhimurium* and *E. coli* with and without metabolic activation. The genotoxicity assays conducted in mammalian cells (i.e. human lymphocytes) with two of the same flavouring agents of this group were also negative. Micronucleus assays conducted in two strains of mice with two of the same flavouring agents were also negative.

(d) Developmental and reproductive toxicity studies

In a teratology study, groups of 6–7 female Sprague-Dawley rats were fed a diet providing *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768) at doses of 0, 10, 30 and 100 mg/kg bw per day for 15 days from day 6 to day 20 during gestation. On day 20, the dams were sacrificed, and gross examination of the females was performed, followed by caesarean section. For each litter, half of the live fetuses were examined for visceral changes and the other half for skeletal abnormalities. In the dams, there were no test material effects on clinical signs, body weight, food consumption or water consumption. There were no gross pathological findings or variations in organ weights when compared with control animals. With respect to the fetuses, there were no differences observed in body weight, placental weight, litter size or external, visceral or skeletal examinations between treatment and control groups. The maternal and fetal NOEL for *N*-(2,4-

dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide was 100 mg/kg bw per day in rats (Tomita, 2007).

In a two-generation reproductive and developmental toxicity study, Sprague-Dawley rats (30 of each sex per group) were fed a diet providing *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768) at doses of 0, 10, 30 and 100 mg/kg bw per day for 10 weeks prior to mating (day 70), throughout the mating process, gestation, lactation and weaning (day 91) and up to sacrifice (day 126) (Weaver, 2007). Parameters evaluated included mortality, clinical signs of toxicity, body weight and body weight gain, food consumption and test article consumption, fertility indices, litter data, gross findings, histopathology of male and female reproductive organs, sperm analysis, ovarian counts, pup weights and landmark data (vaginal opening and preputial separation for F_1 pups).

For the F_0 generation, all animals survived to termination, with no remarkable clinical signs observed. During the first 28 days of the studies, males showed decreases in body weight, body weight gain and food consumption that were most likely attributable to palatability of the test material at such high concentrations. The 100 mg/kg bw per day female group showed a treatment-related decrease in body weights during the premating phase up to day 70. As these changes were less than 10%, they were not considered biologically relevant. There was no evidence of effects on estrous cycle or reproductive performance, pregnancy or litter data (live birth, viability and weaning indices, mean pup weights, vaginal opening or preputial gland separation), the F_0 fertility indices or the macroscopic or microscopic findings in F_0 parents or F_1 pups.

The females in the 100 mg/kg bw per day groups showed increased mean relative uterine weight; however, there were no histopathological findings associated with the increase, so it was not considered relevant to administration of the test material. Also, an increased mean relative liver weight was observed in the 100 mg/kg bw per day male group. No microscopic examination of the liver was performed, so the relevance of this finding is not known. The per cent motility, caudal epididymal sperm count, testicular sperm count, sperm morphology and ovarian primordial follicle count were unaffected by the intake of *N*-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide in the diet.

For the F_0 generation, one high intake level female was found dead on lactation day 0 during delivery. The remaining rats showed no evidence of clinical signs of toxicity. No test article–related effects on mean body weight or body weight gain occurred in males during the premating phase or in females during the gestation, lactation or resting phase, and no effects on mean food consumption in females during the premating, gestation, lactation or resting phase were observed. Prolonged diestrus cycles (longer than 4 days) were observed in 2 out of 30, 9 out of 30, 7 out of 27 and 6 out of 29 females in the 0, 10, 30 and 100 mg/kg bw per day groups, respectively. These rates were not dose related, were not statistically significant and showed no impact on the fertility of the rats.

Pregnancy rates were 93%, 97%, 96% and 86% and delivery rates were 90%, 97%, 96% and 86% for the 0 (control), 10, 30 and 100 mg/kg bw per day groups, respectively. While not statistically significant, the duration of pregnancy

increased with increasing intake level: 21–22 days for control and 21–23 days at 10 and 30 mg/kg bw per day, with a 23-day gestation phase occurring for 1 out of 29 and 3 out of 27 rats, respectively. At 100 mg/kg bw per day, the duration of gestation ranged from 21 to 27 days (1 out of 24 rats each with a gestation phase of 23, 24 and 27 days, respectively). All other litter data were comparable with those of controls. There was no evidence of a test article effect on litter data (live birth, viability and weaning indices, mean pup weights at any dose level) or macroscopic or microscopic findings in F_1 parents or F_2 pups. The 100 mg/kg bw per day females exhibited increased relative uterus weight. This finding, however, did not correlate with any macroscopic or microscopic finding and was found to be not of biological significance.

The per cent motility, caudal epididymal sperm count, testicular sperm count, sperm morphology and ovarian primordial follicle count were not affected by treatment with *N*-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide at a concentration of 100 mg/kg bw per day. A reduction in the mean number of sperm per gram of caudal epididymal tissue in the 100 mg/kg bw per day dietary intake level group, compared with the control of the F_0 generation, was not observed in the F_1 generation. The reduction, therefore, was not considered biologically significant.

The NOAEL is 30 mg/kg bw per day for maternal and paternal toxicity and is based on absence of clinical signs and minimal or no effects on body weight and food consumption at this dose level as well as the single death of a 100 mg/kg bw per day female during delivery. There were no or minimal effects on maternal and paternal parameters at doses of 10 and 30 mg/kg bw per day in F_0 and F_1 rats. The NOAEL for embryo/fetal survival and for growth and development in F_1 and F_2 pups is 100 mg/kg bw per day, as there were no statistically significant decreases in either of these parameters from lactation day 0 through lactation day 21 in either generation. The NOAEL for F_0 and F_1 fertility parameters is 100 mg/kg bw per day for males and 30 mg/kg bw per day for females and is based on the lack of effects on male reproductive tissues in either generation at any dose level tested and the female F_1 generation findings of an extended duration of gestation of up to 27 days at 100 mg/kg bw per day. The 14% reduction in the male fertility index does not appear to be a male reproductive issue based on the sperm analysis data (Weaver, 2007).

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AROMATIC SUBSTITUTED SECONDARY ALCOHOLS, KETONES AND RELATED ESTERS (addendum)

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1. EVALUATION

1.1 Introduction

The Committee was requested to evaluate nine additional flavouring agents that belong to the group of aromatic substituted secondary alcohols, ketones and related esters. This group of nine compounds includes eight ketones (Nos 2040–2045 and 2047–2048) and one diester (No. 2046). The safety of one submitted substance, 2-aminoacetophenone (No. 2043), was not assessed, because the Committee decided that this compound should be evaluated in the future in a group of aliphatic and aromatic amines and amides. The evaluations of the remaining eight were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, Introduction) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

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

Six of the eight flavouring agents (Nos 2040–2042 and 2044–2046) have been reported to occur naturally in various foods and have been detected in honey, milk, tomato, mango, coffee, cloudberry, starfruit, peas, whiskey, papaya, chicken, sherry, beer and white wine (Nijssen, van Ingen-Visscher & Donders, 2009). For No. 2041, the consumption from natural sources is estimated to be 7 times the volume used as a flavouring agent.

1.2 Assessment of dietary exposure

The total annual volumes of production of the eight aromatic substituted secondary alcohols, ketones and related esters are approximately 5 kg in Europe, 52 kg in the USA and 2 kg in Japan (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009). Approximately 80% and 96% of the total annual volumes of production in Europe and the USA, respectively, are accounted for by 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048). In Japan, approximately 50% of the total annual volume of production is accounted for by 4-hydroxyacetophenone (No. 2040).

The estimated dietary exposures for each flavouring agent, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The estimated daily intake is greatest for dihydrogalangal acetate (No. 2046) (10 000 µg, calculated using the SPET obtained from six different food categories). For the other flavouring agents, the estimated daily intakes ranged from 0.01 to 1600 µg, with the SPET yielding the highest estimates for all. Reported annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

1.3 Absorption, distribution, metabolism and elimination

Aromatic secondary alcohols, ketones and related esters are rapidly absorbed from the gut. Hydrolysis of the esters occurs in the intestine and liver. The aromatic secondary alcohols (and aromatic ketones after reduction to the corresponding secondary alcohols) are then either conjugated with glucuronic acid and excreted primarily in the urine or further oxidized to carboxylic acids, which are excreted mainly as glycine conjugates. Studies on absorption, distribution, metabolism and elimination were considered at the fifty-seventh meeting of the Committee (Annex 1, reference *154*).

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

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/High exposure B-side*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
2-Methoxyacetophenone	2042	579-74-8	No, SPET: 1500	NR	Notes 1, 2, 3 and 4	No safety concern
2-Methylacetophenone	2044	577-16-2	No, SPET: 80	NR	Notes 1 and 4	No safety concern
2-Hydroxy-5-methylacetophenone	2045	1450-72-2	No, SPET: 10	NR	Notes 1 and 2	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/High exposure B-side*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Dihydrogalangal acetate	2046	129319-15-9	Yes, SPET: 10 000	A5. No. The NOEL of 15 mg/kg bw per day for the structurally related substance α-methylbenzyl acetate from an oral toxicity study in rats (Gaunt et al., 1974) is at least 86 times greater than the estimated daily dietary exposure to No. 2046 when used as a flavouring agent.	Notes 1 and 5	Additional data required to complete evaluation
2,3,3-Trimethylindan-1-one	2047	54440-17-4	No, SPET: 25	NR	Notes 1 and 4	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step A5/High exposure B-side*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class III						
4-(3,4-Methylenedioxyphenyl)-2-butanone	2048	55418-52-5	Yes, SPET: 640	Yes. The NOEL of 57 mg/kg bw per day for No. 2048 in a 90-day study in rats (Posternak, Linder & Vodoz, 1969) is at least 5000 times its estimated dietary exposure when used as a flavouring agent.	Notes 1, 2 and 3	No safety concern

bw, body weight; CAS, Chemical Abstracts Service; NOEL, no-observed-effect level; NR, not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

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

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

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

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

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

Table 1 (contd)

Notes:

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

Table 2. Annual volumes of production and dietary exposures for aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]	Consumption ratio[e]
		MSDI[b]		SPET[c]			
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day		
4-Hydroxyacetophenone (2040)				300	5	+	NA
Europe	0.1	0.01	0.0002				
USA	ND	ND	ND				
Japan	1	0.4	0.0059				
3-Hydroxy-4-phenylbutan-2-one (2041)				1 600	27	6	7
Europe	0.1	0.01	0.0002				
USA	0.9	0.1	0.002				
Japan	ND	ND	ND				
2-Methoxyacetophenone (2042)				1 500	25	+	NA
Europe	0.1	0.01	0.0002				
USA	ND	ND	ND				
Japan	ND	ND	ND				
2-Methylacetophenone (2044)				80	1	+	NA
Europe	0.1	0.01	0.0002				
USA	0.1	0.01	0.0002				
Japan	ND	ND	ND				
2-Hydroxy-5-methylacetophenone (2045)				10	0.2	+	NA
Europe	ND	ND	ND				
USA	ND	ND	ND				
Japan	0.1	0.03	0.0005				

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]	Consumption ratio[e]
		MSDI[b]		SPET[c]			
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day		
Dihydrogalangal acetate (2046)				10 000	167	−	NA
Europe	ND	ND	ND				
USA	1	0.1	0.001				
Japan	ND	ND	ND				
2,3,3-Trimethylindan-1-one (2047)				25	0.4	−	NA
Europe	ND	ND	ND				
USA	0.4	0.05	0.001				
Japan	ND	ND	ND				
4-(3,4-Methylenedioxyphenyl)-2-butanone (2048)				640	11	−	NA
Europe	4	0.4	0.007				
USA	50	6.1	0.1				
Japan	0.3	0.1	0.001				
Total							
Europe	5						
USA	52						
Japan	2						

bw, body weight; NA, not available; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

Table 2 (contd)

[b] MSDI (μg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009).
MSDI (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (μg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Quantitative data for the USA reported by Stofberg & Grundschober (1987).

[e] The consumption ratio is calculated as follows:
(annual consumption via food, kg)/(most recent reported volume as a flavouring agent, kg).

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned seven flavouring agents (Nos 2040–2042 and 2044–2047) to structural class I. One flavouring agent (No. 2048) was assigned to structural class III (Cramer, Ford & Hall, 1978).

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

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

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

Step A5. The no-observed-effect level (NOEL) of 15 mg/kg body weight (bw) per day for the structurally related substance, α-methylbenzyl acetate, from an oral study of toxicity in rats (Gaunt et al., 1974) provided a margin of safety of less than 100 in relation to the highest estimated dietary exposure to dihydrogalangal acetate (No. 2046) (SPET = 10 000 μg/day) when used as a flavouring agent. The Committee expressed concern that the reported NOEL was insufficient to accommodate any potential differences in toxicity between No. 2046 and the related substance. The Committee therefore concluded that additional data are required to complete the evaluation of this flavouring agent.

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

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

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

A NOEL for 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) of approximately 57 mg/kg bw per day in a 90-day study in rats was identified (Posternak, Linder & Vodoz, 1969). Groups of 10–16 male and female rats per group were fed a diet formulated to provide intake in excess of 100 times the maximum estimated daily human dietary exposure. The animals were monitored for food intake and body weight. End-points evaluated included haematology, clinical chemistry, organ weights and organ pathology. No adverse effects on any of these parameters were observed. The NOEL provides a margin of safety of more than 5000 in relation to the highest estimated dietary exposure to No. 2048 (SPET = 640 μg/day) when used as a flavouring agent. The Committee therefore concluded that 4-(3,4-methylenedioxyphenyl)-2-butanone would not pose a safety concern at current estimated dietary exposures.

Table 1 summarizes the evaluations of the eight aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents (Nos 2040–2042 and 2044–2048) in this group.

1.5 Consideration of combined intakes from use as flavouring agents

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

Flavouring agents in this group with the highest intakes and with the common metabolite α-methylbenzyl alcohol (No. 799), which is in structural class I, are Nos 799, 801, 804, 807 and 810. In the unlikely event that these were to be consumed

concurrently on a daily basis, the estimated combined intakes in Europe, the USA and Japan would be 395, 753 and 76 µg/person per day, respectively, which would not exceed the threshold of concern (i.e. 1800 µg/person per day for class I).

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

1.6 Consideration of secondary components

One member of this group of flavouring agents, 3-hydroxy-4-phenylbutan-2-one (No. 2041), has a minimum assay value of less than 95%. The secondary component of No. 2041, 4-hydroxy-4-phenylbutan-2-one, is expected to undergo rapid absorption, distribution, metabolism and excretion, sharing the same metabolic fate as the primary substance, and is considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary component of this flavouring agent is summarized in Annex 5.

1.7 Conclusion

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

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

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the additional key data relevant to the safety evaluation of the group of eight aromatic substituted secondary alcohols, ketones and related esters, which includes seven ketones (Nos 2040–2042 and 2045–2048) and one diester (No. 2046). These eight flavouring agents are additions to a group of 38 flavouring agents evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposure estimates were made using both the MSDI approach and the SPET.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

No additional information on absorption, distribution, metabolism or elimination of the flavouring agents in this group was identified since the previous evaluation.

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for two of the additional flavouring agents in this group (Table 3). For 2,3,3-trimethylindan-1-one (No. 2047), LD_{50} values in rats were reported as >2000 mg/kg bw (Arcelin, 2004). For 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048), the LD_{50} value in rats was found to be 4000 mg/kg bw (Wohl, 1974).

Table 3. Results of acute oral toxicity studies for aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
2047	2,3,3-Trimethylindan-1-one	Rats; F	>2000	Arcelin (2004)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Rats; NR	4000	Wohl (1974)

F, female; NR, not reported

These studies demonstrate that the acute oral toxicity of aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents is low.

(b) Short-term studies of toxicity

A 90-day study with 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) and a 28-day gavage test with 2,3,3-trimethylindan-1-one (No. 2047) were reported. Further short-term studies of toxicity were also published on benzophenone (No. 831) during the interim period between the original submission of the group of aromatic substituted secondary alcohols, ketones and related esters at the fifty-seventh meeting of the Committee and preparation of the background information for this group of additional flavouring agents. These studies have been summarized in Table 4 and are described below.

(i) 2,3,3-Trimethylindan-1-one (No. 2047)

In a 28-day range-finding study, Wistar rats (five of each sex per dose) were administered 2,3,3-trimethylindan-1-one at doses of 50, 150 and 450 mg/kg bw per day via gavage (Fischer, 2007). The rats were given drinking-water ad libitum. Periodic observations of the animals in their cages were performed to assess

Table 4. Results of short-term studies of toxicity for aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/no. per group[b]	Route	Duration (days)	NOAEL[c]/NOEL[d] (mg/kg bw per day)	Reference
2047	2,3,3-Trimethylindan-1-one	Rat; M, F	3/10	Gavage	28	50[c]	Fischer (2007)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Rat; M, F	1/20–32	Diet	90	57 (M)[d] 66 (F)[d]	Posternak, Linder & Vodoz (1969)
831	Benzophenone	Mouse; M, F	5/20	Diet	90	200 (M)[c] 270 (F)[c]	NTP (2000)
831	Benzophenone	Rat; M, F	5/20	Diet	90	75 (M)[c] 80 (F)[c]	NTP (2000)

F, female; M, male

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

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

[c] No-observed-adverse-effect level.

[d] No-observed-effect level.

mortality and severe signs of toxicity. Locomotor activity and grip strength were assessed during week 4. Following the 28-day treatment period, there was a 14-day treatment-free period. Clinical analyses, including urinalysis, haematology and plasma analysis, were performed following the treatment and recovery periods. All animals were killed, necropsied and examined postmortem. Histological examinations were performed on organs, tissues and gross lesions from all control and high-dose animals. Histological examinations were performed on liver, adrenals, vagina, uterus and all gross lesions from low- and mid-dose animals.

All animals survived to termination without any signs of toxicity. In males, a moderate decrease in absolute food consumption was recorded in the 450 mg/kg bw per day group, and a slight decrease in relative food consumption (based on body weight) was recorded in the 150 and 450 mg/kg bw per day groups, which resulted in slight to moderate decreases in body weights in males of these dose groups. These findings were considered not to be adverse owing to their reversibility after the recovery period. No test item–related changes in food consumption, body weight or body weight gain were noted in males treated with 50 mg/kg bw per day or in females at any dose level. Dose-related decreases in red blood cell counts, haemoglobin and haematocrit were noted in females treated with 50, 150 and 450 mg/kg bw per day. Consequently, the relative and absolute reticulocyte counts were increased for females in this group. All haematological findings were considered by the author not to be adverse owing to their reversibility after the recovery period. Increased triglyceride concentrations and γ-glutamyl transferase activities were noted in animals administered 450 mg/kg bw per day. Males administered 450 mg/kg bw per day and females administered 150 and 450 mg/kg bw per day showed increases in total blood protein and globulin levels and decreases in albumin to globulin ratios. These changes disappeared during the recovery period and were considered by the author to be of no toxicological relevance.

No test item–related macroscopic findings were observed at the end of the treatment period. Microscopic changes observed were hepatocyte hypertrophy, adrenal cell hypertrophy and balloonated cells of the vaginal mucosa, leading to a microcystic appearance of animals treated with 150 and 450 mg/kg bw per day. All of the microscopic changes disappeared during the recovery period and were not considered by the author to be of toxicological relevance. There were dose-dependent increases in absolute and relative liver weights in male and female rats in all treatment groups, as well as increases in adrenal weights in males treated at all dose levels and in females in the 150 and 450 mg/kg bw per day groups. The liver and adrenal weight changes were considered to be adaptive and stress responses, respectively, and were not considered by the author to be toxicologically relevant. In females treated at 150 and 450 mg/kg bw per day, there were dose-related increases in absolute and relative spleen weights. These effects were considered by the author to be secondary to anaemia and were not toxicologically relevant. Females administered 450 mg/kg bw per day showed dose-related decreases in absolute and relative ovary weights, but no microscopic changes were observed, and the decreases were not considered by the author to be toxicologically relevant (Fischer, 2007).

Based on the above findings, the author found the no-observed-adverse-effect level (NOAEL) to be 450 mg/kg bw per day (Fischer, 2007). However, because of the microcystic changes observed in the vagina at 150 mg/kg bw per day, the Committee considered the NOAEL to be 50 mg/kg bw per day.

(ii) 4-(3,4-Methylenedioxyphenyl)-2-butanone (No. 2048)

Groups of 20–32 male and female Charles River CD rats (10–16 of each sex) were fed diets containing 4-(3,4-methylenedioxyphenyl)-2-butanone for a period of 90 days at concentrations resulting in average dietary intakes of 57 and 66 mg/kg bw per day for males and females, respectively. Throughout the study period, body weights and food consumption were monitored on a weekly basis, and the food conversion efficiency was subsequently calculated. Blood samples were taken at weeks 7 and 13 for haematological and clinical chemistry analysis. At study completion, animals were necropsied and subjected to gross examination. The kidneys and liver were excised and weighed, and microscopic examination was performed on a wide range of major organs. In comparison with the basal diet control group, no significant variations were observed in any of the toxicological parameters evaluated. Furthermore, no compound-related histopathological abnormalities were reported (Posternak, Linder & Vodoz, 1969). The NOAELs were considered to be 57 and 66 mg/kg bw per day in males and females, respectively, the only doses tested.

(iii) Benzophenone (No. 831)

Mice

In a 14-week study, benzophenone was provided to groups of B6C3F1 mice (10 of each sex per dose) in the feed at concentrations of 0, 1250, 2500, 5000, 10 000 or 20 000 mg/kg. These concentrations in the feed were estimated by the study authors to provide a daily intake of 0, 200, 400, 800, 1600 or 3300 mg/kg bw for males and 0, 270, 540, 1000, 1900 or 4200 mg/kg bw for females. All animals were observed twice daily, clinical signs were recorded twice weekly and body weights were measured weekly. Necropsies were performed on all animals, including those that died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions, which were examined histopathologically. Histological examinations were performed on prepared tissues, including the adrenal gland, bone and marrow, brain, clitoral gland, oesophagus, eye, gallbladder, heart, large intestine, small intestine, kidney, liver, lung, lymph nodes, mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular stomach), testis, thymus, thyroid gland, trachea, urinary bladder and uterus from all animals in the control, 10 000 mg/kg feed and 20 000 mg/kg feed groups, as well as test animals that died during the study. Owing to the potential photoionizing properties of benzophenone, complete histopathologial examination of the eyes, including the lens, retina and other ocular structures, was performed. Microsomal cytochrome P450 content of the liver was also measured.

One male in the 1250 mg/kg feed group was accidentally killed on day 26. Four males in the 20 000 mg/kg feed group died in week 1, and one male and one female in the 20 000 mg/kg feed group died in week 2. Two males were removed from the study at week 10, three males were removed at week 11 and three females were removed at week 12 in the 20 000 mg/kg feed groups owing to significantly reduced body weights compared with controls. Body weights of the mice in the 5000 and 10 000 mg/kg feed groups were significantly reduced, as were body weights in the surviving females of the 20 000 mg/kg feed group. Clinical observations included lethargy for both males and females in the 20 000 mg/kg feed groups. Food consumption for the 20 000 mg/kg feed groups was reduced compared with controls. The authors attributed this to the lack of palatability of the diet. No haematology or clinical chemistry was performed at week 14 for males in the 20 000 mg/kg feed group. The males in the 5000 and 10 000 mg/kg feed groups showed anaemia, with minimal decreases in haematocrit values, haemoglobin levels and erythrocyte counts. At intakes of 20 000, 10 000 and 5000 mg/kg feed, females showed erythrocytosis. The authors found this consistent with haemo-concentration caused by dehydration, which is supported by reported increases in albumin and total protein concentrations. Total bile salt concentrations and sorbitol dehydrogenase activities were increased in all treated females and in males in the 2500 mg/kg feed or higher groups. The kidney weights of males exposed to 2500 mg/kg feed or higher and the liver weights of all exposed males were significantly increased. The absolute and relative liver weights of all exposed females except those in the 20 000 mg/kg feed group were significantly increased. Females in the 2500 and 10 000 mg/kg feed groups showed a significant increase in absolute kidney weights, and females exposed to 2500 mg/kg feed or higher showed a significant increase in relative kidney weights. The absolute and relative thymus weights of females at the 20 000 mg/kg feed exposure level and absolute thymus weights at the 5000 and 10 000 mg/kg feed levels were significantly lower than those of controls. Other organ weight differences were considered to be due to the overall lower body weights of the exposed mice. No significant changes in the ocular structures or tissues were observed.

There were no exposure-related gross lesions at necropsy. Significant histopathological findings were limited to centrilobular hypertrophy of hepatocytes, which corresponded to an increase in liver weights at all exposure levels in both sexes. No microscopic finding supported the increased kidney weights. Males in the 10 000 mg/kg feed exposure group showed significantly greater hepatic cytochrome P450 activity, which was considered by the authors to be an adaptive response. Ethoxyresorufin de-ethylase activities and pentoxyresorufin dealkylase activities were significantly increased for all exposed male groups compared with controls (NTP, 2000).

Based on the increases observed in kidney weights of both males and females exposed to diets containing 2500 mg of benzophenone per kilogram of feed for 14 weeks, the Committee considered the NOAELs to be 200 mg/kg bw per day (males) and 270 mg/kg bw per day (females).

Rats

In another 14-week study, benzophenone was provided to groups of F344/N rats (10 of each sex per dose) in the feed at concentrations of 0, 1250, 2500, 5000, 10 000 or 20 000 mg/kg. These concentrations in the feed are estimated by the study authors to provide a daily intake of 0, 75, 150, 300, 700 or 800 mg/kg bw for males and 0, 80, 160, 300, 700 or 1000 mg/kg bw for females. The protocol was essentially the same as in the mouse study described above.

One female in the 20 000 mg/kg feed exposure group died on day 12. Owing to the significant decrease in body weights for the remaining females in the 20 000 mg/kg feed group, they were removed from the study at week 6. Body weights for males at 2500 mg/kg feed and higher and all female exposure groups were significantly reduced compared with those of controls. Clinical findings were noted as lethargy for females in the 20 000 mg/kg feed group and thinness in males in the 10 000 mg/kg feed group. Two males in the 20 000 mg/kg feed group had prolapsed penises. Males and females at the 20 000 mg/kg feed exposure level consumed significantly reduced amounts of food compared with controls and other exposure groups. Owing to mortality and early removal from the study, no 14-week clinical chemistry or haematology data were collected for animals at the 20 000 mg/kg feed exposure level.

Haematology data revealed a number of findings. Anaemia was observed in males and females at 2500 mg/kg feed and higher, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte levels. In male rats, increased reticulocyte counts were observed, indicating an erythropoietic response. Platelet count decreases were seen in males in the 10 000 mg/kg feed exposure group and in females in the 5000 and 10 000 mg/kg feed exposure groups. Alanine aminotransferase activity levels were increased for females in the 10 000 mg/kg feed group and in males in the 20 000 mg/kg feed group at 14 weeks and in females in the 20 000 mg/kg feed group at 6 weeks. Sorbitol dehydrogenase activity was increased for females in the 10 000 mg/kg feed group. Minimal to marked increases in bile salts and minimal to mild decreases in alkaline phosphatase activity were reported at various time points in the study for all exposure groups. Hyperproteinaemia was persistent at week 14 for all exposed females and was accompanied by hyperalbuminaemia, which is indicative of dehydration in animals. Plasma creatinine concentration was decreased minimally at 5000 mg/kg feed and higher for males and females at all time points in a concentration-dependent manner. Creatinine levels can be related to muscle mass in rats (Finco, 1989; Ragan, 1989), and rats in the higher exposure groups weighed less and had lower muscle mass than controls.

Kidney and liver weights of all exposed male and female rats were significantly greater than those of controls, with the exception of the kidney weights from females in the 1250 mg/kg feed group. The absolute heart and thymus weights of males in the 10 000 mg/kg feed group and the absolute thymus weights of females in the 5000 and 10 000 mg/kg feed exposure groups were significantly less than those of controls. Other organ weight differences were attributed to lower body weights of the exposed animals.

At necropsy, exposed males had significantly smaller seminal vesicles when compared with controls. There were no microscopic changes accompanying this gross finding. Increased kidney weights were accompanied by a broad range of microscopic findings. Papillary necrosis, as indicated by acute coagulative necrosis of the distal tips of the renal papillae, occurred in 20% of the 10 000 mg/kg feed and 60% ($P = 0.001$) of the 20 000 mg/kg feed exposure groups. In male rats exposed to 2500 mg/kg feed and higher and females at 10 000 and 20 000 mg/kg feed, well-demarcated, wedge-shaped areas of prominent dilatation were observed. These areas were based at the capsular surface and extended deep into the medulla. Within these areas, tubules were dilated and generally empty, but some contained fine granulated eosinophilic material. Increased incidences of severe focal tubule regeneration were observed in all exposure groups. Foci of tubule regeneration are a component of chronic nephropathy in the ageing rat. Exposure-related increases in liver weights were attributed to hypertrophy or cytoplasmic vacuolization of hepatocytes. All exposure groups of females showed increased centrilobular vacuolization of hepatocytes. Vacuolization was observed in all male exposure groups and indicated by a bubbly appearance in the cytosol. Males in the 20 000 mg/kg feed exposure group showed minimal hyperplasia of immature bile ducts in portal areas extending into adjacent sinusoids. Hypercellularity of the bone marrow in males and females and poorly developed seminiferous tubules in males were observed for the 20 000 mg/kg feed exposure groups. These findings were considered by the authors to be secondary to reduced body weight gain and inanition. No microscopic changes were observed for the eye or any substructures or tissues. Males and females exposed to 2500 and 5000 mg/kg feed and females exposed to 1250 mg/kg feed showed significant increases in liver microsomal cytochrome P450. All exposure groups showed increased levels of pentoxyresorufin dealkylase activity (NTP, 2000).

Based on the haematological findings in male and female rats exposed to benzophenone for 14 weeks in the diet at 2500 mg/kg feed, the Committee considered the NOAELs to be 75 mg/kg bw per day (males) and 80 mg/kg bw per day (females).

(c) Long-term studies of toxicity and carcinogenicity

Long-term studies of toxicity and carcinogenicity were published on benzophenone (No. 831) during the interim period between the original submission of the group of aromatic substituted secondary alcohols, ketones and related esters at the fifty-seventh meeting of the Committee and preparation of the background information for this group of additional flavouring agents. These studies have been summarized in Table 5 and are described below.

(i) Benzophenone (No. 831)

Mice

In a National Toxicology Program (NTP) study, groups of 50 male and 50 female B6C3F1 mice were maintained on diets containing benzophenone at concentrations of 0, 312, 625 or 1250 mg/kg for 105 weeks. The administered

Table 5. Results of long-term studies of toxicity and carcinogenicity for aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOAEL/ NOEL (mg/kg bw per day)	Reference
831	Benzophenone	Mouse; M, F	3/100	Diet	730	<40[c]	NTP (2006)
831	Benzophenone	Rat; M, F	3/100	Diet	730	<15[c]	NTP (2006)

F, female; M, male

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

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

[c] The lowest dose tested. See text for relevance to humans.

dietary levels were estimated to provide a daily intake of 0, 40, 80 and 160 mg/kg bw in male mice and 0, 35, 70 and 150 mg/kg bw in female mice (NTP, 2006). Animals were observed twice daily, and clinical signs were recorded monthly. Body weights were measured weekly for the first 12 weeks and monthly thereafter. Necropsies were performed on all animals, including those that died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions, which were histopathologically examined. Histological examinations were performed on prepared tissues from all control and test animals that died during the study. Organs of the treated rats that were targeted for potential neoplastic and non-neoplastic effects were histopathologically examined.

No significant differences in mortality rates were reported between controls and any of the three test groups of male mice. Although there was a slight decrease in survival in females on the 1250 mg/kg diet, there was no significant difference in survival between the control group and any of the three treatment groups. No significant differences in mean body weights were reported between control and test groups in male mice. Mean body weights of females in the 35, 70 and 150 mg/kg bw per day test groups were less than those of controls after weeks 86, 52 and 37, respectively.

Neoplastic and non-neoplastic lesions associated with the dietary administration of benzophenone at 0, 312, 625 or 1250 mg/kg feed to mice developed principally in the liver in males. There was an increase in the incidence of hepatocellular adenomas in males—control, 11/50 (22%); 40 mg/kg bw per day, 15/50 (30%); 80 mg/kg bw per day, 23/50 (46%); 160 mg/kg bw per day, 23/50 (46%)—that was statistically significant at the two highest dietary levels. The incidence of hepatocellular carcinomas was greater in control males than in any group of test group males—control, 8/50 (16%); 40 mg/kg bw per day, 5/50 (10%); 80 mg/kg bw per day, 6/50 (12%); 160 mg/kg bw per day, 6/50 (12%)—whereas the incidence of hepatoblastomas in test groups was less than 6% and not statistically

different from controls. The incidence of combined hepatocellular adenomas, carcinomas and blastomas in males maintained on the 625 and 1250 mg/kg diets was increased ($P < 0.01$) compared with the control group: control, 18/50 (36%); 40 mg/kg bw per day, 20/50 (40%); 80 mg/kg bw per day, 25/50 (50%); 160 mg/kg bw per day, 29/50 (58%).

Non-neoplastic effects in the liver of males included an increase in clear cell foci, eosinophilic cell foci and mixed cell foci and a significant increase in centrilobular hepatocellular hypertrophy, multinucleated cells and hepatic necrosis in all treatment groups and cystic degeneration in the two highest dietary groups. Chronic active inflammation was observed, but the effect was also prevalent in control animals.

Female mice exhibited a low incidence of non-neoplastic and neoplastic lesions compared with male mice. There was no significant difference in the incidence of combined hepatocellular adenomas and carcinomas: control, 5/50 (10%); 35 mg/kg bw per day, 5/50 (10%); 75 mg/kg bw per day, 10/50 (20%); 150 mg/kg bw per day, 9/50 (18%). The historical incidences of adenomas and combined adenomas and carcinomas for mice on the NTP-2000 diet are 9.6% and 11.8%, respectively; a much lower incidence of non-neoplastic effects was observed in females compared with males. The incidence of eosinophilic cell foci and mixed cell foci was slightly increased over controls. Although hypertrophy of hepatocytes was reported in test groups, there was no significant difference in multinucleated cells, necrosis, inflammation or cystic degeneration between test and control groups. These data support the conclusion that the male mouse liver exhibits increased susceptibility to neoplastic changes compared with the female mouse liver.

A statistically significant ($P = 0.03$, 5/50) increase in histiocytic sarcoma was reported in female mice maintained on the 625 mg/kg diet. The incidence was lower (3/50; not statistically significant) in the 1250 mg/kg feed group and was absent in the 312 mg/kg feed and control groups. The authors noted that the historical incidence of histiocytic sarcoma in 2-year feed (NTP-2006 diet) controls was 2/459 (0.4%) and was 18/1258 (1.4%)[1] for all routes of administration.

Under the conditions of these 2-year gavage studies, the NTP (2006) report concluded: “There was some evidence of carcinogenic activity of benzophenone in male B6C3F1 mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was some evidence of carcinogenic activity in female B6C3F1 mice based on increased incidences of histiocytic sarcoma.”

The primary neoplastic effects observed in the treated mice in the 2-year NTP (2006) gavage study were associated with the liver. The high incidence of hepatocellular adenomas and carcinomas in both control and treated groups of male and female mice is indicative of the sensitivity of the B6C3F1 mouse liver to neoplastic changes. The high incidences of hepatocellular adenoma in treated and control male mice were not significantly different. The incidences of non-neoplastic lesions and adenomas in the male control group were higher than the incidences in

[1] Given as 0.3% ± 0.8% (range 0–2%) and 1.5% ± 2.2% (range 0–8%) in NTP (2006).

any group of treated females. Although the incidences of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in all groups of treated male mice were greater than the incidence in the control group, there was no difference in the incidence of malignant neoplasms (hepatocellular carcinomas and hepatoblastomas). The incidence of hepatocellular neoplasms (adenomas and combined adenomas and carcinomas) in treated females was not significantly different from the control group, and the overall incidence of neoplastic and non-neoplastic lesions was significantly less than in males.

The profile of neoplastic responses is consistent with the historically high levels of background hepatocellular neoplasms in male and female B6C3F1 mice (Maronpot et al., 1987). The historical spontaneous incidence of liver neoplasms in control male and female B6C3F1 mice has revealed background incidences of combined hepatocellular adenomas and carcinomas of 32.4% for males, with a range of 20–47%, and 11.8% for females, with a range of 8–16% (NTP, 2006). Similar incidences were reported in the benzophenone study. The high incidence of spontaneous hepatocellular neoplasms (adenomas and carcinomas) in the male B6C3F1 mouse, the absence of consistent dose–response data, the benign nature of the lesion (adenomas), the lack of hepatocellular neoplastic effects in the parallel rat study (discussed below) and the relatively high dose levels administered cast doubt on the applicability of these lesions to humans with respect to current estimated dietary exposures to benzophenone used as a flavouring agent. The comparison of the incidence of histiocytic sarcomas in female mice—5/50 (10%) at 625 mg/kg feed and 3/50 (6%) at 1250 mg/kg feed—with the low historical incidence of these neoplasms in control mice maintained on the NTP-2000 diet—2/459 (0.4%)[1]—was the basis for the conclusion of "some evidence of carcinogenic activity in female B6C3F1 mice" (NTP, 2006). Although the NTP (2006) report did not consider the appearance of histiocytic sarcoma to be sufficient evidence of compound-related carcinogenesis, a low, non-statistically significant increase (i.e. 1/50 at 30 mg/kg bw and 2/50 at 65 mg/kg bw) in histiocytic sarcoma was observed in female rats at the two highest dietary levels.

The female mouse historical database upon which the above comparison was based is relatively small (seven dietary studies containing a total of 359[2] female mice; incidence of 2/359 or 0.6%). When the database is expanded to include control animals maintained on the NTP-2000 diet and administered the test article by all routes of administration, the incidence of histiocytic sarcomas in controls increases (1.5% ± 2.2%, range 0–8%), and the incidences of tumours in the benzophenone study (10% and 6%) are nearly within the historical range.

In order to evaluate the relevance of these tumours to the potential cancer risk for humans consuming benzophenone as a flavouring agent, it is important to note that histiocytic sarcomas were found only at dose levels inducing severe toxicity. Increased incidences of hepatic and renal lesions were recorded in both species, and increased incidences of splenic extramedullary haematopoiesis were reported in female mice. For example, the majority of female mice (four of five) that

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[1] Given as 0.3% ± 0.8% (range 0–2%) in NTP (2006).

[2] Not 459, as reported in the original study.

exhibited histiocytic sarcomas also showed hepatocellular adenomas. In female rats in the middle and high dose groups, there were marked decreases in the incidences of thyroid C-cell hyperplasia and mammary gland fibroadenomas, which may be indicative of high doses of benzophenone interfering with hormones such as calcitonin and estradiol (Boorman, DeLellis & Elwell, 1996).

Clearly, low incidences of histiocytic sarcomas occurred in only one sex, and only then at dose levels inducing toxicity and possibly affecting hormonal balance, rendering it highly likely that the occurrence of these tumours is a high-dose phenomenon that becomes manifest in severely affected female mice. A NOAEL was not defined.

Rats

Groups of 50 male and 50 female F344/N rats were maintained on diets containing benzophenone at concentrations of 0, 312, 625 or 1250 mg/kg for 105 weeks (NTP, 2006). These dietary levels were estimated to provide a daily intake of 0, 15, 30 or 60 mg/kg bw for males and 0, 15, 30 or 65 mg/kg bw for females, respectively. All animals were observed twice daily, and clinical signs were recorded monthly. Body weights were measured weekly for the first 12 weeks and monthly thereafter. Necropsies were performed on all animals, including those that died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions, which were histopathologically examined. Histological examinations were performed on prepared tissues from all control and test animals that died during the study. Organs of the treated rats that were targeted for potential neoplastic and non-neoplastic effects were histopathologically examined.

Mortality rates for male rats approached 100% (96%) in the 1250 mg/kg feed group, but were high in all groups, including controls. A majority of the control animals that died during the study did so by week 93. No significant differences in mortality rates were reported between controls and any of the three test groups of female rats. Mean body weights of males in the control and test groups peaked between weeks 62 (1250 mg/kg feed) and 86 (control) and then steadily decreased until the end of the study.

The kidney was noted to be the principal target organ for toxic and neoplastic responses in male rats. Chronic nephropathy and mineralization of the medulla were reported in essentially all (90–100%) control and test animals. The severity of chronic nephropathy increased with dose. Dose-dependent increases in the incidence of renal tubule hyperplasia (1/50, 5/50, 20/50 and 23/50) and pelvic transitional epithelial hyperplasia (1/50, 11/50, 29/50 and 34/50) were reported. Upon microscopic evaluation of standard sectioning of the kidney, a non-statistically significant increase in the incidence of renal tubule adenomas (1/50, 1/50, 2/50 and 4/50) was reported. When microscopic evaluations were extended to step sectioning, the incidences (2/50, 2/50, 4/50 and 8/50) of renal tubular neoplasm increased to become statistically significant at the two highest dietary levels.

The NTP concluded that “Under the conditions of these 2-year studies, there was some evidence of carcinogenic activity of benzophenone in male F344/N rats

based on increased incidences of renal tubular cell adenoma.” A NOEL was not identified.

The results of this 2-year bioassay for benzophenone in the male F344/N rat mimic those of a number of NTP bioassays for other substances. The classic profile of results involves poor survival, mean body weight changes, chronic nephropathy and associated renal toxicity that are specific to this strain and sex of rat. Analyses by NTP researchers (Haseman, Hailey & Morris, 1998) have shown that the survival rates of F344 male rats, both those in control groups and those in test groups of long-term studies, have decreased significantly over the last several years. One of the major causes of death is severe chronic nephropathy, which has been increasing in incidence in more recent control groups (Eustis et al., 1994). This species-specific phenomenon is male predominant and in all probability reflects the sensitivity of the male rat kidney to chronic progressive nephropathy, focal tubular and pelvic transitional urothelial hyperplasia and specific tumorigenic responses. The interaction of test substances with spontaneous, age-related renal disease in laboratory rats has recently been reviewed (Hard, 1998; Lock & Hard, 2004). Based on a comprehensive review of renal tumours of all types reported in NTP bioassays, it seems that the interaction of chemical agents and spontaneous chronic progressive nephropathy occurs at two levels: one, to exacerbate the rate of chronic progressive nephropathy, and two, to stimulate tubule hyperplasia into foci of atypical hyperplasia, eventually leading to adenomas. The induction of tumours via this pathway normally produces a minimal response in male rats, leading to a low incidence of tumours of relatively small size and low grade.

In the benzophenone study, poor survival, especially in control and high-dose animals, severely reduced the sensitivity of the study for detecting the presence of a carcinogenic response in chemically exposed groups of male rats. Excessive mortality in the control group, which occurred primarily during the last quarter of the study, limited the ability to detect the renal effects resulting from chronic nephropathy. Mean body weights of both control and test males peaked long before study termination (week 86 for control males to week 62 for high-dose males), suggesting that systemic changes related to chronic nephropathy occurred and that the overall health of the animals was adversely affected. These weight changes are similar to those observed in numerous bioassays for other substances (Hard, 1998). Nevertheless, the severity of the chronic nephropathy was significantly greater with increasing dose, as seen by increased renal tubule hyperplasia, increased hyperplasia of the transitional epithelium of the pelvis and increased renal tubule adenoma in both single section evaluation and step section evaluation.

Sex specificity for renal pathology can, in part, be understood in terms of sex-specific differences for renal clearance of the major urinary metabolites. The majority of ingested benzophenone is metabolized via reduction to yield the glucuronic acid conjugate of diphenylmethanol (benzhydrol) (Nakagawa, Suzuki & Tayama, 2000). As for other aliphatic and aromatic ketones (e.g. acetophenone), a steady state develops between the ketone and corresponding alcohol in vivo. Conjugation of the alcohol form and subsequent renal clearance of the conjugate shift the steady state, decreasing the in vivo concentration of the ketone. The fact

that female rats experience higher plasma concentrations of benzophenone compared with male rats suggests that renal clearance is slower for females (NTP, 2006). Conversely, renal tubular concentrations of the conjugate of diphenylmethanol are expected to be higher in males. The observed sex difference is supported by other biochemical evidence.

The severity scores of ageing nephropathy were 1.3, 2.4, 3.3 and 3.8 for the 0, 312, 625 and 1250 mg/kg feed groups of males, respectively. Ageing chronic nephropathy (Seely et al., 2002; Haseman et al., 2003; Hard & Khan, 2004; Hard & Seely, 2005) is considerably more severe in male rats compared with female rats in most strains, including the F344. Although the reason for this difference is not completely understood, the much higher urinary concentration of protein, primarily because of α-2u-globulin, in male rats is considered a major contributing factor.

Several modes of action for renal carcinogenesis in rats and mice have recently been summarized (Lock & Hard, 2004). One of these modes is a marked increase in the severity of ageing chronic nephropathy associated with an increase of tumours, mostly adenomas. One of the characteristics of ageing chronic nephropathy is increased renal tubular degeneration and regenerative hyperplasia. Under normal circumstances, the degree of tubular proliferation is insufficient to generate atypical hyperplastic foci adenomas. However, if the chronic nephropathy increases in severity, as in the 2-year rat study, there is considerably more tubular degeneration, but also, more importantly, an increase in tubular proliferation, hyperplastic foci and atypical hyperplastic foci and an increase in the incidence of adenomas. The findings of the 2-year rat study support a conclusion that increased severity of ageing chronic nephropathy is an explanation for the renal tubular effects produced by benzophenone, together with a correlating dose-related change in tubular proliferation response, a predominant (or exclusive) effect in males compared with females, the lack of other changes (i.e. tubular necrosis, α-2u-globulin, increased apoptosis) that could explain the effect and the lack of such effects in the mouse. Thus, in the 2-year rat study with benzophenone, it can be concluded that the increasing severity of ageing chronic nephropathy is largely responsible for the renal tubular proliferation in the male rat. This mode of action is not relevant to human renal carcinogenesis.

(d) Genotoxicity studies

In vitro and in vivo genotoxicity studies with three flavouring agents in this group are summarized in Table 6 and described below.

(i) In vitro

No genotoxic potential was observed in the Ames test when *Salmonella typhimurium* strain TA100 was incubated in the presence of 4-hydroxyacetophenone (No. 2040) at 0.1–1000 μg/plate (Rapson, Nazar & Butsky, 1980).

No genotoxic potential was observed in the Ames test when *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were incubated in the presence of 2,3,3-trimethylindan-1-one (No. 2047) at concentrations up to 5000 μg/plate (Sokolowski, 2004).

Table 6. Studies of genotoxicity with aromatic substituted secondary alcohols, ketones and related esters used as flavouring agents

No.	Agent	End-point	Test object	Maximum concentration	Results	Reference
In vitro						
2040	4-Hydroxyacetophenone	Reverse mutation	*Salmonella typhimurium* TA100	0.1, 1, 10, 100 and 1000 μg/plate	Negative	Rapson, Nazar & Butsky (1980)
2047	2,3,3-Trimethylindan-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	1, 3, 10, 33, 100, 333, 1000, 2500 and 5000 μg/plate	Negative	Sokolowski (2004)
2047	2,3,3-Trimethylindan-1-one	Chromosomal aberrations	Chinese hamster ovary V79	3.9, 7.8, 15.6, 31.3, 62.5 and 125 μg/ml	Equivocal[a,b] (see text)	Schulz (2006)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10, 33, 100, 333, 900, 1000 and 3333 μg/plate	Negative	Mortelmans et al. (1986)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1538	Up to 3600 μg/plate	Negative	Wild et al. (1983)
In vivo						
2047	2,3,3-Trimethylindan-1-one	Micronucleus assay	Mouse	312.5, 625 and 1250 mg/kg bw	Negative[c,d]	Honarvar (2006)

Table 6 (contd)

No.	Agent	End-point	Test object	Maximum concentration	Results	Reference
2047	2,3,3-Trimethylindan-1-one	Micronucleus assay	Mouse	1250 mg/kg bw	Negative[e]	Honarvar (2006)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Sex-linked lethal mutation	*Drosophila melanogaster*	5 mmol/l	Negative[f]	Wild et al. (1983)
2048	4-(3,4-Methylenedioxyphenyl)-2-butanone	Micronucleus assay	Mouse	384, 576 and 768 mg/kg bw	Negative[g]	Wild et al. (1983)

[a] Cytotoxic above 31.3 μg/ml.
[b] With metabolic activation.
[c] Administered via gavage.
[d] Twenty-four-hour preparation interval.
[e] Forty-eight-hour preparation interval.
[f] Feeding assay.
[g] Administered via intraperitoneal injections.

2,3,3-Trimethylindan-1-one (No. 2047) dissolved in ethanol (at concentrations of 3.9, 7.8, 15.6, 31.3, 62.5, 125 and 250 μg/ml, depending on the experiment) was assessed for its potential to induce structural chromosomal aberrations in V79 cells of the Chinese hamster in vitro. In the absence of S9 metabolic activation, no statistically significant differences in the number of cells carrying structural chromosomal aberrations were observed between cells treated with 2,3,3-trimethylindan-1-one at 7.8, 15.6, 31.3, 62.5, 125 or 250 μg/ml and solvent controls. In the presence of S9 mix, the author reported a statistically significant concentration-dependent increase in the number of cells carrying structural chromosomal aberrations in cells treated with 2,3,3-trimethylindan-1-one at 31.3–125 μg/ml compared with solvent controls. However, at concentrations above 31.3 μg/ml, the results could not be evaluated, and at concentrations below 31.3 μg/ml, the results were not listed as significant. The study author concluded that 2,3,3-trimethylindan-1-one is clastogenic in this chromosomal aberration test in the presence of metabolic activation (Schulz, 2006).

In the above assays with S9 activation, the two highest doses were considered cytotoxic owing to low cell viability compared with solvent controls (37.5% and 21.1%, respectively). The 31.3 μg/ml dose was not considered cytotoxic, despite the fact that the cell viability was only 77.3% and the fact that in pre-study toxicity tests, cells exposed to 2,3,3-trimethylindan-1-one at 27.3 μg/ml and 54.7 μg/ml for 4 h were only 60.2% and 39.9% viable, respectively. Additionally, the mitotic index of the 31.3 μg/ml dose-treated cells was 79% that of solvent controls. At 31.3 μg/ml, it was not determined whether the positive evidence of chromosomal aberration was due to genotoxic effects or to the displayed cytotoxicity. Additionally, the lack of positive effects in the absence of S9 metabolic activation and the uniformly negative results of the other genotoxicity assays using in vitro bacterial and in vivo mammalian systems argue for a more rigorous evaluation by the author (Schulz, 2006).

No genotoxic potential was observed in the Ames test when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were incubated in the presence of 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) at 10–3333 μg/plate (Mortelmans et al., 1986). In a similar group of tests, no genotoxic potential was observed in the Ames test when *S. typhimurium* strains TA98, TA100, TA1535 and TA1538 were incubated in the presence of 4-(3,4-methylenedioxyphenyl)-2-butanone at concentrations up to 3600 μg/plate (Wild et al., 1983).

It was also noted that benzophenone (No. 831) was not mutagenic when evaluated in the Ames test (Mortelmans et al., 1986).

(ii) In vivo

In a standard micronucleus induction assay, mice were administered 2,3,3-trimethylindan-1-one (No. 2047) at doses up to 1250 mg/kg bw by gavage (Honarvar, 2006). Bone marrow isolated from the femur of each animal revealed no increase in micronucleated polychromatic erythrocytes (Honarvar, 2006).

In a standard basc assay, no sex-linked lethal mutations were observed when *Drosophila melanogaster* were fed 5 mmol/l solutions of 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) (Wild et al., 1983).

No increases in micronuclei were observed in bone marrow smears when groups of male NMRI mice (four per dose) were administered 4-(3,4-methylenedioxyphenyl)-2-butanone (No. 2048) at doses of 384, 576 or 768 mg/kg bw by intraperitoneal injection (Wild et al., 1983).

(e) Developmental and reproductive studies of toxicity

New information was provided on the developmental and reproductive effects of two members of this group of flavouring agents that had been evaluated previously.

In a limited developmental toxicity study, groups of 10 female Sprague-Dawley rats were administered acetophenone (No. 806) via gavage at doses of 0, 75, 225 or 750 mg/kg bw per day for a minimum of 14 days through day 3 of lactation. There were no parental deaths. Mating and fertility indices and mean gestation length were unaffected by acetophenone administration up to 750 mg/kg bw per day. The live birth index, pup survival during lactation and pup body weights were decreased for the 750 mg/kg bw per day group offspring. The authors of this abstract concluded that the NOAEL for reproductive effects was 225 mg/kg bw per day (Kapp et al., 2003).

In a developmental toxicity study, 25 female CD rats were administered benzophenone (No. 831) via gavage at a dose of 0, 100, 200 or 300 mg/kg bw per day on gestational days 6 through 19. Dams were monitored for clinical signs, food and water intake and body weight. At necropsy on gestational day 20, maternal clinical condition, body, liver, paired kidney and gravid uterine weights, pregnancy status and number of corpora lutea were recorded. In the gravid uterus, the numbers of resorbed, dead or live fetuses were recorded. All live fetuses were weighed, sexed and examined for external morphological anomalies. Approximately half of the fetuses were examined for visceral anomalies, including internal head structures, and the remainder of the fetuses were examined for skeletal irregularities. Confirmed pregnancy rates were 88–100% per group. No treatment-related maternal deaths occurred. Clinical signs observed were lethargy, piloerection, weight loss and rooting in the bedding after dosing. Maternal body weight gain was reduced for all doses from gestational days 6 to 9 and at the high dose from days 9 to 12. Increased body weight gain was observed for the middle dose group on gestational days 9–12 and 19–20 and for the high dose group on gestational days 18–20. Gestational weight gain corrected for gravid uterine weight was reduced at all doses. Maternal liver and kidney weights were significantly increased at all doses. A significant decrease in feed intake was noted for all high dose groups throughout the study. Relative water intake throughout the study was comparable between test animals and controls. Average fetal weight per litter decreased relative to increasing dose of benzophenone. There was no effect on prenatal viability or overall incidences of fetal malformations. The incidence of unossified sternebrae was increased at all doses, and the incidence of an extra rib

on lumbar I was increased at the middle and high doses. Overall, the authors concluded that evidence of maternal toxicity was present at each dose level, precluding the determination of a NOAEL. The authors explained that this outcome should not be considered atypical. They noted that NOAELs were not established in previous studies of developmental toxicity in female rats with benzophenone (NTP, 2002).

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BENZYL DERIVATIVES (addendum)

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1. EVALUATION

1.1 Introduction

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

The Committee previously evaluated 37 other members of this group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *155*). The

Committee concluded that all 37 flavouring agents in this group were of no safety concern based on estimated dietary exposures.

Three of the additional eight flavouring agents (Nos 2061, 2062 and 2068) have been reported to occur naturally and can be found in passion fruit juice, cinnamon bark, cassia leaf, Tahitian vanilla and raw cabbage (Nijssen, van Ingen-Visscher & Donders, 2009).

1.2 Assessment of dietary exposure

The total annual volumes of production of the eight benzyl derivatives are approximately 27 kg in Europe, 3 kg in the USA and 17 kg in Japan (European Flavour and Fragrance Association, 2004; Japan Flavour and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009). Approximately 70% and 100% of the total annual volumes of production in Europe and in the USA, respectively, are accounted for by *o*-anisaldehyde (No. 2062). In Japan, approximately 50% of the total annual volume of production is accounted for by benzyl levulinate (No. 2064).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest estimate is for benzyl hexanoate (No. 2061) (300 μg, the SPET value obtained for non-alcoholic beverages). For the other flavouring agents in the group, the daily dietary exposures range from 0.004 to 240 μg, with the SPET yielding the highest estimates for all. Annual volumes of production of this group of flavouring agents as well as the daily dietary exposures calculated as the MSDI or using the SPET are summarized in Table 2.

1.3 Absorption, distribution, metabolism and elimination

Metabolic information on this group was considered at the fifty-seventh meeting of the Committee (Annex 1, reference *155*). In general, aromatic esters and acetals are hydrolysed in vivo through the catalytic activity of A-type carboxyl-esterases that predominate in hepatocytes (Heymann, 1980; Anders, 1989). Benzyl esters and acetals are hydrolysed to benzyl alcohol and benzaldehyde, respectively, followed by oxidation to yield benzoic acid. Benzoate esters are hydrolysed to benzoic acid.

Benzyl derivatives have been shown to be rapidly absorbed through the gut, metabolized primarily in the liver and excreted in the urine as glycine conjugates of benzoic acid derivatives (Davison, 1971; Abdo et al., 1985; Temellini, 1993). At high dose levels, formation of the glycine conjugate is glycine limited. When glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted unchanged or as the glucuronic acid conjugate. Alkyl substituents on the aromatic ring have little influence on the principal pathways of metabolism.

Table 1. Summary of the results of the safety evaluations of benzyl derivatives used as flavouring agents[a,b,c]

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Benzyl levulinate	2064	6939-75-9	No, SPET: 240	Note 1	No safety concern
4-Methylbenzyl alcohol	2065	589-18-4	No, SPET: 3	Note 4	No safety concern
Benzyl nonanoate	2066	6471-66-5	No, SPET: 125	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class II					
2-Ethylhexyl benzoate	2068	5444-75-7	No, SPET: 3	Note 3	No safety concern
Structural class III					
4-Methylbenzaldehyde propyleneglycol acetal	2067	58244-29-4	No, SPET: 80	Notes 4 and 5	No safety concern

CAS, Chemical Abstracts Service

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

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

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

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

Table 1 (contd)

Notes:

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

Table 2. Annual volumes of production and dietary exposures for benzyl derivatives used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
Benzyl hexanoate (2061)				300	5	+
Europe	5	0.5	0.008			
USA	ND	ND	ND			
Japan	4	1	0.02			
o-Anisaldehyde (2062)				40	1	+
Europe	19	2	0.03			
USA	3	0.3	0.006			
Japan	0.1	0.03	0.001			
Prenyl benzoate (2063)				180	3	–
Europe	4	0.38	0.0063			
USA	0.1	0.0	0.000			
Japan	ND	ND	ND			
Benzyl levulinate (2064)				240	4	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	8.4	2.2	0.04			
4-Methylbenzyl alcohol (2065)				3	0.1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.2	0.3	0.005			
Benzyl nonanoate (2066)				125	2	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.3	0.3	0.01			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume of natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
4-Methylbenzaldehyde propyleneglycol acetal (2067)				80	1	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	1.5	0.4	0.006			
2-Ethylhexyl benzoate (2068)				3	0.1	25[e]
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.1	0.0009			
Total						
Europe	27					
USA	3					
Japan	17					

bw, body weight; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008), International Organization of the Flavor Industry (2009) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (μg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008; International Organization of the Flavor Industry, 2009).
MSDI (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

Table 2 (contd)

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[c] SPET (μg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Quantitative data for the USA reported by Stofberg & Grundschober (1987).

[e] The consumption ratio (annual consumption via food, kg)/(most recent reported volume of production as a flavouring agent, kg) was not determined, as no volume of production as a flavouring substance was available for the USA.

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned six of the flavouring agents (Nos 2061–2066) to structural class I, one of the flavouring agents (No. 2068) to structural class II and one (No. 2067) to structural class III (Cramer, Ford & Hall, 1978).

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

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

Table 1 summarizes the evaluations of the eight benzyl derivatives (Nos 2061–2068) in this group when used as flavouring agents.

1.5 Consideration of combined intakes from use as flavouring agents

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

Flavouring agents with the highest intakes in this group that have the common metabolite benzyl alcohol, which is in structural class I, are Nos 23–25, 842 and 843. In the unlikely event that these were to be consumed concurrently on a daily basis, the estimated combined intakes in Europe and the USA would be

18 000 and 4700 μg/person per day, respectively, which would exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The majority of this combined intake would be from benzyl alcohol itself (No. 25). All of these agents are expected to be efficiently metabolized and would not saturate metabolic pathways. The Committee concluded that combined intake would not raise concern about safety.

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

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

1.6 Consideration of secondary components

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

1.7 Conclusion

In the previous evaluation of flavouring agents in this group, studies of acute toxicity, short-term toxicity and genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *155*). The Committee concluded that these eight flavouring agents, which are additions to the group of benzyl derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the additional key data relevant to the safety evaluation of the group of eight benzyl derivatives. The structural feature common to all members of the group is a primary oxygenated functional group bonded directly to a benzene ring or a functional group metabolized to a benzyl alcohol or benzoic acid derivative. The ring may also have alkyl substituents. The eight benzyl derivatives are additions to a group of 37 flavouring agents evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *155*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposures were estimated using the MSDI approach or the SPET.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

Relevant data on the absorption, distribution, metabolism and excretion of these flavouring agents have not been reported since the publication of the original monograph (Annex 1, reference *155*).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for three of the eight flavouring agents in this group and for three previously evaluated flavouring agents (Table 3). For *o*-anisaldehyde (No. 2062), LD_{50} values in rats ranged from 500 to 2500 mg/kg body weight (bw) (Bär & Griepentrog, 1967; Bailey, 1976; Moreno, 1977). For prenyl benzoate (No. 2063), LD_{50} values in rats ranged from 2000 to 4700 mg/kg bw (Moreno, 1978; Gardner, 1987). For 4-methylbenzyl alcohol (No. 2065), an LD_{50} of 3900 mg/kg bw in rats was found (Moreno, 1978).

These studies demonstrate that the acute oral toxicity of benzyl derivatives is low.

Table 3. Results of acute oral toxicity studies for benzyl derivatives used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
2062	*o*-Anisaldehyde	Rat; NR	2500	Moreno (1977)
2062	*o*-Anisaldehyde	Rat; NR	1510	Bär & Griepentrog (1967)
2062	*o*-Anisaldehyde	Rat; NR	>500 but <5000	Bailey (1976)
2063	Prenyl benzoate	Rat; NR	4700	Moreno (1978)
2063	Prenyl benzoate	Rat; M, F	>2000 but <5000	Gardner (1987)
2065	4-Methylbenzyl alcohol	Rat; NR	3900	Moreno (1978)
850	Benzoic acid	Mouse; M	>2000[a]	Sasaki et al. (2002)
870	Butyl *p*-hydroxybenzoate	Mouse; NR	230	Sokol (1952)
878	*cis*-3-Hexenyl benzoate	Rat; NR	>5000[a]	Moreno (1976)

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

[a] A single-dose study or the highest dose tested. The actual LD_{50} may be higher.

Table 4. Results of short-term studies of toxicity on benzyl derivatives

No.	Flavouring agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOAEL (mg/kg bw per day)	Reference
2062	*o*-Anisaldehyde	Rat; M, F	1/10	Diet	14	140[c]	Gill & Van Miller (1987)
2062	*o*-Anisaldehyde	Rat; M, F	1/20	Gavage	112	1000[c] (10 000 mg/kg diet)	Hagan et al. (1967)
25	Benzyl alcohol	Rat; M, F	3/20	Gavage	42	300	Foulon et al. (2005)

F, female; M, male; NOAEL, no-observed-adverse-effect level

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

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

[c] Only one dose tested. The actual NOAEL may be higher.

(b) Short-term studies of toxicity

The results of short-term studies of toxicity have been reported for *o*-anisaldehyde (No. 2062). In addition, there is a newly available study on the previously evaluated benzyl alcohol (No. 25) (Annex 1, references *50*, *122* and *155*). The short-term studies of toxicity on the benzyl derivatives are summarized in Table 4.

(i) o-*Anisaldehyde (No. 2062)*

Fischer 344 rats (five of each sex per group) were maintained on diets containing *o-a*nisaldehyde at a level calculated to provide an average daily intake of 0 or 140 mg/kg bw for a period of 14 days. Observations on mortality were made twice a day for the duration of the experiment. Weekly measurement of body weight and food consumption revealed no significant differences between test and control groups. At necropsy, absolute liver and kidney weights were measured, and histopathological examinations were performed. The test material produced no apparent signs of toxicity on a gross or microscopic level (Gill & Van Miller, 1987).

Weanling Osborne-Mendel rats (10 of each sex) were maintained on diets containing *o*-anisaldehyde at a level calculated to provide an average daily intake of 0 or 1000 mg/kg bw per day for 16 weeks (Hagan et al., 1967). Weekly measurement of body weight, general condition and food intake revealed no differences between test rats and controls. At termination, haematological examinations (i.e. white and red cell counts, haemoglobin levels and haematocrits) and histological examination of tissues were performed. No effects in rats were

observed that were attributable to the dietary intake of *o*-anisaldehyde for 16 weeks (Hagan et al., 1967).

An unspecified number and strain of rats were maintained on diets containing *o*-anisaldehyde at a level calculated to provide an average daily intake of 1000 mg/kg bw per day for 15 weeks. No further experimental detail was provided. No adverse effects were reported (Bär & Griepentrog, 1967).

(ii) Benzyl alcohol (No. 25)

In a 6-week study to examine the susceptibility of rat pups to the toxic actions of benzyl alcohol, groups of 10 male and 10 female rat pups were administered benzyl alcohol via oral gavage at dose levels of 100, 300 or 600 mg/kg bw per day from postnatal day 22. In addition to body weight, clinical observations, ophthalmology, haematology, clinical chemistry, urinalysis and histopathology, the study evaluated reactivity, learning and memory, motor activity, sexual development and respiratory function. During the first half of the study, body weight gain was decreased in males (14%, $P < 0.05$) and females (13%) treated with 600 mg/kg bw per day. No other significant treatment-related changes in body weight or food consumption were reported in males or females. No treatment-related effects on haematology or blood biochemistry parameters were reported in either sex. After 3 weeks of treatment, at the 600 mg/kg bw per day dose level, respiratory problems such as audible or loud breathing, dyspnoea and abdominal breathing were observed in males and females. These effects were persistent and so severe at the high dose that premature euthanasia of some animals was necessary. Excessive salivation was seen also, primarily with the high dose. There were no treatment-related histopathology findings in animals killed at the end of the treatment period; specifically, there was no indication of histopathological changes in the lungs at any dose level. Based on significant decreased body weight gain in males at the 600 mg/kg bw per day level, a NOAEL of 300 mg/kg bw per day was established (Foulon et al., 2005).

(c) Genotoxicity studies

In vitro genotoxicity data are available for three of the eight flavouring agents in this group (Nos 2062, 2063 and 2065). Additionally, studies of in vitro genotoxicity have been reported on three flavouring agents in this group that were previously evaluated (Annex 1, reference *155*). The results of all of these studies are summarized in Table 5 and described below.

(i) In vitro

Negative results were reported in the standard assay for reverse mutation in various strains of *Salmonella typhimurium* (strains used were TA97, TA98, TA100, TA102, TA1535 and/or TA1537, depending on the flavouring agent being tested). Specific details, results and references are presented in Table 5.

Table 5. Studies of genotoxicity with benzyl derivatives used as flavouring agents

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
In vitro						
2062	*o*-Anisaldehyde	Reverse mutation	*Salmonella typhimurium* TA97 and TA102	10, 50, 100, 500 and 1000 μg/plate	Negative[a,b]	Fujita & Sasaki (1987)
2062	*o*-Anisaldehyde	Sister chromatid exchange	Human peripheral lymphocytes	0–34 μg/ml (0–0.25 mmol/l)[c]	Positive	Jansson et al. (1988)
2063	Prenyl benzoate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0.5, 5, 50, 500 and 5000 μg/plate	Negative[a,d]	Glover & Richold (1987)
2063	Prenyl benzoate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	1.5, 5, 15, 50 and 150 μg/plate	Negative[e]	Glover & Richold (1987)
2063	Prenyl benzoate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0.15, 0.5, 1.5, 5 and 15 μg/plate	Negative[d]	Glover & Richold (1987)
2063	Prenyl benzoate	Chromosomal aberration	Chinese hamster ovary cells	0, 3.3, 10, 33.3, 100, 333.3 and 1000 μg/plate	Negative[a,f]	Taalman (1988)
2065	4-Methylbenzyl alcohol	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1537	33, 100, 333, 1000, 1666, 3333, 6666 and 10 000 μg/plate	Negative[a]	Zeiger et al. (1992)
2065	4-Methylbenzyl alcohol	Reverse mutation	*S. typhimurium* TA100	50, 100, 200 and 500 μg/plate	Negative[d]	Ball, Foxall-Van Aken & Jensen (1984)

Table 5 (contd)

No.	Flavouring agent	End-point	Test object	Concentration	Results	Reference
22	Benzaldehyde	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 106 µg/plate (0.01 and 1 mmol/l)[g]	Negative[a,b]	Kubo, Urano & Utsumi (2002)
25	Benzyl alcohol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 108 µg/plate (0.01 and 1 mmol/l)[h]	Negative[a,b]	Kubo, Urano & Utsumi (2002)
850	Benzoic acid	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 122 µg/plate	Negative[b,c]	Kubo, Urano & Utsumi (2002)
859	Linalyl benzoate	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	100, 333, 1000, 2500 and 5000 µg/plate	Negative[a,b]	Thompson (2004)

[a] With and without metabolic activation.
[b] Preincubation assay.
[c] Calculated using a relative molecular mass of 136.15.
[d] Cytotoxicity was observed at concentrations greater than 50 µg/plate in the absence of S9 and at 5000 µg/plate in the presence of S9.
[e] With metabolic activation.
[f] Prenyl benzoate was cytotoxic at concentrations greater than 100 µg/ml, as evidenced by lack of cell growth.
[g] Calculated with a relative molecular mass of 106.12.
[h] Calculated with a relative molecular mass of 108.14.

In a sister chromatid exchange assay, human peripheral lymphocytes from non-smokers were incubated in the presence of *o*-anisaldehyde (No. 2062) at 0–0.25 mmol/l (0–34 µg/ml) for 88 h. Under the described conditions, the authors of this study reported a significant increase in the number of sister chromatid exchanges observed (Jansson et al., 1988).

No chromosomal aberration activity was observed when prenyl benzoate (No. 2063) at 0–1000 µg/ml was incubated with Chinese hamster ovary cells for 24 h (Taalman, 1988).

(ii) Conclusion for genotoxicity

The testing of these representative materials in vitro in prokaryotic and eukaryotic test systems indicates some positive results in the sister chromatid exchange assay. However, sister chromatid exchange is considered of limited relevance for the evaluation of genotoxic potential, as this end-point may reflect unspecific interference with DNA replication.

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PHENOL AND PHENOL DERIVATIVES (addendum)

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1. EVALUATION

1.1 Introduction

The Committee evaluated 13 additional flavouring agents belonging to the group of phenol and phenol derivatives used as flavouring agents, which was evaluated previously. The additional substances included an ester of phenol (No. 2019), two polyphenols (Nos 2022 and 2024), a phenol glucoside (No. 2018), alkyl-, alkenyl- or aryl-substituted phenols or their esters (Nos 2012, 2013 and 2023), alkoxyphenols or their esters (Nos 2014–2017) and phenol derivatives with alkyl side-chains containing a ketone function (Nos 2020 and 2021). The group of substances was selected on the basis of the structural criteria that all members either possess an aromatic ring containing one or more free hydroxyl groups or are the esters of phenol derivatives. The evaluations were conducted using the

the esters of phenol derivatives. The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, Introduction) (Annex 1, reference *131*). None of these substances has been evaluated previously by the Committee.

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

Four of the 13 additional flavouring agents (Nos 2012, 2013, 2019 and 2021) in this group have been reported to occur naturally and have been found in dried bonito, apple cider, various cheeses and ginger (Nijssen, van Ingen-Visscher & Donders, 2009).

1.2 Assessment of dietary exposure

The total annual volumes of production of the 13 flavouring agents belonging to the group of phenol and phenol derivatives are approximately 241 kg in Europe, 0.05 kg in Japan and 2602 kg in the USA (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; International Organization of the Flavor Industry, 2009). Approximately 99% of the total annual volume of production in Europe is accounted for by 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024), and approximately 99% of the total annual volume of production in the USA is accounted for by magnolol (No. 2023) and 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024). Approximately 100% of the total annual volume of production in Japan is accounted for by phenyl butyrate (No. 2019).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The highest estimates are for 4-(2-propenyl)phenyl-β-D-glucopyranoside (No. 2018) and magnolol (No. 2023) (6000 μg for both, the SPET value from non-alcoholic beverages for No. 2018 and from chewing gum or other confections for No. 2023). For the other flavouring agents in this group, the daily dietary exposures range from 0.01 to 3000 μg, with the SPET yielding the highest estimates for all except 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024). Reported annual volumes of production and calculated daily dietary exposures (MSDI and SPET) for this group of flavouring agents are summarized in Table 2.

***Table 1. Summary of the results of the safety evaluations of phenol and phenol derivatives used as flavouring agents*[a,b,c]**

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

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Sodium 3-methoxy-4-hydroxycinnamate	2014	24276-84-4	No, SPET: 1500	NR	NR	Notes 1 and 2	No safety concern
Guaicol butyrate	2015	4112-92-9	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern
Guaicol isobutyrate	2016	723759-62-4	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Guaicol propionate	2017	7598-60-9	No, SPET: 60	NR	NR	Notes 1 and 3	No safety concern
4-(2-Propenyl) phenyl-β-D-glucopyranoside	2018	64703-98-6	Yes, SPET: 6000	No	Yes. The NOAEL of 600 mg/kg bw per day for the structurally related eugenol (No. 1529) in a 90-day study in rats (NTP, 1983) is at least 6000 times the estimated daily dietary exposure to No. 2018 when used as a flavouring agent.	Note 1	No safety concern
Phenyl butyrate	2019	4346-18-3	No, SPET: 30	NR	NR	Notes 1 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Hydroxy(4-hydroxy-3-methoxyphenyl)-acetic acid	2020	55-10-7	No, SPET: 1500	NR	NR	Note 1	No safety concern
Structural class II							
1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	2021	27113-22-0	Yes, SPET: 3000	No	Yes. The NOAEL of 70 mg/kg bw per day for the structurally related 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in a 90-day study in rats (Hoffman, 2004) is at least 1400 times the estimated daily dietary exposure to No. 2021 when used as a flavouring agent.	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class III							
3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	2022	60-82-2	Yes, SPET: 480	No	Yes. The NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats (Lina, Dreef-vanderMeulen & Leegwater, 1990) is at least 93 000 times the estimated daily dietary exposure to No. 2022 when used as a flavouring agent.	Note 1	No safety concern
Magnolol	2023	528-43-8	Yes, SPET: 6000	No	Yes. The NOAEL of ≥240 mg/kg bw per day in a 90-day study in rats (Liu et al., 2007) is at least 2400 times the estimated daily dietary exposure to magnolol when used as a flavouring agent.	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5*[e] Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	2024	69097-99-0	Yes, MSDI: Europe 26 USA 153 Japan ND	No	Yes. The NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats (Lina, Dreef-vanderMeulen & Leegwater, 1990) is at least 290 000 times the estimated daily dietary exposure to No. 2024 when used as a flavouring agent.	Note 1	No safety concern

bw, body weight; CAS, Chemical Abstracts Service; ND, no data reported; NOAEL, no-observed-adverse-effect level; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure

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

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

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

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

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

Notes:

1. Detoxication of phenol primarily involves conjugation of the hydroxyl group with sulfate and glucuronic acid and subsequent elimination in the urine.
2. Cinnamic acid derivatives are expected to undergo β-oxidation and are excreted as hippuric acid.
3. The phenolic ester will hydrolyse to phenol and the corresponding carboxylic acid.

Table 2. Annual volumes of production and dietary exposures for phenol and phenol derivatives used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
4-Propenylphenol (2012)				400	7	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	ND	ND	ND			
2,4,6-Trimethylphenol (2013)				300	5	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	ND	ND	ND			
Sodium 3-methoxy-4-hydroxycinnamate (2014)				1500	25	–
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	ND	ND	ND			
Guaiacol butyrate (2015)				60	1	–
Europe	ND	ND	ND			
USA	0.4	0.05	0.001			
Japan	ND	ND	ND			
Guaiacol isobutyrate (2016)				60	1	–
Europe	ND	ND	ND			
USA	0.4	0.05	0.001			
Japan	ND	ND	ND			
Guaiacol propionate (2017)				60	1	–
Europe	ND	ND	ND			
USA	0.4	0.05	0.001			
Japan	ND	ND	ND			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
4-(2-Propenyl)phenyl-β-D-glucopyranoside (2018)				6000	100	–
Europe	ND	ND	ND			
USA	5.0	0.6	0.01			
Japan	ND	ND	ND			
Phenyl butyrate (2019)				30	1	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.05	0.01	0.0002			
Hydroxy(4-hydroxy-3-methoxyphenyl)acetic acid (2020)				1500	25	–
Europe	ND	ND	ND			
USA	0.4	0.05	0.001			
Japan	ND	ND	ND			
1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one (2021)				3000	50	+
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (2022)				480	8	–
Europe	ND	ND	ND			
USA	4.0	0.5	0.008			
Japan	ND	ND	ND			
Magnolol (2023)				6000	100	–
Europe	ND	ND	ND			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)[d]
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
USA	1342	164	3			
Japan	ND	ND	ND			
5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (2024)				140	2	–
Europe	241	26	0.4			
USA	1250	153	3			
Japan	ND	ND	ND			
Total						
Europe	241					
USA	2602					
Japan	0.05					

ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2009), but no quantitative data; –, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Japan Flavor and Fragrance Materials Association (2005) and International Organization of the Flavor Industry (2009). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (μg/person per day) calculated as follows:
(annual volume, kg) × (1 × 10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, “eaters only”) = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; International Organization of the Flavor Industry, 2009).
MSDI (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (μg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

1.3 Absorption, distribution, metabolism and elimination

In the report of the fifty-fifth meeting, biodisposition of flavouring agents in this group was extensively discussed. When ingested as natural or added components of food, phenol and its derivatives are rapidly absorbed from the gastrointestinal tract and participate in common pathways of metabolic detoxication (Hughes & Hall, 1997). Phenol and phenol derivatives are conjugated with sulfate and glucuronic acid and excreted primarily in the urine. Other metabolic pathways, observed mainly at high dose levels, include ring hydroxylation and side-chain oxidation. Phenols containing alkoxy groups and those that contain a ketone function on an alkyl side-chain are also detoxified mainly via conjugation. Alternative detoxication pathways include dealkylation of alkoxyphenols, reduction of side-chain ketones, side-chain oxidation and ring hydroxylation. At very high dose levels, a bioactivation pathway has been characterized; high dose levels of *p*-cresol (i.e. 4-methylphenol; No. 693), *p*-ethylphenol (No. 694), 2-methoxy-4-methylphenol (No. 715), 2-methoxy-4-propylphenol (No. 717), 2-methoxy-4-vinylphenol (No. 725) and 4-allyl-2,6-dimethoxyphenol (No. 726) are oxidized to reactive quinone methide intermediates (Thompson et al., 1994, 1995; Thompson, Perera & London, 1995).

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned nine flavouring agents (Nos 2012–2020) to structural class I. One flavouring agent (No. 2021) was assigned to structural class II, and three flavouring agents (Nos 2022–2024) were assigned to structural class III (Cramer, Ford & Hall, 1978).

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

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

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

Step A5. For 4-(2-propenyl)phenyl-β-D-glucopyranoside (No. 2018), the no-observed-adverse-effect level (NOAEL) of 600 mg/kg body weight (bw) per day for the structurally related eugenol (No. 1529) in a 90-day study in rats (NTP, 1983) provides a margin of safety of 6000 in relation to the highest estimated dietary exposure to No. 2018 (SPET = 6000 μg/person per day) when used as a flavouring agent.

For 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one (No. 2021), the NOAEL of 70 mg/kg bw per day for the structurally related 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) in a 90-day study in rats (Hoffman, 2004) provides a margin of safety of 1400 in relation to the highest estimated dietary exposure to No. 2021 (SPET = 3000 μg/person per day) when used as a flavouring agent.

For 3-(4-hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (No. 2022), the NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats (Lina, Dreef-vanderMeulen & Leegwater, 1990) provides a margin of safety of greater than 93 000 in relation to the highest estimated dietary exposure to No. 2022 (SPET = 480 μg/person per day) when used as a flavouring agent.

The NOAEL of 240 mg/kg bw per day for magnolol (No. 2023) in a 90-day study in rats (Liu et al., 2007) provides a margin of safety of 2400 in relation to the highest estimated dietary exposure to No. 2023 (SPET = 6000 μg/person per day) when used as a flavouring agent.

For 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024), the NOAEL of approximately 750 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats (Lina, Dreef-vanderMeulen & Leegwater, 1990) provides a margin of safety of greater than 290 000 in relation to the highest estimated dietary exposure to No. 2024 (MSDI = 153 μg/person per day) when used as a flavouring agent.

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

Table 1 summarizes the evaluations of the 13 phenol and phenol derivatives (Nos 2012–2024) in this group.

1.5 Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined exposures to flavouring agents was undertaken based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*). In addition, at this meeting, the Committee also considered combined intakes for structurally closely related series of flavouring agents.

Flavouring agents in this series that are members of a structurally closely related series of simple phenols or alkylphenols or predicted to be metabolized to such compounds, in structural class I, are Nos 2012, 2013, 2018 and 2019. The five related flavouring agents with the highest intakes in Europe are Nos 690, 691, 694,

697 and 705 and in the USA are Nos 693, 695, 698, 699 and 703. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 316 μg/person in Europe and 81 μg/person in the USA, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

Flavouring agents in this series that are members of a structurally closely related series of methoxyphenols or predicted to be metabolized to such compounds, in structural class I, are Nos 2015, 2016 and 2017. The five related compounds with the highest intakes in Europe are Nos 713, 715, 717, 721 and 725 and in the USA are Nos 711, 713, 715, 721 and 726. In the unlikely event that these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined intakes would be 307 μg/person in Europe and 43 μg/person in the USA, which would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I). The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

Flavouring agents in this series that are members of a structurally closely related series of phenols or methoxyphenols containing an additional oxygenated functional group or predicted to be metabolized to such compounds, in structural class I, are Nos 2014 and 2020. The related compounds with the highest intakes in Europe are Nos 727, 728, 736 and 731 and in the USA are Nos 727, 728, 736, 730 and 731. In the unlikely event that these substances were to be consumed concurrently on a daily basis, the estimated combined intakes would be approximately 3000 μg/person in Europe and approximately 4000 μg/person in the USA, which would exceed the threshold of concern (i.e. 1800 μg/person per day for class I). However, all five flavouring agents in this group are expected to be efficiently metabolized and would not saturate metabolic pathways. The Committee concluded that the combined intake of these substances, when used as flavouring agents, would not raise safety concerns.

The remaining flavouring agents (Nos 2022–2024) do not share close structural characteristics with others in the group, and consideration of combined intake is not indicated.

The Committee concluded that the combined intakes of these substances, when used as flavouring agents, would not raise safety concerns.

1.6 Consideration of secondary components

Two members of this group of flavouring agents, sodium 3-methoxy-4-hydroxycinnamate (No. 2014) and magnolol (No. 2023), have minimum assay values of less than 95%. The secondary component in No. 2014, vanillin (No. 889), was previously evaluated and found to be of no concern. The secondary components of magnolol (No. 2023), honokiol and eudesmol, are expected to share the same metabolic fate as the flavouring agent and are considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 5.

1.7 Conclusion

In the previous evaluations of substances in this group of flavouring agents, studies of biological properties, acute toxicity, short-term toxicity and genotoxicity were available. None raised safety concerns. The additional biochemical and toxicological data available for this evaluation supported those from the previous evaluation (Annex 1, reference *149*).

The Committee concluded that these 13 flavouring agents, which are additions to the group of phenol and phenol derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the additional key data relevant to the safety evaluation of a group of 13 phenol and phenol derivatives (see Table 1), including 1 ester of phenol, 3 polyhydroxyphenols, 3 alkyl-, alkenyl- or aryl-substituted phenols and 6 alkoxyphenols or phenol derivatives with alkyl side-chains containing a ketone function. These flavouring agents are additions to the group of 48 phenol and phenol derivatives evaluated previously by the Committee at its fifty-fifth meeting (Annex 1, reference *149*).

2.2 Additional considerations on intake

There is no additional information on intake. Dietary exposure estimates were made using the SPET in addition to the MSDI approach.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

Additional studies related to the absorption, distribution, metabolism and elimination of the additional and previously evaluated flavouring agents have been reported since the preparation of the original monograph and are described below.

In a study designed to extrapolate oral systemic toxicity results to the inhalation route for phenol exposure, male and female F344 rats were administered single daily doses of [^{14}C]phenol by 1) intragastric instillation (1.5, 15 and 150 mg/kg bw per day), 2) drinking-water (5000 mg/l) or 3) inhalation (96 mg/m^3 for 6 h) for 8 consecutive days. For all exposure routes, radioactivity was rapidly eliminated in urine (>94% in 24 h), and less than 1% remained in tissues and carcass. The urinary metabolite profile was dose dependent. The ratio of glucuronide to sulfate conjugates of phenol was 0.61 after oral gavage administration for 8 days of 1.5 or 15 mg/kg bw per day and increased to 1.16 following a 150 mg/kg bw per day dose, suggesting saturation of the sulfate conjugation pathway at the high dose level. Metabolic profiles obtained after drinking-water exposure were equivalent to the

high-dose oral gavage profile, and results for male and female rats were equivalent in all studies (Hiser et al., 1993).

The kinetic behaviour of *p*-cresol (No. 693) was investigated in a group of control (n = 6) and dosed (n = 7) male OFA rats (mean body weight, 310 g) that received an intravenous bolus of either 2 ml isotonic saline or 3 mg *p*-cresol in 2 ml isotonic saline, respectively. Serum samples were collected at 0, 5, 30, 60, 120, 180 and 240 min after administration of *p*-cresol, and urine was collected at 1 h intervals. The maximum serum concentration of *p*-cresol at 5 min was 6.7 ± 1.4 mg/l, and the concentration decreased gradually to 0.6 ± 0.3 mg/l at 240 min post-administration. No *p*-cresol was detected in the serum of control rats. Urinary excretion of *p*-cresol was 23 ± 10% of the administered dose, and the excretion half-life was 1.5 ± 0.8 h. Total clearance was 23.2 ± 4.5 ml/min per kilogram body weight, but renal clearance was substantially lower, at 4.8 ± 2.0 ml/min per kilogram body weight. The volume of distribution was determined to be 2.9 ± 1.4 l/kg (Lesaffer et al., 2001).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal dose (LD_{50}) studies have been reported for 3 of the 13 additional flavouring agents in this group. New LD_{50} values have been published for two flavouring agents previously evaluated by the Committee at its fifty-fifth meeting. In rats, LD_{50} values ranged from 632 mg/kg bw for 2-methoxy-4-methylphenol (No. 715) to 1320 mg/kg bw for 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) and greater than 2000 mg/kg bw for 3-(4-hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (No. 2022) and 5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024) (Scheerbaum, 2001; Merkel, 2003; Vaeth, 2005, 2006). In mice, an LD_{50} value of 270 mg/kg bw for 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one (No. 2021) was reported (Lee et al., 1991). Results of oral acute toxicity studies are summarized in Table 3.

These studies demonstrate that the acute oral toxicity of phenol and phenol derivatives is low. The hepatotoxicity of *p*-cresol (No. 693), *p*-[α,α,α-d_3]-cresol, 4-methylanisole (No. 1243), 2,4-dimethylphenol, 2,4,6-trimethylphenol (No. 2013) and 2-methoxy-4-methylphenol was studied using rat liver slices and liver microsomes. The liver slices were exposed to varying concentrations of *p*-cresol analogues for 6 h, and the median lethal concentration (LC_{50}) was measured by loss of intracellular potassium. 2,4,6-Trimethylphenol (LC_{50} 2.22 mmol/l) was less toxic than *p*-cresol (LC_{50} 1.32 mmol/l) and 2,4-dimethylphenol (LC_{50} 1.33 mmol/l). 4-Methylanisole showed the least toxicity, with an LC_{50} of 4.35 mmol/l. The reduced cellular toxicity is consistent with increased glutathione conjugation when incubated with either liver microsomal fractions or liver slices (Table 4). Presumably, the increased rate of glutathione conjugation of the reactive quinone methide metabolite is in part responsible for the decrease in toxicity. In the case of 4-methylanisole, no glutathione conjugates were detected (Thompson, Perera & London, 1996).

Table 3. Results of oral acute toxicity studies with phenol and phenol derivatives used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
715	2-Methoxy-4-methylphenol	Rat; M, F	632	Scheerbaum (2001)
728	4-(*p*-Hydroxyphenyl)-2-butanone	Rat; F	1320	Merkel (2003)
2021	1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	Mouse; M, F	270	Lee et al. (1991)
2022	3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	Rat; M, F	>2000	Vaeth (2006)
2024	5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	Rat; M, F	>2000	Vaeth (2005)

F, female; M, male

Table 4. Data summary of the comparative rates of glutathione conjugate formation from 4-methylphenol analogues in rat liver microsomes and slices

Test substance	Glutathione conjugate formation	
	Microsomes (nmol/min per milligram protein)	Slices (nmol/h per slice)
p-Cresol	0.39 ± 0.02	2.31 ± 0.14
4-Methylanisole	ND	ND
2,4-Dimethylphenol	0.24 ± 0.04	2.18 ± 0.21
2,4,6-Trimethylphenol	0.13 ± 0.03	1.00 ± 0.10
p-[α,α,α-d_3]-Cresol	0.13 ± 0.02	1.39 ± 0.07

ND, not detected
Source: Thompson, Perera & London (1996)

(b) Short-term studies of toxicity

The results of short-term toxicological studies have been reported for one additional member of this group, magnolol (No. 2023). Studies with phenol (No. 690), *o*-, *m*- and *p*-cresol (Nos 691–693), *p*-ethylphenol (No. 694), resorcinol (No. 712) and 4-(*p*-hydroxyphenyl)-2-butanone (No. 728) were described in detail by the Committee at its fifty-fifth meeting (Annex 1, reference *149*). Only the results of studies on the flavouring agents in the group that were not reported at the fifty-fifth meeting are presented below. The short-term studies of toxicity on phenol and phenol derivatives are summarized in Table 5.

Table 5. Results of short-term studies on phenol and phenol derivatives

No.	Agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOAEL[c]/ NOEL[d] (mg/kg bw per day)	Reference
690	Phenol	Rat; M, F	3/30	Drinking-water	91	308.2[d] (M) 359.8[d] (F)	Beyrouty (1998)
692	*m*-Cresol	Rat; M, F	3/24	Gavage	17	30[c]	Koizumi et al. (2003)
692	*m*-Cresol	Rat; M, F	3/14	Gavage	28	300[c]	Koizumi et al. (2003)
692/693	*m/p*-Cresol	Rat; M	3/50	Diet	735	230[c]	NTP (2008)
692/693	*m/p*-Cresol	Mouse; F	3/50	Diet	735	300[c]	NTP (2008)
694	*p*-Ethylphenol	Rat; M, F	4/6	Gavage	18	30[c]	Takahashi et al. (2006)
694	*p*-Ethylphenol	Rat; M, F	4/7	Gavage	28	100[c]	Takahashi et al. (2006)
712	Resorcinol	Rat; M, F	4/30	Drinking-water	126	1000[d] (mg/l)	Welsch, Nemec & Lawrence (2006)
728	4-(*p*-Hydroxyphenyl)-2-butanone	Rat; M, F	3/10	Diet	90	70[c]	Hoffman (2004)
2023	Magnolol	Rat; M, F	5/5	Diet	21	≥480[c,e]	Liu et al. (2007)
2023	Magnolol	Rat; M, F	4/20	Diet	90	≥240[c,e]	Liu et al. (2007)

F, female; M, male
[a] Total number of test groups does not include control animals.
[b] Total number per test group includes both male and female animals.
[c] No-observed-adverse-effect level.
[d] No-observed-effect level.
[e] Highest dose tested. The actual NOAEL/NOEL may be higher.

(i) Phenol (No. 690)

The potential neurotoxicity of phenol was investigated in three groups of 15 male and 15 female Sprague-Dawley rats dosed for 13 weeks with phenol (purity 100%) in the drinking-water at concentrations of 0 (control), 200, 1000 or 5000 mg/l, which correspond to 0, 18.1, 83.1 and 308.2 mg/kg bw per day for males and 0, 24.6, 107.0 and 359.8 mg/kg bw per day for females, respectively (USFDA, 1993), followed by a 4-week recovery phase. Body weights and food consumption were determined weekly, water intake was measured daily and clinical signs were recorded daily. A functional observational battery (FOB) and motor activity test were conducted before initiation of exposures and once during each of weeks 4, 8, 13 and 17 (recovery animals). At the end of 13 weeks of dosing, five rats of each sex per group were anaesthetized and subjected to whole-body perfusion with a washout solution followed by a fixative for neuropathological evaluation. The remaining animals were terminated at the end of the recovery period. Five rats of each sex per group were subjected to fixation perfusion but not further examined, and the remainder underwent necropsy.

One high-dose female was euthanized at 14 days owing to poor condition. All others survived for the study duration. Food consumption in 5000 mg/l males and females was reduced, and mean body weights were lower for these groups from days 64 and 8, respectively, until dosing termination. Marked body weight gains occurred during the recovery period for both sexes, resulting in no significant difference between dosed and control groups. Mean food consumption and daily water intake were significantly lower for high dose group males and females during various intervals of the dosing period, and mean food consumption and daily water intake for high-dose animals were increased during the recovery period.

No differences in group mean motor activity counts were detected for males in any of the dosed groups; some reductions in activity counts were observed in females, but were attributed to reduction in water or food intake. The FOB evaluation did not reveal any findings of neurotoxicological significance following qualitative (observations in home cage, removal from home cage, observations in area, handling observations on surface and on top of box) or quantitative (grip strength, hind limb splay or body temperature) measurements throughout dosing or following the recovery period. No macroscopic or histopathological lesions in nervous tissue attributed to dosing were noted. A no-observed-effect level (NOEL) for neurotoxicity was established as 5000 mg/l in drinking-water (308.2 and 359.8 mg/kg bw per day for males and females, respectively) under the conditions of this study (Beyrouty, 1998).

(ii) m-*Cresol (No. 692)*

In order to determine the susceptibility of neonates to *m*-cresol (referred to as 3-methylphenol), newborn Sprague-Dawley rats (12 of each sex per group) were administered *m*-cresol (purity 99.13%) at doses of 0, 30, 100 or 300 mg/kg bw per day on postnatal days 4–21 by intragastric instillation in olive oil. At 300 mg/kg bw per day, clinical signs, including deep respiration, hypersensitivity on handling and tremors under contact stimulus, and depressed body weight gain were observed.

Hypersensitivity and tremors were also reported occasionally during the dosing period in a small number of males. There were no abnormalities in physical development, sexual maturation or reflex ontogeny at any dose level, and no adverse effects were reported in the 30 mg/kg bw per day dose group. In the same report, a 28-day toxicity study was described in which 5-week-old Sprague-Dawley rats (seven of each sex per group) were administered *m*-cresol at doses of 0, 100, 300 or 1000 mg/kg bw per day by intragastric instillation in olive oil with a 2-week recovery period. At 1000 mg/kg bw per day, clinical signs and body weight depression occurred in both sexes, as was observed in the newborn animals. No changes were observed at lower doses. At the end of the recovery period, no significant change in any parameter was observed. The authors concluded that newborn rats are 3–4 times more susceptible than adult rats to toxicity from exposure to *m*-cresol (Koizumi et al., 2003).

(iii) m/p*-cresol (Nos 692 and 693)*

In a 2-year multiple dose study, groups of 50 male F344/N rats approximately 6 weeks of age were maintained on diets containing 0, 1500, 5000 or 15 000 mg/kg (equivalent to average daily doses of approximately 0, 70, 230 and 720 mg/kg bw per day) of a 60:40 mixture of *m*/*p*-cresol (overall purity 99.5%). Food consumption and body weights were measured weekly for the first 13 weeks and at 4-week intervals thereafter. Clinical signs were measured at week 5 and at 4-week intervals thereafter. Following necropsy, complete histopathology was performed on all animals, including examination of macroscopic lesions and tissue masses.

Mean body weights of the 720 mg/kg bw per day group were less than those of the control group throughout the study. Owing to lack of palatability, food consumption by the 720 mg/kg bw per day group was less than that by the control group during the first week of the study but increased to control levels by the second week of the study. No clinical signs of toxicity were observed. At necropsy and histopathology, the severity of nephropathy was slightly increased in the 720 mg/kg bw per day group. Renal tubule adenomas in the kidney were reported in animals maintained at this dietary level (720 mg/kg bw per day). The incidence of hyperplasia of the transitional epithelium of the renal pelvis was significantly increased in the 720 mg/kg bw per day group as well. Examination of the liver showed that the incidences of eosinophilic foci were significantly increased in the 720 mg/kg bw per day group. Exposure to cresols increased the incidences of hyperplasia of the goblet cells and respiratory epithelium of the nose in all exposed groups of rats in this study. The incidences of squamous metaplasia of the respiratory epithelium were significantly increased at the 230 and 720 mg/kg bw per day dose levels, and inflammation was significantly increased in the 720 mg/kg bw per day group. Based on increased incidences of renal tubule adenomas in male rats at 720 mg/kg bw per day, a NOAEL of 230 mg/kg bw per day (5000 mg/kg) was reported (NTP, 2008).

In a similar 2-year multiple dose study, groups of 50 6-week-old female B6C3F1 mice were maintained on diets calculated to provide an average daily intake of *m*/*p*-cresol (40:60 mixture; overall purity 99.5%) of 0, 1000, 3000 or 10 000 mg/kg (equivalent to average daily doses of approximately 0, 100, 300 or

1040 mg/kg bw per day). Food consumption and body weights were measured weekly for the first 13 weeks and at 4-week intervals thereafter. Clinical signs were measured at week 5 and at 4-week intervals thereafter. Complete necropsies and histopathology were performed on all animals. At necropsy, all organs and tissues were examined for visible lesions. Mean body weights of the animals maintained at the 300 and 1040 mg/kg bw per day doses were less than those of the control group after weeks 12 and 9, respectively. Food consumption was temporarily decreased in animals treated in the 1040 mg/kg bw per day group. In females maintained at the 1040 mg/kg bw per day dose, the incidence of squamous cell papilloma of the forestomach was significantly greater when compared with the control group. In the 300 and 1040 mg/kg bw per day groups, increased epithelial hyperplasia of the nose was reported, and occurrences of minimal to moderate bronchiolar hyperplasia were significantly increased in all exposed groups, but the severity increased with increasing concentrations. In the 1040 mg/kg bw per day group, increased incidences of eosinophilic foci of the liver were reported. Based on the increased incidence of forestomach squamous cell papilloma in the females maintained at a dose of 1040 mg/kg bw per day, a NOAEL of 300 mg/kg bw per day (3000 mg/kg diet) was reported (NTP, 2008).

The development of forestomach tumours in rodents in these studies, together with their relevance to the potential for human carcinogenicity, has been the subject of much consideration (Grice, 1988; Wester & Kroes, 1988; Clayson et al., 1990; IARC, 2003). The mucosa of the rodent forestomach is not similar to that of the human oesophagus; it is partially composed of a keratinizing squamous epithelial layer. The rodent forestomach stores food and is constantly exposed to acidic gastric juice. Conversely, the human distal oesophagus is not involved in storage and does not have constant contact with the acidic gastric medium. The human oesophagus contains a non-keratinizing squamous epithelium that is adversely affected by strongly acidic medium (Adams et al., 2008). Therefore, the incidences of forestomach lesions in rodent studies where high concentrations of test material were administered by intragastric instillation are not relevant to humans, as these lesions occur at the contact site (forestomach) and arise from the irritating effect of a bolus dose of the irritant given intragastrically.

In the NTP (2008) studies described above, the papillomas in the rodent forestomach were likely due to prolonged contact with high concentrations of irritating cresols (Adams et al., 2008).

(iv) p-*Ethylphenol (No. 694)*

In an 18-day repeated-dose study, newborn Sprague-Dawley rats (six of each sex per dose) were administered *p*-ethylphenol (98.4% pure) at doses of 0, 30, 100 or 300 mg/kg bw per day by intragastric instillation in olive oil on postnatal days 4–21. Recovery groups (six of each sex per dose) were maintained for 9 weeks and examined at 12 weeks. General condition, body weights, haematology, blood chemistry, necropsy and organ weights were examined. Clinical signs of hypoactivity, hypothermia, tremor, deep respiration, emaciation and decreased body weights occurred in both sexes at 300 mg/kg bw per day. Two out of the 12 females dosed with 300 mg/kg bw per day were found dead on days 10 and 12 of

dosing. One showed dark red lungs and congestive oedema of the lung, and the other showed distension of the gastrointestinal tract and atrophy of the thymic cortex at necropsy. A delay in the righting reflex was observed in 4 out of 12 males at 300 mg/kg bw per day and in 1 out of 12 females at 100 mg/kg bw per day as well as in 1 out of 10 females at 300 mg/kg bw per day. At necropsy at the end of dosing, an increase in the relative weights of the livers was observed in males and females at 300 mg/kg bw per day. There were no changes in blood biochemistry or histopathological findings related to liver damage. No effects of dosing were found at the end of the recovery period. A NOAEL of 30 mg/kg bw per day in the newborn rat was based on the delay in the development of the righting reflex at 100 mg/kg bw per day (Takahashi et al., 2006).

In a 28-day repeated-dose study, 5-week-old Sprague-Dawley rats (seven of each sex per dose) were given *p*-ethylphenol (98.4% pure) at doses of 0, 100, 300 or 1000 mg/kg bw per day by intragastric instillation in olive oil. Recovery groups (0 or 1000 mg/kg bw per day) (seven of each sex per dose) were maintained for 2 weeks and examined at 11 weeks of age. At 1000 mg/kg bw per day, clinical signs of toxicity, including salivation, staggering gait, lateral position and soiled perigenital fur, were observed in 11 out of 14 males and 9 out of 14 females. Decreased body weights were also reported at this dose level. A high volume of urine was seen in females at 1000 mg/kg bw per day. Compared with controls, clinical chemistry revealed elevated alanine aminotransferase activities in males and total cholesterol levels in females at 1000 mg/kg bw per day. At necropsy at the end of dosing, thinning of the mucosa in the glandular stomach in both sexes and reddish spots in the glandular stomach in females were evident. In males dosed with 300 and 1000 mg/kg bw per day, increases in relative liver weights were observed. Males dosed with 1000 mg/kg bw per day showed a significant increase in relative kidney weight. Females dosed with 1000 mg/kg bw per day showed an increase in liver weight. Erosion, hyperplasia of squamous cells, degeneration of squamous cells or oedema of the submucosa in the forestomach was observed in all seven males at 1000 mg/kg bw per day. Hyperplasia of squamous cells in the forestomach was observed in one out of seven males at 300 mg/kg bw per day. Hyperplasia of squamous cells in the oesophagus, degeneration of squamous cells, oedema of the submucosa, granulation of the submucosa, hyperplasia of squamous cells or ulcer in the forestomach was observed in six out of seven females at 1000 mg/kg bw per day. The NOAEL in the 5-week-old rat was 100 mg/kg bw per day based on the lesions in the forestomach at 300 mg/kg bw per day. At the end of the recovery period, the only effect of dosing was lowered body weight of males (Takahashi et al., 2006).

(v) Resorcinol (No. 712)

In order to assess the potential adverse effects of resorcinol (No. 712) on reproduction, test guideline criteria (Organisation for Economic Co-operation and Development [OECD], United States Environmental Protection Agency) with expanded thyroid gland end-points were evaluated at termination of either the F_0 or F_1 generation. Male and female Sprague-Dawley rats, 30 of each sex per group, were provided concentrations of resorcinol (purity not stated) of 0, 120, 360, 1000 and 3000 mg/l in the drinking-water for 18 weeks starting at 6 weeks of age and at

least 70 days before mating. Body weights and food consumption were measured weekly. Thyroid hormone function assessment was performed at study termination. None of the reproductive end-points assessed according to OECD Test Guideline 416 was affected by resorcinol exposure at any concentration. There was a slight decrease in mean body weights among the 3000 mg/l F_0 animals of both sexes at certain times during the study and in F_1 males during the entire 18 weeks. There were no statistically significant changes in the mean concentrations of thyroid stimulating hormone (TSH), triiodothyronine (T_3) or thyroxine (T_4) in either food intake or food conversion efficiency. Resorcinol-induced colloid depletion was marginal in the 1000 mg/l males and not detectable in the females. Because there were no detectable adverse effects on the set of reproductive toxicity end-points or on the thyroid end-points, it was concluded that the NOAEL for continuous exposure was 3000 mg/l. A NOEL for histopathology and stereomicroscopy of follicular colloid for resorcinol intake in the drinking-water was concluded to be 1000 mg/l (Welsch, Nemec & Lawrence, 2006).

*(vi) 4-(*p*-Hydroxyphenyl)-2-butanone (No. 728)*

In a 90-day study, male and female Sprague-Dawley rats approximately 8 weeks of age were fed diets containing 4-(*p*-hydroxyphenyl)-2-butanone (purity 12%),[1] referred to as Raspberry Ketone GMP, at levels calculated to provide an average intake of 0, 70, 275 or 700 mg/kg bw per day. Body weights and food consumption were measured weekly. A FOB and motor activity test were conducted on all animals at weeks 12 and 13. At the end of the 90 days, haematology, clinical chemistry, organ weight measurements, macroscopic examination and histopathology were performed. Males in the highest dietary intake group (700 mg/kg bw per day) showed dose-related decreases in body weight gain. There were no test substance–related or statistically significant differences in body weights in the 70 and 275 mg/kg bw per day dose groups for males or in any dose group for females when compared with the controls. Minimal decreases in food consumption were reported in males only in the 275 and 700 mg/kg bw per day groups during the first and second weeks of dosing. There were no test substance–related differences in food consumption during the remainder of the study in males and females compared with the control animals. No effect on motor activity was reported at any dietary level. The only dosing-related effect on organ weights occurred with liver weights; statistically significant but mild increases in liver weights were noted in the 275 and 700 mg/kg bw per day dose groups, which may correlate with serum enzyme increases. Haematology revealed a decrease in reticulocyte counts in males at 700 mg/kg bw per day compared with controls. However, the differences were not considered biologically significant, because the majority of the individual values were within the range for the control group. No other haematology parameters were affected in the males or females. Significant increases in alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities were noted in the 700 mg/kg bw per day male and female groups. The increases in

[1] Raspberry ketone from a commercial dietary supplement preparation was used to incorporate the test material into the feed. The intake levels were calculated based on 12% of 4-(*p*-hydroxyphenyl)-2-butanone in the preparation.

alanine aminotransferase and aspartate aminotransferase activities were also noted in the 275 mg/kg bw per day group of females. However, as no histopathological effects were observed for the liver, the increased enzyme activities may represent an adaptive metabolic response of the liver to the test article. Statistically significant decreases from control values in serum creatinine levels were noted in females in the 275 and 700 mg/kg bw per day dose groups, but the absolute differences were minimal and considered not to be test substance related. No significant dosing-related macroscopic findings were noted. A NOAEL of 70 mg/kg bw per day was reported for both male and female rats (Hoffman, 2004).

(vii) Magnolol (No. 2023)

In a 21-day pilot study, groups of five Sprague-Dawley rats, 6–7 weeks old, were administered magnolia bark extract (94% magnolol and 1.5% honokiol) in the diet at doses of 0, 60, 120, 240 or 480 mg/kg bw per day. No deaths occurred, and there were no dosing-related effects on body weight gain, clinical observations, haematology, clinical chemistry, urinalysis, organ weight measurements or macroscopic or microscopic findings. Similarly, in a 90-day study, groups of five rats were fed magnolia bark extract at doses of 0, 60, 120 or 240 mg/kg bw per day. No mortality, ophthalmic abnormalities or dosing-related findings in clinical observations, haematology, coagulation or organ weight measurements were observed. There were no test substance–related macroscopic or microscopic findings. Differences between dosed and control groups in body weight, body weight gain, food consumption and food conversion efficiency, clinical chemistry and urinalysis parameters were not considered toxicologically significant, as they were not dose related or because values remained within historical control ranges. A NOAEL of 240 mg/kg bw per day, the highest dose tested, was established based on the findings of the 90-day study (Liu et al., 2007).

(c) Genotoxicity studies

Genotoxicity testing has been performed on six additional flavouring agents and seven previously evaluated flavouring agents (Annex 1, reference *149*). The results of these tests are summarized in Table 6 and are described below.

(i) In vitro

2,4,6-Trimethylphenol (No. 2013) was tested for mutagenicity by incubation with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 either alone or with an exogenous induced male rat liver bioactivation system using a spot test and subsequently in TA98 using the plate incorporation assay. The test substance alone and with bioactivation was toxic at 4086 µg (30 µmol) per plate. At concentrations up to 4086 µg/plate, the test substance was reported to produce no increase in revertant mutants in any strain (data not given), whereas appropriate control substances established the responsiveness of the tester strains (Florin et al., 1980). Negative results were also reported (data not given) in strains TA98 and TA100 (Epler, Rao & Guerin, 1979; Kubo, Urano & Utsumi, 2002).

Table 6. Studies of genotoxicity of phenol and phenol derivatives used as flavouring agents

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
2013	2,4,6-Trimethylphenol	Reverse mutation	*Salmonella typhimurium* TA98 and TA100	Up to 1000 μg/plate	Negative[a]	Epler, Rao & Guerin (1979)
2013	2,4,6-Trimethylphenol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	4, 41, 409 and 4086 μg/plate[b] (0.03, 0.3, 3 and 30 μmol/plate)	Negative[a,c]	Florin et al. (1980)
2013	2,4,6-Trimethylphenol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 136 μg/plate[b] (0.01 and 1 mmol/plate)	Negative[a]	Kubo, Urano & Utsumi (2002)
2013	2,4,6-Trimethylphenol	Chromosomal aberration	CHO cells	0–100 μg/ml[d] 1–200 μg/ml[e]	Negative[d] Positive[e]	Gudi & Brown (2002)
2019	4-(2-Propenyl)phenyl-β-D-glucopyranoside	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	100, 316, 1000, 3160 and 5000 μg/plate	Negative[a]	August (2007)
2019	4-(2-Propenyl)phenyl-β-D-glucopyranoside	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	100, 316, 1000, 3160 and 5000 μg/plate	Negative[a,f]	August (2007)
2021	1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	DNA damage	*Bacillus subtilis* rec^+ and rec^-	3000 μg/plate	Equivocal	Kim et al. (1998)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
2021	1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0.069, 0.69, 6.9 and 13.8 µg/plate	Negative[a,f]	Kim et al. (1998)
2022	3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	3.16, 10, 31.6, 100 and 316 µg/plate	Negative[a]	August (2006)
2022	3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	3.16, 10, 31.6, 100 and 316 µg/plate	Negative[a,f]	August (2006)
2023	Magnolol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	18.5, 37.5, 75, 150 and 300 µg/plate	Negative[a]	Li et al. (2007)
2023	Magnolol	Reverse mutation	*Escherichia coli* WP2 *uvrA*	18.5, 37.5, 75, 150 and 300 µg/plate	Negative[a]	Li et al. (2007)
2023	Magnolol	Chromosomal aberration	CHO cells WB1	2.2, 7, 20, 25 and 30 µg/ml	Negative[f,g]	Zhang et al. (2008)
2023	Magnolol	Chromosomal aberration	CHO cells WB1	1.25, 2.5 and 7 µg/ml	Negative[e]	Zhang et al. (2008)
2023	Magnolol	Chromosomal aberration	CHO cells WB1	0.6, 1.7, 5 and 15 µg/ml	Negative[d,f]	Zhang et al. (2008)
2023	Magnolol	Chromosomal aberration	V79 Chinese hamster lung cells	6.5, 13, 26 and 52 µg/ml[d] 7.5, 15, 30 and 59 µg/ml[e]	Negative[a]	Zhang et al. (2008)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
2024	5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	10, 31.6, 100, 316 and 1000 µg/plate	Negative[a,h]	Stien (2005)
2024	5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	10, 31.6, 100, 316 and 1000 µg/plate	Negative[a,f,h]	Stien (2005)
690	Phenol	UDS	Mouse lymphoma cells	0.1–94.1 µg/ml[i] (1.0 µmol/l – 1.0 mmol/l)	Negative	Pellack-Walker & Blumer (1986)
690	Phenol	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104 and TA1535	100–5000 µg/plate[d] 20–5000 µg/plate[e]	Negative[a]	Glatt et al. (1989)
690	Phenol	SCE	Human peripheral blood lymphocytes	5, 50, 500, 1000, 5000 and 7000 µg/ml	Positive	Erexson, Wilmer & Kligerman (1985)
690	Phenol	SCE	Chinese hamster V79 cells	94 µg/ml[j] (1000 µmol/l)	Negative	Glatt et al. (1989)
690	Phenol	Micronuclei assay	Chinese hamster V79 cells	376 µg/ml[j] (4000 µmol/l)	Negative	Glatt et al. (1989)
690	Phenol	Micronuclei assay	CHO cells (CHO-K5)	10, 50, 100, 175 and 250 µg/ml[g,e] 350, 475, 600, 800, 1000, 1500 and 2000 µg/ml[d]	Weakly positive[k]	Miller, Pujadas & Gocke (1995)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
690	Phenol	Chromosomal aberration	Syrian hamster embryo cells	0.3, 1, 3 and 9 µg/ml[j] (3, 10, 30 and 100 µmol/l)	Positive	Tsutsui et al. (1997)
690	Phenol	SCE	Syrian hamster embryo cells	1, 9, 28, 94 and 282 µg/ml[j] (10, 100, 300, 1000 and 3000 µmol/l)	Positive	Tsutsui et al. (1997)
690	Phenol	UDS	Syrian hamster embryo cells	0.09, 0.3, 1, 3 and 9 µg/ml[j] (1, 3, 10, 30 and 100 µmol/l)	Positive	Tsutsui et al. (1997)
690	Phenol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 94 µg/plate[j] (0.01 and 1 mmol/plate)	Negative[a]	Kubo, Urano & Utsumi (2002)
691	*o*-Cresol	Reverse mutation	*E. coli* W3110 (polA$^+$) and p3478 (polA$^-$)	0, 50, 100, 500, 1000 and 5000 µg/plate	Negative[a]	Pepper, Hamilton & Scheetz (1980)
691	*o*-Cresol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 108 µg/plate[l] (0.01–1 mmol/plate)	Negative[a]	Kubo, Urano & Utsumi (2002)
691	*o*-Cresol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0.01, 0.1, 1, 5, 10, 26 and 52 µg/plate[m] (0.01, 0.1, 1, 5, 10, 25 and 50 µl/plate)	Negative[a]	Jagannath & Brusick (1981)
691	*o*-Cresol	SCE	CHO cells	0.01, 0.03, 0.05, 0.08 and 0.1 µg/ml[d,m] (12.5, 25.0, 50.6, 75.0 and 100 nl/ml) 0.002, 0.003, 0.006, 0.01 and 0.02 µg/ml[e,m] (1.56, 3.13, 6.25, 12.5 and 15 nl/ml)	Positive[a]	Galloway & Brusick (1981)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
691	*o*-Cresol	SCE	CHO cells	0.4, 0.5, 0.6, 0.7 and 0.8 μg/ml[e,m] (400, 500, 600, 700 and 800 nl/ml)	Positive[n]	Galloway & Brusick (1981)
691, 692, 693	*o*-, *m*-, *p*-Cresol (33.3% each, mixture)	SCE	CHO cells	0.005, 0.001, 0.005, 0.01, 0.05 and 0.1 μg/ml[d,m] (0.5, 1, 5, 10, 50 and 100 nl/ml) 0.0007, 0.001, 0.003, 0.005, 0.01 and 0.02 μg/ml[e,m] (0.625, 1.25, 2.5, 5, 10 and 15 nl/ml)	Positive	Galloway & Brusick (1980)
691, 692, 693	*o*-, *m*-, *p*-Cresol (33.3% each, mixture)	SCE	CHO cells	0.05, 0.08, 0.1 and 0.13 μg/ml[d,m] (50, 75, 100 and 125 nl/ml) 0.01, 0.02, 0.03, 0.04, 0.05, 0.8 and 0.1 μg/ml[e,m] (10, 20, 30, 40, 50, 75 and 100 nl/ml)	Positive	Galloway & Brusick (1980)
691, 692, 693	*o*-, *m*-, *p*-Cresol (33.3% each, mixture)	UDS	Rat primary hepatocytes	0.0005, 0.001, 0.003, 0.005, 0.01, 0.03, 0.05 and 0.1 μg/ml[d,m] (0.5, 1, 2.5, 5, 10, 25, 50 and 100 nl/ml)	Slightly positive	Myhr & Brusick (1980)
692	*m*-Cresol	UDS	Rat primary hepatocytes	0.251, 0.502, 1, 2.51, 5.02, 10, 25.1, 50.2, 100, 251 and 502 μg/ml	Negative[o]	Cifone (1988)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
693	*p*-Cresol	UDS	Human embryonic lung fibroblast cells	10, 80 and 800 µg/ml	Positive[p]	Crowley & Margard (1978)
693	*p*-Cresol	UDS	Human peripheral lymphocytes	0.5–3 µg/ml[l] (5–25 µmol/l)	Negative	Daugherty & Franks (1986)
693	*p*-Cresol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0.008, 0.04, 0.2 and 1 µg/plate[m] (0.008, 0.04, 0.2 and 1 µl/plate)	Negative[a]	Crowley & Margard (1978)
693	*p*-Cresol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 108 µg/plate[l] (0.01 and 1 mmol/plate)	Negative[a]	Kubo, Urano & Utsumi (2002)
693	*p*-Cresol	Transformation	C3H10t1/2 mouse fibroblasts	6–6000 µg/plate[m] (0.006–6.0 µl/plate)	Negative	Crowley & Margard (1978)
709	Thymol	Chromosomal aberration	Chinese hamster lung cells	Up to 15 000 µg/ml	Slightly positive[d] Negative[e]	Kusakabe et al. (2002)
712	Resorcinol	Reverse mutation	*S. typhimurium* TA98 and TA100	1 and 110 µg/plate[q] (0.01 and 1 mmol/plate)	Negative[a]	Kubo, Urano & Utsumi (2002)
733	4-(1,1-Dimethyl)-ethyl phenol	Chromosomal aberration	Chinese hamster lung cells	Up to 30 000 µg/ml	Slightly positive[d] Negative[e]	Kusakabe et al. (2002)

Table 6 (contd)

No.	Flavouring agent	End-point	Test object	Dose or concentration	Results	Reference
In vivo						
2013	2,4,6-Trimethylphenol	Micronuclei	Mice bone marrow	0–1600 mg/kg bw	Negative	Gudi & Krsmanovic (2002)
2023	Magnolol	Micronuclei	Swiss albino CD-1 mice bone marrow	625, 1250 and 2500 mg/kg bw[r]	Negative[s]	Li et al. (2007)
690	Phenol	Sex-linked lethal mutation	*Drosophila melanogaster*	2000[t] and 5250[u] µg/ml	Negative	Woodruff et al. (1985)
690	Phenol	Micronuclei	Pregnant mouse bone marrow	265 mg/kg bw	Negative	Ciranni et al. (1988a)
690	Phenol	Micronuclei	Fetal mouse liver	265 mg/kg bw to dams	Negative	Ciranni et al. (1988a)
690	Phenol	Micronuclei	Mouse bone marrow	265 mg/kg bw	Positive[r]	Ciranni et al. (1988b)
690	Phenol	Micronuclei	Mouse bone marrow	265 mg/kg bw	Positive[v]	Ciranni et al. (1988b)
691	*o*-Cresol	Sex-linked lethal mutation	*D. melanogaster*	100, 500 and 1000 µg/ml	Negative[r]	Sernau (1989)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; SCE, sister chromatid exchange; UDS, unscheduled DNA synthesis

[a] With and without an exogenous bioactivation system.

[b] Calculated using a relative molecular mass of 136.19.

[c] Toxic at 30 µmol/plate.

[d] Without exogenous bioactivation system.

[e] With exogenous bioactivation system.

[f] Preincubation assay.

[g] Media supplemented with rat serum.

Table 6 (contd)

[h] Scarce background lawn observed at 1000 µg/plate.
[i] Toxicity observed at concentrations of 175 µl/ml and higher without exogenous bioactivation system.
[j] Calculated with a relative molecular mass of 94.11.
[k] Observed only in the absence of exogenous bioactivation system.
[l] Calculated using a relative molecular mass of 108.14.
[m] Calculated using a density of 1.044 g/ml.
[n] Toxic at 800 nl/ml.
[o] Excessive toxicity observed at concentrations greater than 10 µg/ml.
[p] Positive only at the highest concentration tested, which is more than double the median effective concentration (EC_{50}) of 310 µg/ml.
[q] Calculated using a relative molecular mass of 110.11.
[r] Short-term, 24 h treatment.
[s] Administered via oral gavage.
[t] Twenty-four-hour and 48 h sampling.
[u] Feeding route of administration.
[v] Injection route of administration.

2,4,6-Trimethylphenol (No. 2013) was tested for induction of chromosomal aberration in the cultured Chinese hamster ovary (CHO) cell line, either alone or with an exogenous induced rat liver bioactivation system. Data were provided for the cells exposed to test substance, either alone at three concentrations up to 300 µg/ml for 4 h or up to 100 µg/ml for 20 h or with the bioactivation system at three concentrations up to 200 µg/ml for 4 h. Increases in the percentages of cells with structural aberrations above solvent control levels were observed at concentrations of 100 and 200 µg/ml with bioactivation (Gudi & Brown, 2002).

4-(2-Propenyl)phenyl-β-D-glucopyranoside (No. 2019), referred to as Chavicol-β-*O*-D-glucoside, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, either alone or with an exogenous induced male rat liver bioactivation system, using both the plate incorporation and preincubation assays. At five concentrations up to 5000 µg/plate, the test substance produced no cytotoxicity and no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (August, 2007).

1-(4′-Hydroxy-3′-methoxyphenyl)-decan-3-one (No. 2021), referred to as 6-paradol, was tested for deoxyribonucleic acid (DNA) damaging activity to bacteria in the spore rec assay using *Bacillus subtilis* tester strains H17 rec^+ (repair proficient) and M45 rec^- (repair deficient). At three doses up to 3 mg/disc, the test substance produced no difference in the surrounding growth zone of the rec^- strain compared with the rec^+ strain, whereas the positive control was active (Kim et al., 1998).

1-(4′-Hydroxy-3′-methoxyphenyl)-decan-3-one (No. 2021) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537, either alone or with an exogenous induced male rat liver bioactivation system, using a preincubation assay. Either alone at four concentrations up to 13.8 µg/plate or with the bioactivation system at four concentrations up to 1.38 µg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Kim et al., 1998).

3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one (No. 2022), referred to as Phoretin Kadem, was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, either alone or with an exogenous induced male rat liver bioactivation system, using both plate incorporation and preincubation assays. The test substance either alone or with the bioactivation system was toxic at 316 µg/plate. At five concentrations up to 316 µg/plate, the test substance either alone or with the bioactivation system produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (August, 2006).

Magnolol (No. 2023) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537, as well as *Escherichia coli* strain WP2 *uvrA*, either alone or with an exogenous induced male rat liver bioactivation system, using a plate incorporation assay. At five concentrations up to 300 µg/plate, the test substance either alone or with the

bioactivation system produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Li et al., 2007).

Magnolia bark extract, which contained 40–90% magnolol (No. 2023), was tested for induction of chromosomal aberration in CHO WB1 and V79 with rat serum and no exogenous rat liver bioactivation system or with an exogenous rat liver bioactivation system. In the first experiment, concentrations of 0, 2.2, 7, 20 and 30 μg/ml of magnolia bark extract were used; one aberrant chromosome was observed at 2.2 μg/ml, and three aberrant chromosomes were observed at 30 μg/ml. In the second experiment, 0, 1.25, 2.5 and 7 μg/ml of magnolia bark extract were used; one chromatid break and one chromosome break were observed at 7 μg/ml. In experiment 3, 0, 0.6, 1.7, 5 and 15 μg/ml of magnolia bark extract were used; one isochromatid break and one ring were observed at 0.6 μg/ml. In all three experiments, the levels of structural aberrations were not concentration related and were not consistent with spontaneous mutation. Structural aberrations in test substance–dosed groups were not observed under any conditions. In a fourth experiment with V79 cells using magnolol concentrations of 6.5, 13, 26 and 52 μg/ml with exogenous bioactivation and 7.5, 15, 30 and 59 μg/ml alone, no statistically significant structural aberrations were observed (Zhang et al., 2008).

5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one (No. 2024) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 either alone or with an exogenous induced male rat liver bioactivation system, using both plate incorporation and preincubation assay. The test substance alone and with bioactivation was toxic at 1000 μg/plate. At five concentrations up to 1000 μg/plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Stien, 2005).

Phenol (No. 690) was tested for induction of unscheduled DNA synthesis (UDS) in cultured mouse lymphoma cells (L5178YS). Phenol concentrations of 1 μmol/l – 1 mmol/l were used in the mouse lymphoma cells. No increase in UDS was observed (Pellack-Walker & Blumer, 1986).

Phenol (No. 690) was tested for induction of UDS in tertiary cultures of Syrian hamster embryo cells. Phenol concentrations of 1–30 μmol/l for 1 h in the presence of hydroxyurea were used. UDS was induced in a concentration-dependent manner (Tsutsui et al., 1997).

Phenol (No. 690) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA97, TA98, TA100, TA102, TA104 and TA1535 either alone or with an exogenous induced rat liver bioactivation system, using the preincubation assay. At concentrations up to 100–5000 μg/plate alone or 20–5000 μg/plate with bioactivation, the test substance was reported to produce no increase in revertant mutants in any strain (data not given), whereas appropriate control substances established the responsiveness of the tester strains (Glatt et al., 1989).

Phenol (No. 690) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98 and TA100 either alone or with an exogenous induced rat liver bioactivation system, using the plate incorporation assay. At two concentrations up to 94 μg/plate, the test substance was reported to produce no increase in revertant mutants in any strain (data not given), whereas appropriate control substances established the responsiveness of the tester strains (Kubo, Urano & Utsumi, 2002).

Phenol (No. 690) was tested for mutagenicity to Syrian hamster embryo cells. Mutations were assessed at the hypoxanthine–guanine–phosphoribosyl transferase (HGPRT) (6-thioguanine resistance and ouabain resistance) loci. At concentrations of 3, 10 and 30 μmol/l for 48 h, the test substance induced dose-related increases in mutations (Tsutsui et al., 1997).

Phenol (No. 690) was tested for induction of sister chromatid exchange (SCE) in human peripheral blood lymphocyte cells. No exogenous metabolic bioactivation system was used. Phenol concentrations of 5, 50, 500, 700, 1000 and 3000 μmol/l were used. At increasing concentrations, an increase in SCE was observed (Erexson, Wilmer & Kligerman, 1985).

Phenol (No. 690) was tested for induction of SCE in Chinese hamster V79 cultured cells. No exogenous metabolic bioactivation system was used. A test substance concentration of 94 μg/ml (1000 μmol/l) was used. No increase in SCE was observed (Glatt et al., 1989).

Phenol (No. 690) was tested in an in vitro micronucleus assay in Chinese hamster V79 cultured cells. A phenol concentration of 376 μg/ml (4000 μmol/l) was used. No significant increase in micronucleated cells was observed (Glatt et al., 1989).

Phenol (No. 690) was tested in an in vitro micronucleus assay in cultured CHO cells either alone or with an exogenous rat liver bioactivation system. Phenol concentrations of 10, 50, 100, 175 and 250 μg/ml alone and 350, 475, 600, 800, 1000, 1500 and 2000 μg/ml with the bioactivation system were used. A weak increase in the occurrence of micronucleated cells was observed with high concentrations of phenol (Miller, Pujadas & Gocke, 1995).

Phenol (No. 690) was tested for induction of morphological transformation in Syrian hamster embryo cells. No effect was found with exposure for 48 h at 3 μmol/l, but 10, 30 and 100 μmol/l induced increases without dose dependence (Tsutsui et al., 1997).

o-Cresol (No. 691) (as Guaiacol Special C, which is 60% by weight *o*-cresol) was tested for DNA damaging activity in *E. coli*. At five concentrations up to 5000 μg/ml, no preferential killing of the pol A^- repair-deficient strain was found. Thus, there was no evidence of DNA damage (Pepper, Hamilton & Scheetz, 1980).

o-Cresol (No. 691) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538, either alone or with an exogenous induced male rat liver bioactivation system, using a plate incorporation assay. At greater than 1.044 mg (10 μl) per plate, the test

substance killed all strains. At four concentrations up to 5220 µg (5.0 µl) per plate, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Jagannath & Brusick, 1981). Negative results were also reported (data not given) in TA98 and TA100 strains (Kubo, Urano & Utsumi, 2002).

o-Cresol (No. 691) was tested for induction of SCE in cultured CHO cells alone and with an exogenous rat liver bioactivation system. The test substance either alone or with the bioactivation system was toxic at 0.8 µg/ml. A concentration-related increase in SCE was observed with the test substance alone and with the exogenous bioactivation system (Galloway & Brusick, 1981).

m-Cresol (No. 692) was tested for induction of UDS in primary rat hepatocyte cells. *m*-Cresol concentrations of 0.251, 0.502, 1, 2.51, 5.02 and 10 µg/ml were used; the test substance was toxic at concentrations greater than 10 µg/ml. There was no increase in nuclear labelling among the cells treated with the test substance. *m*-Cresol did not induce UDS in primary rat hepatocyte cells (Cifone, 1988).

p-Cresol (No. 693) was tested for induction of UDS in human embryonic lung fibroblast WI-38 cells. *p*-Cresol concentrations of 0.01, 0.08 and 0.8 mg/ml were used. There was an increase in nuclear labelling among the cells dosed with the test substance (Crowley & Margard, 1978).

p-Cresol (No. 693) was tested for induction of UDS in primary human peripheral blood lymphocyte cells. *p*-Cresol concentrations of 5–25 µmol/l were used. There was no increase in nuclear labelling among the cells dosed with the test substance (Daugherty & Franks, 1986).

p-Cresol (No. 693) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538, either alone or with an exogenous induced male rat liver bioactivation system, using a plate incorporation assay. At four concentrations up to 108 µg (1 µl) per plate, either alone or with the bioactivation system, the test substance produced no increase in revertant mutants in any strain, whereas appropriate control substances established the responsiveness of the tester strains (Crowley & Magard, 1978). Negative results were also reported (data not given) for strains TA98 and TA100 by Kubo, Urano & Utsumi (2002).

p-Cresol (No. 693) was tested for morphological transformation of C3H10T1/2 cells. Cells were exposed to test substance at up to 6000 µg/plate (6.0 µl/plate) for 24 h and then maintained for 6 weeks before assessment of foci of transformed cells. No increase in transformation was found (Crowley & Margard, 1978).

A mixture of cresols (33.3% of each of *p*-cresol, *m*-cresol and *o*-cresol; Nos 691, 692 and 693, respectively) was tested for UDS in primary rat hepatocyte cells. Based on a preliminary cytotoxicity study, the test substance mixture at concentrations of 0.0005, 0.001, 0.003, 0.005, 0.01, 0.03, 0.05 and 0.1 µg/ml (0.5, 1, 2.5, 5, 10, 25, 50 and 100 nl/ml) was applied for 1 h followed by incubation with [^{3}H]thymidine for 3 h for incorporation during repair synthesis. The test substances were toxic at the highest concentration. The criteria for UDS assessed by

autoradiography were increases in the nuclear grain count exceeding 7.74, at least 17.3% of nuclei containing 6 or more grains or at least 2% of the nuclei containing 20 or more grains. The latter two were observed at 0.0005 and 0.001 μg/ml. However, this trend was not continued in a concentration-dependent manner. The test substance was considered weakly active in the primary rat hepatocyte UDS assay (Myhr & Brusick, 1980).

A mixture of cresols (33.3% of each of *p*-cresol, *m*-cresol and *o*-cresol; Nos 691, 692 and 693, respectively) was tested for SCE in cultured CHO cells alone and with an exogenous rat liver metabolic activation system. In the first experiment, cresols were used alone at 0.5, 1, 5, 10, 50 and 100 nl/ml or with an exogenous metabolic bioactivation system at 0.625, 1.25, 2.50, 5, 10 and 15 nl/ml. The test substance caused a marked cell cycle delay in the test without the exogenous metabolic bioactivation system, but no cell cycle delay was observed in the test with the exogenous metabolic bioactivation system. At 50 nl/ml, there was a small increase in SCE, and in the test with exogenous metabolic bioactivation, an increase in SCE was observed at 10 nl/ml. In the second experiment, cresols were used alone at 50, 75, 100 and 125 nl/ml or with an exogenous metabolic bioactivation system at 10, 20, 30, 40, 50, 75 and 100 nl/ml. A significant concentration-dependent increase in SCE was observed. In the third experiment, cresols were used with an exogenous metabolic bioactivation system at 100, 150, 200, 250, 300, 400 and 500 nl/ml. Significant increases in SCE were observed at concentrations of 200 nl/ml and above. The authors of the study concluded that cresols induce increases in SCE but are less effective in the presence of an exogenous metabolic bioactivation system (Galloway & Brusick, 1980).

Thymol (No. 709) was tested for induction of chromosomal aberration in Chinese hamster lung cells (CHL/IU) alone and with an exogenous rat liver metabolic bioactivation system. Exposures were for 6, 24 or 48 h alone or 6 h with bioactivation at concentrations up to 15 000 μg/ml. The test substance alone did not induce structural chromosomal aberrations, but it induced 10–20% aberrations with bioactivation (Kusakabe et al., 2002).

Resorcinol (No. 712) was tested for mutagenicity to bacteria by incubation with *S. typhimurium* tester strains TA98 and TA100 either alone or with an exogenous induced rat liver bioactivation system, using the plate incorporation assay. At two concentrations up to 110 μg (1 mmol) per plate, either alone or with the bioactivation system, the test substance was reported to produce no increase in revertant mutants in any strain (data not given), whereas appropriate control substances established the responsiveness of the tester strains (Kubo, Urano & Utsumi, 2002).

4-(1,1-Dimethyl)-ethyl phenol (No. 733), referred to as *p-tert*-butylphenol, was tested for induction of chromosomal aberration in Chinese hamster lung cells (CHL/IU) alone and with an exogenous rat liver metabolic bioactivation system. Exposures were for 6, 24 or 48 h alone or 6 h with bioactivation at concentrations up to 30 000 μg/ml. The test substance alone did not induce structural chromosomal aberration, but it induced 10–20% aberrations with bioactivation. Also, polyploidy up to 93% was observed with 48 h of exposure (Kusakabe et al., 2002).

(ii) In vivo

2,4,6-Trimethylphenol (No. 2013) was tested for mutagenicity in the mouse bone marrow micronucleus assay. ICR mice (five of each sex per dose) were administered 0, 125, 250 or 500 mg/kg bw of the test substance by intragastric instillation in corn oil. Twenty-four hours after dosing, the bone marrow from the femur was harvested, fixed and stained. There was no indication of an increase in the numbers of micronucleated polychromatic erythrocytes (Gudi & Krsmanovic, 2002).

Magnolol (No. 2023) was tested for mutagenicity in the mouse bone marrow micronucleus assay. Male and female Swiss albino CD-1 mice (five of each sex per group) were administered test substance at a dose of 625, 1250 or 2500 mg/kg bw by intragastric instillation. At 24 or 48 h after dosing, the bone marrow from the femur was harvested, fixed and stained. There was no indication of an increase in the numbers of micronucleated polychromatic or normochromatic erythrocytes (Li et al., 2007).

Phenol (No. 690) was tested for mutagenicity by assessment of micronucleus induction in erythrocytes in the bone marrow of pregnant mice and livers of fetuses. Pregnant CD-1 mice were administered phenol at 265 mg/kg bw by intragastric instillation on day 13 of gestation. At six intervals of 15–40 h, bone marrow cells of pregnant mice were harvested, and at six intervals of 9–24 h, fetal liver cells were harvested. Both were analysed for micronucleated polychromatic erythrocytes. Statistically significant ($P < 0.05$) differences of about 2-fold from the controls in micronucleated polychromatic erythrocytes were observed at the three early time points (15–24 h) for the pregnant female mice, but statistically significant differences were not observed in liver polychromatic erythrocytes. The transient increases in micronucleated polychromatic erythrocytes observed in the pregnant female mice were not considered to be biologically significant (Ciranni et al., 1988a).

Phenol (No. 690) was tested for mutagenicity in the mouse bone marrow micronucleus assay. Male Swiss CD-1 mice were administered phenol at 265 mg/kg bw by intragastric instillation and were killed at 18–48 h after dosing. Bone marrow cells were harvested and analysed for micronucleated polychromatic erythrocytes. Dosing produced a 3- to 4-fold increase ($P < 0.05$) in micronucleated cells at 24 h, but not at 18, 42 or 48 h, despite immediate and severe bone marrow cellularity depression that persisted for 48 h after dosing. In the same study, when phenol was administered to male Swiss CD-1 mice by intraperitoneal injection at 265 mg/kg bw, an increase in micronucleated polychromatic erythrocytes was observed at 18 h, which rapidly decreased and was no longer significant at 42 h. Bone marrow depression was higher and longer lasting following injection compared with oral administration (Ciranni et al., 1988b).

Phenol (No. 690) was tested for mutagenicity in the sex-linked recessive lethal assay in adult *Drosophila melanogaster.* The test substance was fed for 3 days at 2000 µg/ml or injected at 5250 µg/ml in males before mating. These doses were intended to achieve 30% mortality. No increase in mutations in broods was found, whereas the positive control was active (Woodruff et al., 1985).

o-Cresol (No. 691) was tested for mutagenicity in the sex-linked recessive lethal assay in adult *D. melanogaster.* The test substance was fed to males for 3 days at 100, 500 and 1000 µg/ml in water before mating. The high dose approximated an LD_{50}. No increase in mutations in broods was found, whereas the positive control was active (Sernau, 1989).

(d) Reproductive toxicology

Phenol (No. 690) was studied in a standard two-generation reproductive toxicity study. Groups of 30 male and 30 female Sprague-Dawley rats were administered phenol (100% pure) in the drinking-water at target doses of 0 (control), 20, 100 and 500 mg/kg bw per day beginning 10 weeks prior to mating and continuing uninterrupted for two generations (P_1 and P_2). The average daily intake was calculated to be 0, 14.7, 70.9 and 301.0 mg/kg bw per day for parental (P_1) males and 0, 20.0, 93.0 and 320.5 mg/kg bw per day for females for control, low, middle and high dose groups, respectively. Significant reductions in water consumption (probably due to taste aversion) and, to a lesser extent, food consumption were observed in the 500 mg/kg bw per day group across both generations, with concomitant reductions in body weight and body weight gain. Mating performance and fertility in both generations were similar in dosed groups compared with control animals. Vaginal cytology/cyclicity and male reproductive functions (sperm counts, motility and morphology) were unaffected by dosing or the effects were not biologically significant, and no adverse dosing-related findings were observed microscopically in the testes, ovaries, uterus, prostate or any other tissues examined in either generation. Litter survival and offspring body weight were lowered in the 500 mg/kg bw per day group across both generations. The NOEL for reproductive toxicity of phenol in drinking-water was determined to be 70.9 and 93.0 mg/kg bw per day for males and females, respectively, based on decreased pup survival (probably due to taste aversion) at the highest administered dose, 320.5 mg/kg bw per day (Ryan et al., 2000, 2001).

Phenol (No. 690) was tested for developmental toxicity in rats. Phenol (90% United States Pharmacopeia [USP]) was administered to 25 presumed pregnant female Sprague-Dawley rats at doses of 0, 20, 40 and 120 mg/kg bw per dose 3 times daily by instragastric instillation on days 6 through 15 of gestation for daily doses of 0, 60, 120 and 360 mg/kg bw. The maternal NOEL is 60 mg/kg bw per day based on significantly reduced body weights and body weight gains at doses greater than or equal to 120 mg/kg bw per day. The NOEL for developmental toxicity is 120 mg/kg bw per day based on significantly reduced fetal body weights and reduced average number of ossification sites for the metatarsals in the high dose group (York, 1997).

o-Cresol (No. 691), *m*-cresol (No. 692) or *p*-cresol (No. 693) was tested for developmental toxicity in rabbits. Groups of 28 control and 14 treated timed-pregnant primiparous New Zealand White rabbits were administered 0, 5, 50 or 100 mg *o*-cresol (99.7% pure), *m*-cresol (99.4% pure) or *p*-cresol (98.93% pure) per kilogram body weight in corn oil by gastric instillation on gestation days 6–18. The pregnant animals were observed twice daily for clinical signs of toxicity, and all were weighed on gestation days 0, 6, 12, 18, 24 and 29. All does were killed on day 29

of gestation, and liver and uterine contents were weighed and/or examined. All live fetuses were removed from the uterus, counted, weighed, sexed and examined for external malformations and variations. The heads of approximately one half of the fetuses were preserved and prepared for assessment of soft tissue craniofacial malformations, and all of the fetuses were preserved and prepared for skeletal examinations. Oral administration of *o*-cresol, *m*-cresol or *p*-cresol during organogenesis resulted in maternal toxicity at 50 and 100 mg/kg bw per day for all three compounds. No embryotoxicity or teratogenicity was observed at any of the dose levels employed for the three cresol isomers. Slight fetotoxicity was reported at 100 mg/kg bw per day for *o*-cresol only. The NOEL for maternal toxicity was 5 mg/kg bw per day for all three cresol isomers, and the NOEL for developmental toxicity was 50 mg/kg bw per day for *o*-cresol and 100 mg/kg bw per day for *m*-cresol and *p*-cresol (Tyl, 1988b).

o-Cresol (No. 691) was tested in a standard two-generation reproductive toxicity assay in rats. Groups of 25 male and 25 female Sprague-Dawley rats were administered test substance (97% pure) by intragastric instillation in corn oil at doses of 0 (control), 30, 175 and 450 mg/kg bw per day, 5 days/week, beginning 10 weeks prior to mating and continuing uninterrupted for two generations (P_1 and P_2). Dosing of *o*-cresol at 450 mg/kg bw per day resulted in significant parental mortality or other signs of toxicity in the P_1 and P_2 generations. The observed clinical signs of toxicity in F_1 males and females (i.e. P_2 generation) at 175 mg/kg bw per day resulted in a NOEL for *o*-cresol of 30 mg/kg bw per day for the parental animals. F_1 male offspring dosed at 450 mg/kg bw per day exhibited reduced body weights prior to initiation of their premating treatment; therefore, for offspring, the NOEL for *o*-cresol was determined to be 175 mg/kg bw per day. There were no dosing-related reproductive effects observed in this study (Tyl & Neeper-Bradley, 1989a).

m-Cresol (No. 692) was tested in a standard two-generation reproductive toxicity assay in rats. Test substance (99.4% pure) was administered by intragastric instillation in corn oil to 25 male and 25 female Sprague-Dawley rats at target doses of 0 (control), 30, 175 and 450 mg/kg bw per day, 5 days/week, beginning 10 weeks prior to mating and continuing uninterrupted for two generations (P_1 and P_2). Dosing of *m*-cresol at 450 mg/kg bw per day resulted in significant parental mortality or other signs of toxicity in the P_1 and P_2 generations. Indications of concomitant perinatal toxicity were observed in F_1 and F_2 pups at 450 mg/kg bw per day. F_1 parental males at approximately 4–6 weeks of age exhibited reduced body weights at all dose levels, as did females at 30 and 450 mg/kg bw per day. Because of the consistently reduced body weights of F_1 males and females (i.e. P_2 generation) at all dose levels, a NOEL could not be determined. For offspring, the NOEL for *m*-cresol was determined to be 175 mg/kg bw per day. There were no dosing-related reproductive effects observed in this study (Tyl & Neeper-Bradley, 1989b).

p-Cresol (No. 693) was tested for reproductive toxicity in a standard two-generation assay in rats. *p*-Cresol (98.9% pure) was administered by intragastric instillation in corn oil to 25 male and 25 female Sprague-Dawley rats at target doses of 0 (control), 30, 175 and 450 mg/kg bw per day, 5 days/week, beginning 10 weeks prior to mating and continuing uninterrupted for two generations (F_1 and F_2). Administration of *p*-cresol at 450 mg/kg bw per day resulted in significant parental

mortality or other signs of toxicity in the F_1 and F_2 generations. F_2 pups exhibited occasionally reduced body weight gains at 450 mg/kg bw per day. The observed clinical signs of toxicity in F_0 and F_1 males and F_1 females (i.e. F_2 generation) at 175 mg/kg bw per day resulted in a NOEL for *p*-cresol of 30 mg/kg bw per day for the parental animals. For offspring, the NOEL for *p*-cresol was determined to be 175 mg/kg bw per day. There were no treatment-related reproductive effects observed in this study (Tyl & Neeper-Bradley, 1989c).

o-Cresol (No. 691), *m*-cresol (No. 692) or *p*-cresol (No. 693) was tested for developmental toxicity in rats. Groups of 50 control and 25 treated timed-pregnant primiparous Sprague-Dawley rats were administered 0, 30, 175 or 450 mg *o*-cresol (99.7% pure), *m*-cresol (99.4% pure) or *p*-cresol (98.93% pure) per kilogram body weight by intragastric instillation in corn oil on gestation days 6–15. The pregnant animals were observed twice daily for clinical signs of toxicity, and all were weighed on gestation days 0, 6, 11, 15 and 21. All dams were killed on day 21 of gestation, and liver and uterine contents were weighed and/or examined. All live fetuses were removed from the uterus, counted, weighed, sexed and examined for external malformations and variations. Approximately one half of the fetuses were preserved and prepared for visceral examinations, and the remaining fetuses were preserved and prepared for skeletal examinations. Oral administration of *o*-cresol, *m*-cresol or *p*-cresol during organogenesis resulted in maternal toxicity at 450 mg/kg bw per day for all three compounds. No embryotoxicity or teratogenicity was observed at any of the dose levels employed for the three cresol isomers. Slight toxicity was reported at 450 mg/kg bw per day for *o*-cresol and *p*-cresol. The NOEL for maternal toxicity was 175 mg/kg bw per day for all three cresol isomers, and the NOEL for developmental toxicity was 175 mg/kg bw per day for *o*-cresol and *p*-cresol and 450 mg/kg bw per day for *m*-cresol (Tyl, 1988a).

A mixture of *m*- and *p*-cresol isomers (58.7% and 41.3%, respectively) was tested for reproductive toxicity in mice using the Fertility Assessment by Continuous Breeding protocol (Heindel et al., 1997), which consisted of four related tasks, as follows:

- Task 1: Dose range–finding phase
- Task 2: Continuous breeding phase
- Task 3: Crossover mating trial
- Task 4: Offspring reproductive performance phase.

After the dose range–finding phase, the *m*-cresol/*p*-cresol mixture (purity not given) was administered in the diet to three groups of 20 male and 20 female mice at concentrations of 0%, 0.25%, 1.0% and 1.5% in feed (calculated to be 0, 370, 1500 and 2100 mg/kg bw per day for 18 weeks during the continuous breeding phase). A small number of deaths occurred in all groups, but not in a dose-related manner. There was no effect on the mean number of litters per mating pair at the low and middle doses, whereas in the high dose group, the number of live pups per litter was reduced by approximately 20%, and the cumulative days to deliver each litter was increased by 1–4 days. Although pup viability was unaffected by exposure to *m*-cresol/*p*-cresol in the diet, pup body weights were significantly lower than those

of controls after postnatal day 7 in the middle and high dose groups, and by postnatal day 21, these mean pup body weights were reduced 28% and 23%, respectively.

In Task 3, animals from the control and high dose groups were tested in a crossover mating trial to determine whether males, females or both sexes had compromised reproductive performance when mated with control animals. The results indicated that there were no changes in any fertility or reproductive end-point, except that body weight for the pups from either treated males or treated females was reduced by 6–8%; however, pup number and viability were unaffected.

In Task 4, the reproductive performance of the offspring from litters of all dose groups exposed to *m*-cresol/*p*-cresol via lactation and diet and control groups was evaluated. No significant differences were seen for mating index, fertility indices, litter size, proportion of pups born alive or sex ratio. The only adverse effect observed was a 13% reduction in adjusted pup weight in the high dose group. In the F_1 adults, there were reductions in the weights of the reproductive organs at necropsy at the middle and high dose levels. However, in these animals, changes in pup growth and weights of somatic organs occurred at all dose levels (Heindel et al., 1997).

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SIMPLE ALIPHATIC AND AROMATIC SULFIDES AND THIOLS (addendum)

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1. EVALUATION

1.1 Introduction

The Committee evaluated 36 additional flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols that was evaluated previously. This group included 4 simple sulfides (Nos 1909–1911 and 1939), 13 acyclic sulfides with oxidized side-chains (Nos 1912, 1913, 1915–1922 and 1940–1942), 3 cyclic sulfides (Nos 1923, 1943 and 1944), 1 simple thiol (No. 1924), 8 thiols with oxidized side-chains (Nos 1914, 1925–1929, 1936 and 1938), 5 simple disulfides (Nos 1930–1933 and 1935), 1 trisulfide (No. 1934) and 1 thioester (No. 1937). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Figure 1, Introduction) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 137 other members of this group of flavouring agents at its fifty-third meeting (Annex 1, reference *143*). The group was divided into 12 subgroups on the basis of the position of the sulfur atom, in order to facilitate the assessment of the relevant data on metabolism and toxicity. The Committee concluded that all 137 flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee also evaluated 12 additional members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The Committee concluded that all 12 additional flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee evaluated another 51 additional members of this group of flavouring agents at its sixty-eighth meeting (Annex 1, reference *187*). The Committee concluded that all 51 additional flavouring agents in that group were of no safety concern at estimated dietary exposures.

Ten of the 36 flavouring agents evaluated at the current meeting are natural components of foods (Nos 1909, 1910, 1913, 1915, 1916, 1918, 1923, 1932, 1933 and 1937) and have been detected in beef, fish oil, onion, shallot, potato chips, cabbage, peanut, apple, pineapple, melon, yellow passion fruit, coffee and beer (Nijssen, van Ingen-Visscher & Donders, 2008).

1.2 Assessment of dietary exposure

The total annual volumes of production of the 36 flavouring agents in this group are approximately 0.3 kg in Europe (European Flavour and Fragrance Association, 2004), 2 kg in the USA (Gavin, Williams & Hallagan, 2008) and 19 kg in Japan (Japan Flavor and Fragrance Materials Association, 2005). In Europe, only methyl 1-propenyl sulfide (No. 1910), 2-(methylthio)ethyl acetate (No. 1913) and 3-mercaptohexanal (No. 1929) are produced (each accounts for one third of the total annual volume of production). Only four are produced in the USA, with (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928) and 3-(methylthio)propyl hexanoate (No. 1941) accounting for the largest part of the total annual volume of production (42% each). All but five of these flavouring agents are produced in Japan, with methyl octyl sulfide (No. 1909) and 2-ethylhexyl 3-mercaptopropionate (No. 1938) making the largest contribution to the total annual volume of production (32% each).

The estimated dietary exposures for each of the flavouring agents, calculated either as the maximized survey-derived intake (MSDI) or using the single portion exposure technique (SPET), are reported in Table 1. The estimated daily dietary exposure is the highest for 3-(methylthio)propyl hexanoate (No. 1941) (1500 µg, the SPET value obtained for composite foods). For the other flavouring agents, the estimated daily per capita dietary exposures varied from 0.1 from 400 µg. For all of these flavouring agents except (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928) and 3-mercaptopropionic acid (No. 1936), the SPET gave the highest estimate. Annual volumes of production of this group of flavouring agents and the calculated daily dietary exposures (MSDI and SPET) are summarized in Table 2.

Table 1. Summary of the results of the safety evaluations of simple aliphatic and aromatic sulfides and thiols used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup i: Simple sulfides							
Structural class I							
Methyl octyl sulfide	1909	3698-95-1	No, SPET: 400	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) (Butterworth et al., 1975) is at least 37 500 times the estimated daily dietary exposure to No. 1909 when used as a flavouring agent.	NR	Note 1	No safety concern
Methyl 1-propenyl sulfide	1910	0152-77-9	No, SPET: 2	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) (Butterworth et al., 1975) is at least 7 500 000 times the estimated daily dietary exposure to No. 1910 when used as a flavouring agent.	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Di-(1-propenyl)-sulfide (mixture of isomers)	1911	65819-74-1;37981-37-6; 37981-36-5	No, SPET: 80	B4. Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) (Butterworth et al., 1975) is at least 187 500 times the estimated daily dietary exposure to No. 1911 when used as a flavouring agent.	NR	Note 1	No safety concern
Structural class III							
Butanal dibenzyl thioacetal	1939	101780-73-8	No, SPET: 40	B4. No.	Yes.	Note 1	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup ii: Acyclic sulfides with oxidized side-chains							
Structural class I							
Ethyl 2-hydroxyethyl sulfide	1912	110-77-0	No, SPET: 3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 28 000 times the estimated daily dietary exposure to No. 1912 when used as a flavouring agent.	NR	Notes 1 and 2	No safety concern
2-(Methylthio)-ethyl acetate	1913	5862-47-5	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 280 times the estimated daily dietary exposure to No. 1913 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Ethyl 3-(methylthio)-(2*Z*)-propenoate	1915	136115-66-7	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 280 times the estimated daily dietary exposure to No. 1915 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
Ethyl 3-(methylthio)-(2*E*)-propenoate	1916	136115-65-6	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 280 times the estimated daily dietary exposure to No. 1916 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Ethyl 3-(methylthio)-2-propenoate (mixture of isomers)	1917	77105-51-2	No, SPET: 300	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 280 times the estimated daily dietary exposure to No. 1917 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
4-Methyl-2-(methylthiomethyl)-2-pentenal	1918	40878-73-7	No, SPET: 0.125	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 672 000 times the estimated daily dietary exposure to No. 1918 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
4-Methyl-2-(methylthiomethyl)-2-hexenal	1919	99910-84-6	No, SPET: 1.5	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 56 000 times the estimated daily dietary exposure to No. 1919 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern
5-Methyl-2-(methylthiomethyl)-2-hexenal	1920	85407-25-6	No, SPET: 3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 28 000 times the estimated daily dietary exposure to No. 1920 when used as a flavouring agent.	NR	Notes 1 and 4	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Butyl β-(methylthio)-acrylate	1921	77105-53-4	No, SPET: 0.3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 280 000 times the estimated daily dietary exposure to No. 1921 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern
Ethyl 3-(ethylthio)-butyrate	1922	90201-28-8	No, SPET: 24	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance ethyl 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 3500 times the estimated daily dietary exposure to No. 1922 when used as a flavouring agent.	NR	Notes 1 and 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/ day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Methional diethyl acetal	1940	16630-61-8	No, SPET: 6	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance ethyl 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 14 000 times the estimated daily dietary exposure to No. 1940 when used as a flavouring agent.	NR	Note 1	No safety concern
3-(Methylthio)-propyl hexanoate	1941	906079-63-8	No, SPET: 1500	B4. No.	Yes.	Notes 1 and 3	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Structural class III							
1-(3-(Methylthio)-butyryl)-2,6,6-trimethylcyclohexene	1942	68697-67-6	No, SPET: 0.25	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (Cox, Rucci & Babish, 1979) is at least 336 000 times the estimated daily dietary exposure to No. 1942 when used as a flavouring agent.	NR	Notes 1 and 5	No safety concern
Subgroup iii: Cyclic sulfides							
Structural class II							
2-Oxothiolane	1923	1003-10-7	No, SPET: 6	B4. Yes. The NOEL of 9.2 mg/kg bw per day for the related substance 4,5-dihydro-3(2H)-thiophenone (No. 498) (Morgareidge, 1970) is at least 92 000 times the estimated daily dietary exposure to No. 1923 when used as a flavouring agent.	NR	Note 1	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Structural class III							
(±)-*cis*- and *trans*-2-Pentyl-4-propyl-1,3-oxathiane	1943	59323-81-8	Yes, SPET: 300	Additional data: No.	NR	Note 1	Additional data required to complete evaluation
2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	1944	1094004-39-3	Yes, SPET: 300	Additional data: No.	NR	Note 1	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup iv: Simple thiols							
Structural class I							
Dodecanethiol	1924	112-55-0 SH	No, SPET: 1.5	B4. Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) (Morgareidge & Oser, 1970a) is at least 22 400 times the estimated daily dietary exposure to No. 1924 when used as a flavouring agent.	NR	Notes 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup v: Thiols with oxidized side-chains							
Structural class I							
2-Hydroxyethanethiol	1925	60-24-2	No, SPET: 600	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 190–280 times the estimated daily dietary exposure to No. 1925 when used as a flavouring agent.	NR	Notes 2, 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
4-Mercapto-4-methyl-2-hexanone	1926	851768-52-0	No, SPET: 0.3	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 380 000–560 000 times the estimated daily dietary exposure to No. 1926 when used as a flavouring agent.	NR	Notes 5, 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
3-Mercapto-3-methylbutyl isovalerate	1927	612071-27-9 (structure: SH, O, O)	No, SPET: 20	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 5700–8400 times the estimated daily dietary exposure to No. 1927 when used as a flavouring agent.	NR	Notes 3, 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
(±)-Ethyl 3-mercapto-2-methylbutanoate	1928	888021-82-7	No, MSDI: Europe ND USA 0.1 Japan ND	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 1 140 000–1 680 000 times the estimated daily dietary exposure to No. 1928 when used as a flavouring agent.	NR	Notes 3, 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
3-Mercaptohexanal	1929	51755-72-7	No, SPET: 3	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 38 000–56 000 times the estimated daily dietary exposure to No. 1929 when used as a flavouring agent.	NR	Notes 4, 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
3-Mercaptopropionic acid	1936	107-96-0	No, MSDI: Europe ND USA ND Japan 0.5	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 228 000–336 000 times the estimated daily dietary exposure to No. 1936 when used as a flavouring agent.	NR	Notes 6 and 7	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
2-Ethylhexyl 3-mercaptopropionate	1938	50448-95-8	No, SPET: 30	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) are at least 3800–5600 times the estimated daily dietary exposure to No. 1938 when used as a flavouring agent.	NR	Notes 3, 6 and 7	No safety concern
Structural class III							
3-(Methylthio)propyl mercaptoacetate	1914	852997-30-9	Yes, SPET: 300	Additional data: No.	NR	Notes 1, 3, 6 and 7	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 µg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup vii: Simple disulfides							
Structural class I							
Diisoamyl disulfide	1930	2051-04-9	No, SPET: 10	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) (Posternak, Linder & Vodoz, 1969) is at least 43 800 times the estimated daily dietary exposure to No. 1930 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Butyl propyl disulfide	1932	72437-64-0	No, SPET: 0.2	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) (Posternak, Linder & Vodoz, 1969) is at least 2 190 000 times the estimated daily dietary exposure to No. 1932 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Di-*sec*-butyl disulfide	1933	5943-30-6	No, SPET: 50	B4. Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) (Posternak, Linder & Vodoz, 1969) is at least 8760 times the estimated daily dietary exposure to No. 1933 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Structural class III							
Bis(2-methylphenyl) disulfide	1931	4032-80-8	Yes, SPET: 350	Additional data: No.	NR	Notes 7, 8 and 9	Additional data required to complete evaluation

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Methyl 2-methylphenyl disulfide	1935	35379-09-0	No, SPET: 0.2	B4. Yes. The NOEL of 3.4 mg/kg bw per day for the related substance 2-naphthalenethiol (No. 531) (Morgareidge, 1971b) is at least 1 020 000 times the estimated daily dietary exposure to No. 1935 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern
Subgroup ix: Trisulfides							
Structural class I							
Diisoamyl trisulfide	1934	955371-64-9	No, SPET: 2	B4. Yes. The NOEL of 4.8 mg/kg bw per day for the related substance dipropyl trisulfide (No. 585) (Morgareidge & Oser, 1970b) is at least 144 000 times the estimated daily dietary exposure to No. 1934 when used as a flavouring agent.	NR	Notes 7, 8 and 9	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4*[e] Adequate margin of safety for the flavouring agent or related substances? / Are additional data available for substances with an estimated intake exceeding the threshold of concern?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusion based on current dietary exposure
Subgroup xi: Thioesters							
Structural class I							
Methyl isobutanethioate	1937	42075-42-3	No, SPET: 60	B4. Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) (Shellenberger, 1970) is at least 6500 times the estimated daily dietary exposure to No. 1937 when used as a flavouring agent.	NR	Note 10	No safety concern

bw, body weight; CAS, Chemical Abstracts Service; ND, no data reported; NOEL, no-observed-effect level; NR, not required for evaluation

[a] One hundred and thirty-seven flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols were previously evaluated by the Committee at its fifty-third meeting (Annex 1, reference *143*), 12 additional members at its sixty-first meeting (Annex 1, reference *166*) and 51 additional members at its sixty-eighth meeting (Annex 1, reference *187*).

[b] *Step 1:* Twenty-eight flavouring agents in this group are in structural class I (Nos 1909–1913, 1915–1922, 1924–1930, 1932–1934, 1936–1938, 1940 and 1941), 1 is in structural class II (No. 1923) and the remaining 7 are in structural class III (Nos 1914, 1931, 1935, 1939 and 1942–1944).

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

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

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

Table 1 (contd)

Notes:

1. The sulfur is expected to be oxidized to the sulfoxide and sulfone.
2. The hydroxy group is expected to undergo oxidation to the carboxylic acid and/or conjugation with glucuronic acid, followed by excretion.
3. The ester is expected to undergo hydrolysis to the corresponding carboxylic acid and alcohol.
4. The aldehyde group is expected to be oxidized to the corresponding carboxylic acid, conjugated and subsequently excreted.
5. The ketone group is expected to be reduced to the alcohol, conjugated and subsequently excreted.
6. The sulfur is expected to be oxidized to sulfonic acid and/or undergo methylation, followed by excretion.
7. Free thiols may form mixed disulfides with glutathione or cysteine.
8. The disulfides or trisulfides are expected to be reduced to free thiols.
9. The geminal dithiols are expected to be hydrolysed to yield their parent aldehydes and hydrogen sulfide.
10. The thioester is expected to undergo hydrolysis to acetate and the corresponding thiol, which will be further oxidized.

Table 2. Annual volumes of production and daily dietary exposures for simple aliphatic and aromatic sulfides and thiols used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Methyl octyl sulfide (1909)				400	6.7	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	6	1.5	0.02			
Methyl 1-propenyl sulfide (1910)				2	0.03	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.4	0.1	0.002			
Di-(1-propenyl)-sulfide (mixture of isomers) (1911)				80	1.3	−
Europe	ND	ND	ND			
USA	0.2	0.02	0.0004			
Japan	ND	ND	ND			
Ethyl 2-hydroxyethyl sulfide (1912)				3	0.05	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
2-(Methylthio)-ethyl acetate (1913)				300	5.0	+
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.2	0.06	0.001			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
3-(Methylthio)propyl mercaptoacetate (1914)				300	5.0	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.4	0.09	0.002			
Ethyl 3-(methylthio)-(2*Z*)-propenoate (1915)				300	5.0	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.05	0.001			
Ethyl 3-(methylthio)-(2*E*)-propenoate (1916)				300	5.0	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.05	0.001			
Ethyl 3-(methylthio)-2-propenoate (1917)				300	5.0	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.05	0.001			
4-Methyl-2-(methylthiomethyl)-2-pentenal (1918)				0.1	0.002	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
4-Methyl-2-(methylthiomethyl)-2-hexenal (1919)				1.5	0.03	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
5-Methyl-2-(methylthiomethyl)-2-hexenal (1920)				3	0.05	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.8	0.2	0.004			
Butyl β-(methylthio)-acrylate (1921)				0.3	0.01	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Ethyl 3-(ethylthio)-butyrate (1922)				24	0.4	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
2-Oxothiolane (1923)				6	0.1	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Dodecanethiol (1924)				1.5	0.025	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.06	0.0009			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
2-Hydroxyethanethiol (1925)				600	10	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.5	0.1	0.002			
4-Mercapto-4-methyl-2-hexanone (1926)				0.3	0.005	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
3-Mercapto-3-methylbutyl isovalerate (1927)				20	0.3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
(±)-Ethyl 3-mercapto-2-methylbutanoate (1928)				0.001	0.000 02	–
Europe	ND	ND	ND			
USA	1	0.1	0.002			
Japan	ND	ND	ND			
3-Mercaptohexanal (1929)				3	0.05	–
Europe	0.1	0.01	0.0002			
USA	ND	ND	ND			
Japan	0.8	0.2	0.004			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	
Diisoamyl disulfide (1930)				10	0.2	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Bis(2-methylphenyl) disulfide (1931)				350	5.8	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Butyl propyl disulfide (1932)				0.2	0.003	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Di-*sec*-butyl disulfide (1933)				50	0.8	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Diisoamyl trisulfide (1934)				2	0.03	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
Methyl 2-methylphenyl disulfide (1935)				0.2	0.003	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
3-Mercaptopropionic acid (1936)				0.1	0.002	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	2	0.5	0.008			
Methyl isobutanethioate (1937)				60	1.0	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.4	0.1	0.002			
2-Ethylhexyl 3-mercaptopropionate (1938)				30	0.5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	6	1.5	0.03			
Butanal dibenzyl thioacetal (1939)				40	0.7	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.04	0.0007			
Methional diethyl acetal (1940)				6	0.1	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
3-(Methylthio)propyl hexanoate (1941)				1500	25	–
Europe	ND	ND	ND			
USA	1	0.1	0.002			
Japan	ND	ND	ND			

Table 2 (contd)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Annual volume from natural occurrence in foods (kg)
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
1-(3-(Methylthio)-butyryl)-2,6,6-trimethylcyclohexene (1942)				0.25	0.004	−
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0004			
(±)-*cis*- and *trans*-2-Pentyl-4-propyl-1,3-oxathiane (1943)				300	5.0	−
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (1944)				300	5.0	−
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Total						
Europe	0.3					
USA	2					
Japan	19					

bw, body weight; ND, no data reported; +, reported to occur naturally in foods (Nijssen, van Ingen-Visscher & Donders, 2008), but no quantitative data; −, not reported to occur naturally in foods

[a] From European Flavour and Fragrance Association (2004), Gavin, Williams & Hallagan (2008) and Japan Flavor and Fragrance Materials Association (2005). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:

Table 2 (contd)

(annual volume, kg) × (1 × 10^9 μg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (European Flavour and Fragrance Association, 2004; Japan Flavor and Fragrance Materials Association, 2005; Gavin, Williams & Hallagan, 2008).
MSDI (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (μg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (International Organization of the Flavor Industry, 2009). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

1.3 Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols has previously been described in the monographs of the fifty-third, sixty-first and sixty-eighth meetings (Annex 1, references *144*, *167* and *188*). No additional relevant data have been reported since these meetings.

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 36 flavouring agents in this group of simple aliphatic and aromatic sulfides and thiols, the Committee assigned 28 flavouring agents to structural class I (Nos 1909–1913, 1915–1922, 1924–1930, 1932–1934, 1936–1938, 1940 and 1941), 1 flavouring agent to structural class II (No. 1923) and 7 flavouring agents to structural class III (Nos 1914, 1931, 1935, 1939 and 1942–1944) (Cramer, Ford & Hall, 1978).

Step 2. None of the flavouring agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the B-side of the Procedure.

Step B3. The highest estimated daily per capita intakes of the 28 flavouring agents in structural class I and the 1 flavouring agent in structural class II are below the respective thresholds of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II). Accordingly, the evaluation of these 29 flavouring agents proceeded to step B4.

The highest estimated daily per capita intakes of three flavouring agents in structural class III (Nos 1935, 1939 and 1942) are below the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of these three

flavouring agents proceeded to step B4. The highest estimated daily per capita intakes of the four remaining flavouring agents in structural class III (Nos 1914, 1931, 1943 and 1944) are 350 μg for No. 1931 and 300 μg for Nos 1914, 1943 and 1944 (calculated using the SPET) and are above the threshold of concern (i.e. 90 μg/person per day for class III). Therefore, additional data are necessary for the evaluation of these flavouring agents.

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

In accordance with the Procedure, additional data were evaluated for 3-(methylthio)propyl mercaptoacetate (No. 1914), bis(2-methylphenyl) disulfide (No. 1931), (±)-*cis*- and *trans*-2-pentyl-4-propyl-1,3-oxathiane (No. 1943) and 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944), as the estimated intakes exceeded the threshold of concern for structural class III (90 μg/person per day).

No. 1914

No data are available for 3-(methylthio)propyl mercaptoacetate (No. 1914) or closely related substances with which to perform a safety evaluation. Therefore, the Committee determined that additional metabolic or toxicological data would be necessary to complete the evaluation of No. 1914 at current estimated dietary exposures.

No. 1931

No data are available for bis(2-methylphenyl) disulfide (No. 1931) or closely related substances with which to perform a safety evaluation. Bis(2-methylphenyl) disulfide is expected to be reduced rapidly to a thiophenol analogue; however, the rate and extent of reduction are unknown. Therefore, the Committee determined that additional metabolic or toxicological data would be necessary to complete the evaluation of No. 1931 at current estimated dietary exposures.

No. 1943

No data are available for (±)-*cis*- and *trans*-2-pentyl-4-propyl-1,3-oxathiane (No. 1943). The no-observed-effect level (NOEL) of 0.44 mg/kg body weight (bw) per day for the closely related substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) from a 90-day study in rats (British Industrial Biological Research Association, 1976) provides a margin of safety of 88 (SPET for No. 1943 = 300 μg/day). The Committee considered that this margin of safety is inadequate and that additional data would be necessary to complete the evaluation of No. 1943 at current estimated dietary exposures.

No. 1944

No data are available for 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944). The NOEL of 0.44 mg/kg bw per day for the closely related

substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) from a 90-day study in rats (British Industrial Biological Research Association, 1976) provides a margin of safety of 88 (SPET for No. 1944 = 300 μg/day). The Committee considered that this margin of safety is inadequate and that additional data would be necessary to complete the evaluation of No. 1944 at current estimated dietary exposures.

Step B4. Subgroup i: Simple sulfides. The NOEL of 250 mg/kg bw per day for the structurally related substance methyl sulfide (No. 452) from a 14-week oral gavage study in rats (Butterworth et al., 1975) provides adequate margins of safety (ranging from 37 500 to 7 500 000) for methyl octyl sulfide (No. 1909; SPET = 400 μg/day), methyl 1-propenyl sulfide (No. 1910; SPET = 2 μg/day) and di-(1-propenyl)-sulfide (mixture of isomers) (No. 1911; SPET = 80 μg/day) when used as flavouring agents. The Committee therefore concluded that these three flavouring agents are not of safety concern at current estimated dietary exposures.

No NOEL is available for butanal dibenzyl thioacetal (No. 1939). Although the thioacetal group in butanal dibenzyl thioacetal can be expected to be hydrolysed, the rate and extent of hydrolysis are unknown. A NOEL was not available for a structurally related substance. Accordingly, the evaluation of butanal dibenzyl thioacetal proceeded to step B5.

Subgroup ii: Acyclic sulfides with oxidized side-chains. The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from a 90-day oral study in rats (Cox, Rucci & Babish, 1979) provides adequate margins of safety, ranging from 3500 to 672 000, for ethyl 2-hydroxyethyl sulfide (No. 1912; SPET = 3 μg/day), 4-methyl-2-(methylthiomethyl)-2-pentenal (No. 1918; SPET = 0.125 μg/day), 4-methyl-2-(methylthiomethyl)-2-hexenal (No. 1919; SPET = 1.5 μg/day), 5-methyl-2-(methylthiomethyl)-2-hexenal (No. 1920; SPET = 3 μg/day), butyl β-(methylthio)acrylate (No. 1921; SPET = 0.3 μg/day), ethyl 3-(ethylthio)butyrate (No. 1922; SPET = 24 μg/day), methional diethyl acetal (No. 1940; SPET = 6 μg/day) and 1-(3-(methylthio)-butyryl)-2,6,6-trimethylcyclohexene (No. 1942; SPET = 0.25 μg/day) when used as flavouring agents. The Committee therefore concluded that these eight flavouring agents are not of safety concern at current estimated dietary exposures.

The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) provides a margin of safety of 280 for 2-(methylthio)ethyl acetate (No. 1913), ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915), ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) and ethyl 3-(methylthio)-2-propenoate (No. 1917) (SPET for Nos 1913 and 1915–1917 = 300 μg/day) when used as flavouring agents. This margin of safety is lower than the value of 1000 proposed at the forty-fourth meeting of the Committee as an adequate margin for flavouring agents on the B-side of the Procedure (Annex 1, reference *116*). However, No. 505 bears more structural alerts for toxicity compared with Nos 1913 and 1915–1917 because of its more complex molecular structure. Also, the value of 1000 was based on the comparison of the no-observed-adverse-effect level (NOAEL) with the MSDI. The Committee noted that the margin of safety for these compounds based on the MSDI (range 0.05–0.06 μg/day) is about 1 400 000. The Committee concluded that the values of 280 (based on the SPET) and about

1 400 000 (based on the MSDI) provide an adequate margin of safety and concluded that these four flavouring agents are not of safety concern at current estimated dietary exposures.

The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from a 90-day oral study in rats (Cox, Rucci & Babish, 1979) provides a margin of safety of 56 for 3-(methylthio)-propyl hexanoate (No. 1941; SPET = 1500 μg/day). This margin of safety is approximately 20 times lower than the value of 1000 proposed at the forty-fourth meeting of the Committee (Annex 1, reference *116*) and is not considered adequate. Accordingly, the evaluation of 3-(methylthio)propyl hexanoate proceeded to step B5.

Subgroup iii: Cyclic sulfides. The NOEL of 9.2 mg/kg bw per day for the structurally related substance 4,5-dihydro-3(2H)-thiophenone (No. 498) from a 90-day study in rats (Morgareidge, 1970) provides an adequate margin of safety of 92 000 for 2-oxothiolane (No. 1923; SPET = 6 μg/day). The Committee concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup iv: Simple thiols. The NOEL of 0.56 mg/kg bw per day for the structurally related substance cyclopentanethiol (No. 516) from a 90-day study in rats (Morgareidge & Oser, 1970a) provides an adequate margin of safety of 22 400 for dodecanethiol (No. 1924; SPET = 1.5 μg/day) when used as a flavouring agent. The Committee concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup v: Thiols with oxidized side-chains. For 2-hydroxyethanethiol (No. 1925), several studies of short-term toxicity were available, but it was not possible to derive an overall NOAEL for this compound. From the limitedly reported studies available, the NOAEL appears to be lower than 11 mg/kg bw per day. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, the structurally related substances 2-mercapto-3-butanol (No. 546), α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (Morgareidge, 1971a; Cox, Bailey & Morgareidge, 1974; Morgareidge, Bailey & Cox, 1974) provide a margin of safety of at least 190 for No. 1925 (SPET = 600 μg/day). This margin of safety is lower than the value of 1000 proposed at the forty-fourth meeting of the Committee (Annex 1, reference *116*). However, the value of 1000 was based on the comparison of the NOAEL with the MSDI. The Committee noted that the margin of safety for No. 1925 based on the MSDI of 0.1 μg/person per day is at least 950 000. The Committee concluded that the values of at least 190 (based on the SPET) and at least 950 000 (based on the MSDI) provide an adequate margin of safety. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, Nos 546, 547 and 560 provide adequate margins of safety, ranging from 3800 to 1 680 000, for 4-mercapto-4-methyl-2-hexanone (No. 1926; SPET = 0.3 μg/day), 3-mercapto-3-methylbutyl isovalerate (No. 1927; SPET = 20 μg/day), (±)-ethyl 3-mercapto-2-methylbutanoate (No. 1928; MSDI = 0.1 μg/day), 3-mercaptohexanal

(No. 1929; SPET = 3 μg/day), 3-mercaptopropionic acid (No. 1936; MSDI = 0.5 μg/day) and 2-ethylhexyl 3-mercaptopropionate (No. 1938; SPET = 30 μg/day) when used as flavouring agents. The Committee therefore concluded that these six flavouring agents are not of safety concern at current estimated dietary exposures.

Subgroup vii: Simple disulfides. The NOEL of 7.3 mg/kg bw per day for the structurally related substance propyl disulfide (No. 566) from a 90-day study in rats (Posternak, Linder & Vodoz, 1969) provides adequate margins of safety (range 8760–2 190 000) for diisoamyl disulfide (No. 1930; SPET = 10 μg/day), butyl propyl disulfide (No. 1932; SPET = 0.2 μg/day) and di-*sec*-butyl disulfide (No. 1933; SPET = 50 μg/day) when used as flavouring agents. The NOEL of 3.4 mg/kg bw per day for 2-naphthalenethiol (No. 531) from a 90-day study in rats (Morgareidge, 1971b) provides an adequate margin of safety (1 020 000) for methyl 2-methylphenyl disulfide (No. 1935; SPET = 0.2 μg/day) when used as a flavouring agent. No. 1935 is predicted to be reduced rapidly to the corresponding thiophenol. The Committee therefore concluded that these four flavouring agents are not of safety concern at current estimated dietary exposures.

Subgroup ix: Trisulfides. The NOEL of 4.8 mg/kg bw per day for the structurally related substance dipropyl trisulfide (No. 585) from a 90-day study in rats (Morgareidge & Oser, 1970b) provides an adequate margin of safety of 144 000 for diisoamyl trisulfide (No. 1934; SPET = 2 μg/day) when used as a flavouring agent. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Subgroup xi: Thioesters. The NOEL of 6.5 mg/kg bw per day for the structurally related substance ethyl thioacetate (No. 483) from a 90-day study in rats (Shellenberger, 1970) provides an adequate margin of safety of 6500 for methyl isobutanethioate (No. 1937; SPET = 60 μg/day) when used as a flavouring agent. The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures.

Step B5. The conditions of use for butanal dibenzyl thioacetal (No. 1939; SPET = 40) result in an intake greater than 1.5 μg/day. Therefore, the Committee determined that additional data would be necessary to complete the evaluation of this flavouring agent.

The conditions of use for 3-(methylthio)propyl hexanoate (No. 1941; SPET = 1500 μg/day) result in an intake greater than 1.5 μg/day. Therefore, the Committee determined that additional data would be necessary to complete the evaluation of this flavouring agent.

Table 1 summarizes the evaluations of the 36 additional members of the group of simple aliphatic and aromatic sulfides and thiols (Nos 1909–1944).

1.5 Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the combined intakes of the five compounds with the highest estimated dietary exposure in each subgroup in which additional compounds were evaluated, using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

Subgroup i: Simple sulfides

In the unlikely event that the flavouring agents belonging to the subgroup of simple sulfides, of which the highest estimated intakes are for Nos 452, 454, 455, 533 and 1909 (all structural class I) in Europe, the USA and Japan, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup ii: Acyclic sulfides with oxidized side-chains

In the unlikely event that the flavouring agents belonging to the subgroup of acyclic sulfides with oxidized side-chains, of which the highest estimated intakes are for Nos 466, 472, 476, 478 and 481 (all structural class I) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup iii: Cyclic sulfides

In the unlikely event that the flavouring agents belonging to the subgroup of cyclic sulfides, of which the highest estimated intakes correspond to Nos 464, 498, 499, 534 and 543 (all structural class II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

Subgroup iv: Simple thiols

In the unlikely event that the flavouring agents belonging to the subgroup of simple thiols, of which the highest estimated intakes correspond to Nos 508, 509, 520, 525 and 528 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup v: Thiols with oxidized side-chains

In the unlikely event that the flavouring agents in the subgroup of thiols with oxidized side-chains, of which the highest estimated intakes are for Nos 546, 551, 553, 558 and 561 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup vii: Simple disulfides

In the unlikely event that the flavouring agents in the subgroup of simple disulfides, of which the highest estimated intakes are for Nos 564, 565, 567, 570 and 572 (belonging to structural class I or II) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed either threshold of concern (i.e. 1800 μg/person per day for class I and 540 μg/person per day for class II).

Subgroup ix: Trisulfides

In the unlikely event that the flavouring agents in the subgroup of trisulfides, of which the highest estimated intakes are for Nos 582, 585, 587, 588 and 1701 (all structural class I) in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

Subgroup xi: Thioesters

In the unlikely event that the flavouring agents in the subgroup of thioesters, of which the highest estimated intakes correspond to Nos 484, 492, 493, 1295 and 1676 in Europe, the USA and Japan (all structural class I), were to be consumed concurrently on a daily basis, the estimated combined intakes of 5 and 14 μg/person in Europe and the USA, respectively, would not exceed the threshold of concern (i.e. 1800 μg/person per day for class I).

1.6 Consideration of secondary components

Four flavouring agents in this group (Nos 1915, 1916, 1932 and 1944) have assay values of less than 95%. The secondary component of ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915) is ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916), and the secondary component of ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) is ethyl 3-(methylthio)-(*2Z*)-propenoate (No. 1915). These compounds are expected to share the same metabolic fate and are considered not to present a safety concern at current estimated dietary exposures. The secondary components of butyl propyl disulfide (No. 1932) are dipropyl disulfide and dibutyl disulfide. They are both expected to share the same metabolic fate as the primary substance and are considered not to present a safety concern at current estimated dietary exposures. The secondary components of 2-pentenyl-4-propyl-1,3-oxathiane (mixture of isomers) (No. 1944), (2-[(2*E*)-pent-2-en-1-yl]-4-propyl-1,3-oxathiane and 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane), are expected to share the same metabolic fate as the primary substance and are considered not to present a safety concern at current estimated dietary exposures. Information on the safety of the secondary components of these flavouring agents is summarized in Annex 5.

1.7 Conclusion

In the previous evaluations of flavouring agents in the group of simple aliphatic and aromatic sulfides and thiols, studies of biological properties, acute toxicity, short-term and long-term toxicity, genotoxicity and developmental toxicity as well as observations in humans were available (Annex 1, references *144*, *167* and *188*). The toxicity data available for this evaluation supported those from previous evaluations.

The Committee concluded that 30 flavouring agents (Nos 1909–1913, 1915–1930, 1932–1938, 1940 and 1942), which are additions to the group of simple aliphatic and aromatic sulfides and thiols, would not give rise to safety concerns at current estimated dietary exposures. For the other six flavouring agents (Nos 1914, 1931, 1939, 1941, 1943 and 1944), the Committee concluded that the evaluations

could not be completed and that additional data would be necessary to complete these evaluations at current estimated dietary exposures.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes key aspects relevant to the safety evaluation of 36 simple aliphatic and aromatic sulfides and thiols, which are additions to a group of, in total, 200 flavouring agents evaluated previously by the Committee at its fifty-third, sixty-first and sixty-eighth meetings (Annex 1, references *143, 166* and *187*).

2.2 Additional considerations on intake

Annual volumes of production and dietary exposures estimated both as the MSDI and using the SPET for each flavouring agent are reported in Table 2.

Ten of the 36 flavouring agents in the group have been reported to occur naturally in traditional foods (Nijssen, van Ingen-Visscher & Donders, 2008; Table 2), but quantitative data on their natural occurrence have not been reported.

2.3 Biological data

2.3.1 Biochemical data: absorption, distribution, metabolism and elimination

No relevant information additional to that available and described in the monographs of the fifty-third, sixty-first and sixty-eighth meetings (Annex 1, references *144, 167* and *188*) was available on the absorption, distribution, metabolism or elimination of flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols.

2.3.2 Toxicological studies

(a) Acute toxicity

Oral median lethal doses (LD_{50} values) have been reported for 1 of the 36 flavouring agents evaluated in this group and are summarized in Table 3. Oral LD_{50} values ranging from 244 to 330 and from 190 to 345 mg/kg bw have been reported for 2-hydroxyethanethiol (No. 1925) in rats and mice, respectively (Smyth & Carpenter, 1944; BASF AG, 1964; Pugaeva et al., 1971; White, Bruckner & Guess, 1973; Sax et al., 1979). These results support the findings in the previous evaluations (Annex 1, references *144*, *167* and *188*) that the oral acute toxicity of simple aliphatic and aromatic sulfides and thiols is low to moderate.

(b) Short-term and long-term studies of toxicity

Only for 1 of the 36 flavouring agents evaluated in this group have results of studies of short-term toxicity been reported, although the reporting was very limited in all cases. The results of these studies with 2-hydroxyethanediol (No. 1925) are summarized in Table 4 and described below. No additional studies of short-term or

Table 3. Results of studies of oral acute toxicity with simple aliphatic and aromatic sulfides and thiols used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	References
1925	2-Hydroxyethanethiol	Rat; NR	244	Pugaeva et al. (1971)
1925	2-Hydroxyethanethiol	Rat; NR	330	BASF AG (1964)
1925	2-Hydroxyethanethiol	Rat; M	300	Smyth & Carpenter (1944)
1925	2-Hydroxyethanethiol	Rat; NR	300	Sax et al. (1979)
1925	2-Hydroxyethanethiol	Mouse; M	345	White, Bruckner & Guess (1973)
1925	2-Hydroxyethanethiol	Mouse; NR	190	Pugaeva et al. (1971)

M, male; NR, not reported

Table 4. Results of studies of oral short-term toxicity with simple aliphatic and aromatic sulfides and thiols used as flavouring agents

No.	Flavouring agent	Species; sex	No. of test groups[a]/ no. per group[b]	Route	Duration (days)	NOAEL (mg/kg bw per day)	Reference
1925	2-Hydroxyethanethiol	Rat; M, F	3/15	Oral gavage	49	15	Regnier et al. (2006)
1925	2-Hydroxyethanethiol	Rabbit; NR	3/NR	Oral gavage	NR	<11[c]	BASF AG (1967); BASF Corporation (1992)
1925	2-Hydroxyethanethiol	Cat; NR	1/NR	Oral[d]	10–19[e]	28	BASF AG (1967)
1925	2-Hydroxyethanethiol	Guinea-pig; NR	3/NR	Oral[d]	28[e]	22[c]	BASF AG (1967); BASF Corporation (1992)

F, female; M, male; NR, not reported
[a] Total number of test groups does not include control animals.
[b] Total number per test group includes both male and female animals.
[c] Lowest dose tested.
[d] Route not further specified.
[e] Number of doses; frequency not stated.

long-term toxicity of any of the related flavouring agents evaluated previously have been submitted (Annex 1, references *144*, *167* and *188*).

In a combined repeated-dose oral toxicity study and reproductive/ developmental toxicity screening test, groups of 10 male and 5 female Sprague-Dawley rats were given 2-hydroxyethanediol (No. 1925) by gavage at doses of 0, 15, 50 or 75 mg/kg bw per day for 7 weeks. Groups of 10 female rats were treated with the same doses from premating to day 21 of lactation. Only a brief abstract of this study was available. The study was reported to be conducted under good laboratory practice (GLP) and according to Organisation for Economic Co-operation and Development (OECD) Test Guidelines 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) and 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents). The reproductive/ developmental toxicity part of this study is described below in section (d).

Males in the two highest dose groups had significantly lower cholesterol and triglyceride levels, accompanied by increased liver weights and slight hepatocellular hypertrophy. Males from the highest dose group also had marked degenerative cardiomyopathy. Females from the two highest dose groups had moderately increased liver weights with minimal to slight hepatocellular hypertrophy and high incidences of vacuolated hepatocytes. In addition, these animals showed cardiomyopathy. It is not completely clear from the abstract whether these effects were observed in the females of the general toxicity study or from the reproductive/ developmental toxicity study. The NOAEL for general toxicity reported in the abstract was 15 mg/kg bw per day in both sexes (Regnier et al., 2006).

In the International Uniform Chemical Information Database (IUCLID) of the European Commission, an additional three oral short-term studies of toxicity with 2-hydroxyethanediol have been summarized briefly (European Commission, 2000), but the original study reports were not submitted. These summaries have been provided by the European chemicals industry, but they have not undergone any evaluation by the European Commission.

Rabbits (sex and number not specified) were given doses of 2-hydroxyethanediol (No. 1925) at approximately 11, 28 or 56 mg/kg bw by gavage for an unknown period of time. No rabbits survived after three doses of 56 mg/kg bw, and 3 out of 11 rabbits died after two doses of 11 mg/kg bw. Effects on white blood cell counts and liver and kidney function were observed. Body weights were decreased by about 10%. Necropsy showed fatty liver and kidney plus oedema of the lungs (BASF AG, 1967; BASF Corporation, 1992). From this limited description, the NOAEL that can be established for this study was less than 11 mg/kg bw, the lowest dose tested.

Cats (sex and number not specified) were orally administered 10 or 19 doses of 2-hydroxyethanediol (No. 1925) at approximately 28 mg/kg bw (frequency of treatment not stated). The administration of the test compound induced vomiting. No decrease in body weight was observed. Blood values and liver function were normal. After 19 doses of 2-hydroxyethanediol, blood urea was elevated. There were no abnormal pathological findings (BASF AG, 1967). From this limited description, the NOAEL in this study was probably 28 mg/kg bw, the only dose tested.

Guinea-pigs (sex and number not specified) were administered 2-hydroxyethanediol (No. 1925) at approximately 22, 56 or 112 mg/kg bw orally for up to 28 doses (frequency of treatment not stated). At 112 mg/kg bw, all animals died overnight. After 19–28 doses of 56 mg/kg bw, there were no clinical signs until the last dose, when, shortly before death, animals displayed atonia, difficulty breathing and lateral position. Animals at the lowest dose survived and showed no clinical signs. Minimal weight loss and unspecific changes in blood parameters, liver function and blood urea were reported after several doses. There were no abnormal pathological findings (BASF AG, 1967; BASF Corporation, 1992). From this limited description, the NOAEL in this study was probably 22 mg/kg bw, the lowest dose tested.

(c) Genotoxicity

(i) In vitro

Studies of genotoxicity in vitro have been reported for 3 of the 36 flavouring agents evaluated in this group (Nos 1911, 1924 and 1925). The results of these studies are summarized in Table 5 and described below. All studies were from the public literature and were not performed according to OECD guidelines, except for the study of Stien (2005).

No evidence of mutagenicity was observed in Ames assays when di-(1-propenyl)-sulfide (No. 1911), docedanethiol (No. 1924) and 2-hydroxyethanethiol (No. 1925) were incubated with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 or TA1537 with or without metabolic activation at concentrations up to 10 000 μg/plate (Carter & Josephy, 1986; Zeiger et al., 1987; Stien, 2005).

No evidence of induction of chromosomal malsegregation or recombination/mutation was observed when 2-hydroxyethanethiol (No. 1925; up to 560 μg/ml) was incubated with *Saccharomyces cerevisiae* D61.M (Albertini, Brunner & Würgler, 1993).

In two limited chromosomal aberration assays with 2-hydroxyethanethiol in human lymphocytes, an increase in gaps but not in breaks or exchanges was observed. The gaps could be attributed to a denaturing effect on the protein moiety of the chromosome, and therefore 2-hydroxyethanethiol was concluded to be negative in these assays (Brøgger, 1975; Brøgger & Waksvik, 1978). In another chromosomal aberration assay, 2-hydroxyethanethiol (No. 1925) gave inconclusive results in Chinese hamster V79 cells (Speit, Wolf & Vogel, 1980).

2-Hydroxyethanethiol (No. 1925) was negative in a sister chromatid exchange assay with human lymphocytes (Brøgger & Waksvik, 1978), whereas inconclusive results were obtained in a sister chromatid exchange assay with Chinese hamster V79 cells (Speit, Wolf & Vogel, 1980).

Table 5. Results of studies of genotoxicity in vitro with simple aliphatic and aromatic sulfides and thiols used as flavouring agents

No.	Flavouring agent	End-point	Test system	Concentration	Results	Reference
1911	Di-(1-propenyl)-sulfide	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537	1–100 µg/plate, ±S9[a]	Negative[b]	Stien (2005)
1924	Dodecanethiol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0.01–3.3 µg/plate, −S9[c]; 100–10 000 µg/plate, +S9	Negative	Zeiger et al. (1987)
1925	2-Hydroxyethanethiol	Reverse mutation	*S. typhimurium* TA100	Approximately 40–1170 µg/plate, −S9[d] (approximately 0.5–15 µmol/plate)	Negative	Carter & Josephy (1986)
1925	2-Hydroxyethanethiol	Chromosomal malsegregation or recombination/mutation	*Saccharomyces cerevisiae* D61.M	60–560 µg/ml, −S9[e,f] (0.05–0.5 µl/ml) 60–450 µg/ml, −S9[e,g] (0.05–0.4 µl/ml)	Negative	Albertini, Brunner & Würgler (1993)
1925	2-Hydroxyethanethiol	Chromosomal aberration	Human lymphocytes	0.8–78 µg/ml, −S9[d] (0.01–10 mmol/l)	Negative[h]	Brøgger (1975)
1925	2-Hydroxyethanethiol	Chromosomal aberration	Human lymphocytes	8 µg/ml, −S9[d] (1 mmol/l)	Negative	Brøgger & Waksvik (1978)
1925	2-Hydroxyethanethiol	Chromosomal aberration	Chinese hamster V79 cells	0.8 and 8 µg/ml, −S9[d] (0.1 and 1 mmol/l)	Inconclusive[i]	Speit, Wolf & Vogel (1980)
1925	2-Hydroxyethanethiol	Sister chromatid exchange	Human lymphocytes	0.8–8 µg/ml, −S9[d] (0.1–1 mmol/l)	Negative	Brøgger & Waksvik (1978)
1925	2-Hydroxyethanethiol	Sister chromatid exchange	Chinese hamster V79 cells	0.08–8 µg/ml, −S9[d] (0.01–1 mmol/l)	Inconclusive[j]	Speit, Wolf & Vogel (1980)

Table 5 (contd)

[a] Two independent experiments using the preincubation method and the plate incorporation method, respectively.
[b] Toxicity was observed in all strains, with and without metabolic activation, at the highest concentration tested.
[c] Test concentrations were 0.01–1 μg/plate for strains TA100 and TA1535 and 0.03–3.3 μg/plate for strains TA98 and TA1537.
[d] Calculated using the molecular weight of 2-hydroxyethanethiol = 78.13 g/mol.
[e] Calculated using the density of 2-hydroxyethanethiol = 1.117 g/ml.
[f] Incubated for 16 h at 28 °C.
[g] Incubated for 4 h at 28 °C, followed by 16 h at 4 °C and another 4 h at 28 °C.
[h] Increases in chromatid constrictions and gaps were observed, as well as a very small increase in breaks, but no exchanges were observed.
[i] At both concentrations, an increase in chromatid aberrations and aberrant metaphases was observed, but the effects were not dose related.
[j] At concentrations from 0.1 mmol/l onwards, the number of sister chromatid exchanges per cell was nearly doubled, but no clear dose–response relationship was observed.

(ii) Conclusion

The available data for the current evaluation are in line with the conclusion in the previous evaluation of the group of simple aliphatic and aromatic sulfides and thiols that this group is not expected to exhibit any mutagenic or genotoxic properties.

(d) Developmental/reproductive toxicity

The results of one developmental/reproductive screening test and two teratogenicity studies with 2-hydroxyethanediol (No. 1925) are described below.

In a combined repeated-dose oral toxicity study and reproductive/ developmental toxicity screening test, groups of 10 male and 5 female Sprague-Dawley rats were given 2-hydroxyethanediol (No. 1925) by gavage at doses of 0, 15, 50 or 75 mg/kg bw per day for 7 weeks. Groups of 10 female rats were treated with the same doses from premating to day 21 of lactation. Only a brief abstract of this study was available. The study was reported to be conducted under GLP and according to OECD Test Guidelines 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) and 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents). The repeated-dose toxicity part of this study is described above in section (b).

At the two highest dose levels, some pregnant rats (number not specified) were found dead or had to be euthanized on days 19–23 of gestation owing to poor clinical conditions, with no sign of delivery seen at that time. Surviving females in these groups had a prolonged mean duration of gestation, resulting in higher pup birth weights (statistical significance not stated). 2-Hydroxyethanediol treatment was stopped at the end of pregnancy to allow delivery and was resumed afterwards. At the highest dose, there were high post-implantation losses associated with low number of live births; pup survival was decreased (statistical significance not stated). As reported in the abstract, the NOAEL was 15 mg/kg bw per day for maternal toxicity and parturition and 75 mg/kg bw per day for male reproductive performance and fertility (Regnier et al., 2006).

Two teratogenicity studies were limitedly reported in IUCLID (European Commission, 2000).

Five pregnant Sprague-Dawley rats were gavaged with 2-hydroxyethanediol (No. 1925) at 168 mg/kg bw per day on gestation days 12 and 13 (BASF AG, 1965; BASF Corporation, 1992). Three of the treated rats died within 2 days of treatment. Of the two surviving rats, one had severely diminished body weight at the end of the study compared with controls. No further details were given.

Six pregnant Sprague-Dawley rats were gavaged with 2-hydroxyethanediol (No. 1925) at 67 mg/kg bw per day on gestation days 12–16 (BASF AG, 1965). A concurrent control group received no treatment. Maternal body weights were slightly lower than those of controls. The mean number of fetuses, fetal weight, fetal length and the incidence of malformations were similar to those of controls. The resorption rate (18.7%) was higher than in controls, but 10 out of the 12 observed resorptions were from one animal.

(e) Other studies

A few studies were submitted on the supposed beneficial effects of 2-hydroxyethanethiol (No. 1925) on ageing. A review of these studies is, however, beyond the scope of this monograph, which deals with the safety aspects of flavouring agents.

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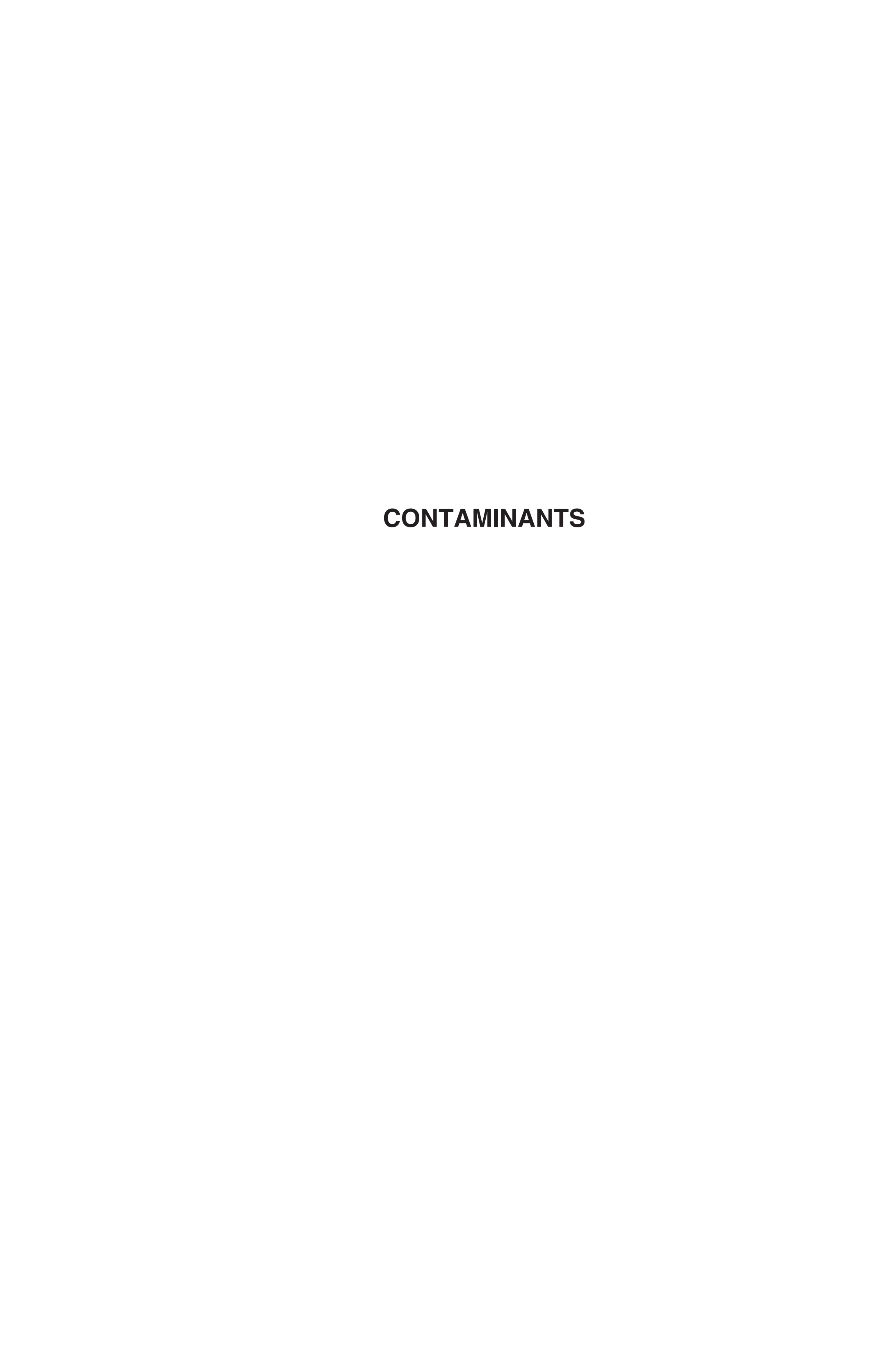

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1. EXPLANATION

The presence of cadmium (Cd) in food results from contamination of soil and water both from natural sources and from anthropogenic activities. Crops differ with respect to absorption of cadmium, and cadmium is known to accumulate in the tissues (particularly the liver and kidney) of terrestrial animals and in aquatic animals (particularly detritus feeders, such as molluscs).

Cadmium was evaluated by the Committee at its sixteenth, thirty-third, forty-first, fifty-fifth, sixty-first and sixty-fourth meetings (Annex 1, references *30*, *83*, *107*, *149*, *166* and *176*). At the thirty-third meeting, a provisional tolerable weekly intake (PTWI) of 400–500 μg or 7 μg/kg body weight (bw) (assuming a body weight of 60 kg) was derived from a critical concentration of cadmium in the kidneys (200 mg/kg tissue), which caused an increase in β_2-microglobulin (β2MG) concentration in urine, and a toxicokinetic model that related cadmium bio-accumulation in the kidneys to dietary exposure. In 1992, Environmental Health Criteria 134 (IPCS, 1992a) provided a detailed description of the model on which the PTWI was based and its various assumptions. At the forty-first meeting, the Committee concluded that the model estimates used to derive the PTWI were conservative, but it did not include a safety factor and reiterated that there was only a small margin of safety between exposure via the diet and the exposure that would result in deleterious effects.

At its fifty-fifth meeting, the Committee concluded that the prevalences of renal tubular dysfunction that correspond to various dietary exposures to cadmium were still appropriate for risk assessment and that the risk of renal tubular dysfunction in the general population would be negligible below a urinary cadmium excretion of 2.5 μg/g creatinine. The estimate of 2.5 μg/g creatinine was based on occupational data and involved a number of assumptions about creatinine excretion, cadmium absorption and bioavailability and the ratio of dietary exposure to cadmium to excreted cadmium.

At the sixty-first meeting, the Committee considered studies including epidemiological investigations of environmental exposure to cadmium, such as the CadmiBel studies from Belgium and a series of Japanese reports. The Committee reaffirmed that renal tubular dysfunction remained the critical health outcome with regard to the toxicity of cadmium and that an excess prevalence of renal tubular dysfunction would not be expected to occur if the urinary cadmium concentration did not exceed 2.5 μg/g creatinine. The Committee concluded that the new data did not provide a sufficient basis for revising the PTWI and therefore maintained the PTWI of 7 μg/kg bw.

At its sixty-fourth meeting, the Committee evaluated the impact of different maximum levels (MLs) for cadmium in commodities that contribute to dietary exposure. The dietary assessment took into account the potential impact of different MLs on the distribution of concentrations of cadmium in each commodity and the dietary exposures to cadmium from each individual commodity. The Committee concluded that a change in the proposed Codex Alimentarius Commission MLs would result in a change of only 1–6% in the dietary exposure to cadmium and therefore was of no significance in terms of risk to human health, considering that the total dietary exposure to cadmium was only 40–60% of the PTWI of 7 μg/kg bw.

At the request of the Codex Committee on Contaminants in Food, the Committee considered new information that had become available since cadmium was last evaluated, together with the data it had previously reviewed. The Committee also considered new information on cadmium levels in food and dietary exposure. As it is now acknowledged that renal dysfunction is the most sensitive toxicological end-point arising from cadmium exposure, most of the new data involved the use of urinary biomarkers to estimate risk based on statistical modelling. The Committee considered whether these recent modelled risk estimates for cadmium would support the current PTWI.

2. BIOLOGICAL DATA

A number of detailed evaluations have reviewed data on the kinetics and toxicity of cadmium (IPCS, 1992a; ATSDR, 2008; EFSA, 2009a). Overall, the absorption or bioavailability of cadmium from the gastrointestinal tract is generally considered to be slightly lower (0.5–3.0%) in experimental animals (mice, rats and monkeys) than in humans (1–10%). Previous reviews have described how the composition of the diet, including fibre, protein (sunflower seeds) and carbohydrates (rice), can also affect the bioavailability of cadmium (Annex 1, references *149* and *166*). Following absorption, cadmium binds to metallothionein, but this binding can be overloaded at relatively moderate doses. In adult experimental animals, cadmium concentrations in liver and kidneys are comparable after short-term exposure, but the kidney concentration generally exceeds the liver concentration following prolonged exposure, except at very high exposures. Cadmium is virtually absent at birth but accumulates with time, especially in the liver and kidneys; renal concentration occurs mainly during the early years of life, and 50–75% of the total body burden is found in these two organs at autopsy. An additional 20% is found in muscle, whereas the quantity of cadmium in bone is small. In human cadavers, no statistically significant relationship has been observed linking bone cadmium content with demineralization (Knuuttila et al., 1982).

2.1 Biochemical aspects

2.1.1 Absorption

Groups of 12 male ICR mice were fed a diet with sufficient iron (120 mg/kg) or insufficient iron (2–6 mg/kg) for 4 weeks. Tissue iron concentrations were analysed in half the animals at the end of the study, whereas tissue cadmium

concentrations were analysed in the remaining mice 24 h after a single oral dose of $^{109}CdCl_2$ at 100 μg/kg bw. The expression of divalent metal transporter 1 (*DMT1*) and metal transporter protein 1 (*MTP1*) genes was also analysed in various tissues. Iron stores were shown to be depleted in mice on the iron-deficient diet. The majority of the tissues analysed from the iron-deficient mice contained significantly higher ($P < 0.05$) concentrations of cadmium compared with the tissues from the iron-sufficient mice. In particular, the duodenums of the iron-deficient mice contained approximately 3-fold more cadmium than those of the iron-sufficient mice. The body burden of cadmium was also approximately 3-fold higher in iron-deficient than in iron-sufficient mice. The *DMT1* and *MTP1* genes were expressed in all of the tissues analysed in both groups of mice. However, in the iron-deficient mice, there was a 65- and 8-fold upregulation of the *DMT1* and *MTP1* genes, respectively, in the duodenum. These results indicate that iron deficiency increases the uptake of cadmium from the digestive tract, which appears to be associated with the increased expression of *DMT1* and *MTP1* in the duodenum (Kim et al., 2007).

Reeves & Chaney (2008) reviewed the effect of marginal deficiencies of zinc, iron and calcium on the gastrointestinal absorption of cadmium in rats. Absorption and retention of cadmium (present at 0.25–0.45 mg/kg) from diets containing suboptimal concentrations of zinc, iron and calcium were increased up to 10-fold following repeated dosing.

2.1.2 Elimination

From previously evaluated reports, the retention of cadmium in various tissues is variable, and its release appears to be multiphasic. The apparent half-life estimates range between 200 and 700 days for mice and rats and up to 2 years in the squirrel monkey. In humans, the reported half-life of cadmium in kidneys ranges from 10 to 33 years, and in liver, from 4 to 19 years.

Recently, Amzal et al. (2009) estimated the apparent half-life of kidney cadmium in 680 Swedish women aged between 56 and 70 years who had never smoked to be lognormally distributed, with a mean of 11.6 years (95% confidence interval [CI] 10.1–14.7) and a standard deviation of 3.0 years. The half-life of cadmium in kidneys was calculated using toxicokinetic modelling based on a one-compartment model (IPCS, 1992a). By assuming that cadmium concentration in urine was proportional to the cadmium concentration in the kidney cortex, that gastrointestinal absorption among females ranged between 1% and 10% and that approximately one third of the absorbed fraction was transported to the kidney, the model accurately predicted the observed level of cadmium in the urine except at the lowest concentrations (<0.1 μg/g creatinine).

The Amzal et al. (2009) study was the first to investigate, on a population level, variability in cadmium kinetics using paired individual data on dietary intake and urinary excretion. A determination of intervariability in a population is considered important in risk assessment because it overcomes the need to use uncertainty or safety factors to protect a portion of the population. Of particular value in this study was the availability of data matching dietary exposure with urinary cadmium levels on three separate occasions over a 20-year period (i.e. 1987, 1997

and 2007). The overall average daily dietary exposure from the three measurements was 14 µg (0.2 µg/kg bw; range 0.1–0.4 µg/kg bw) after an adjustment for a total daily energy intake of 1700 kcal. The mean urinary cadmium level was 0.34 µg/g creatinine (median 0.31 µg/g; range 0.09–1.23 µg/g).

2.2 Toxicological studies

2.2.1 Acute toxicity

Oral median lethal doses (LD_{50} values) for experimental animals (mainly rodents) range from approximately 100 to 300 mg/kg bw and are dependent on the form of cadmium administered (Annex 1, reference *166*).

2.2.2 Long-term studies of toxicity and carcinogenicity

The kidney is the critical organ in humans and other mammals exposed for long periods to the relatively small amounts of cadmium that might occur in foods and feed, respectively. Many studies in experimental animals have demonstrated an association between morphological and/or functional changes in the kidney and the renal concentration of cadmium. In most of these studies, cadmium was given parenterally rather than in food or water. The many studies of this type performed before 1990 were reported in detail and tabulated by IPCS (1992a) and are only summarized here. Although a variety of toxicological end-points have been observed in experimental animals (reproductive toxicity, neurotoxicity, carcinogenicity), those of relevance to humans are the renal effects that manifest after low-level, long-term exposure to cadmium and accumulation of cadmium to a critical concentration in the kidney (Annex 1, reference *166*).

(a) Renal effects

Long-term exposure to cadmium eventually results in a variety of renal changes involving damage to proximal tubule epithelial cells, degeneration and apoptosis. Morphological changes observed include atrophy, interstitial fibrosis, glomerular sclerosis and focal necrosis. Earlier indications usually associated with renal damage include low molecular weight proteinuria, glucosuria and amino-aciduria. The results of relevant experimental studies are summarized in Table 1. In general, although some variability exists, renal effects in laboratory animals are associated with kidney concentrations of cadmium of between 200 and 300 µg/g, resulting from long-term exposures of, on average, between 1 and 10 mg/kg bw per day.

(b) Carcinogenicity

Studies in experimental animals treated by injection or inhalation have provided considerable evidence that cadmium is carcinogenic. In rats, cadmium causes a variety of tumours, including malignant tumours at the site of injection and in the lungs after inhalation. Oral intake is associated with proliferative lesions of the ventral lobe of the prostate gland in rats fed diets that are adequate in zinc, whereas deficiency in zinc in the diet appears to inhibit the tumorigenic effect of

Table 1. NOAELs and LOAELs for renal effects of cadmium chloride administered in drinking-water

Species	Strain	Sex	Effect	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rabbit	New Zealand/ Flemish Giant	Male	Tubular necrosis Interstitial necrosis at 200 days	ND	15
Rat	Sprague-Dawley	Male and female	Cloudy swelling of tubular epithelium at 92 weeks (males) and 84 weeks (females)	0.8	1.5
	Wistar	Male	Increased urinary metallothionein; no tubular dysfunction	2.6	ND
	Sprague-Dawley	Female	Albuminuria	ND	13
Monkey	Rhesus	Male	Tubular dysfunction	0.4	4.0

LOAEL, lowest-observed-adverse-effect level; ND, not determined; NOAEL, no-observed-adverse-effect level
Source: Adapted from Annex 1, reference *150*

cadmium. The relevance of these studies to carcinogenesis in the human prostate gland is questionable because of anatomical differences between the prostate gland in humans and that in rodents.

2.2.3 Developmental toxicity

Groups of 7–9 pregnant Wistar rats were given drinking-water containing cadmium chloride (10 mg/l) from day 0 to day 21 of gestation. A control group received deionized water. At the end of the dosing period, concentrations of cadmium and lead were measured in placenta, fetal brain, fetal blood and maternal blood. Metallothionein was analysed in placenta and fetal brain. The average fetal weight of the cadmium-treated group was approximately 20% lower than that of the control group ($P < 0.05$). The average dam weight of the cadmium-treated group was approximately 14% lower than that of the control group, whereas the number of fetuses per dam was slightly lower (10 versus 13, respectively); neither difference was statistically significant. Fetal brain weight and placenta weight were similar between control and treated groups. Significantly elevated ($P < 0.05$) concentrations of cadmium were detected in dam blood, fetal blood, placenta and fetal brain (approximately 13-, 2.5-, 17- and 3-fold higher than the controls, respectively). Metallothionein concentrations in placenta, but not in brain, in the cadmium-treated group were significantly higher ($P < 0.05$) than those of the controls (4.39 μg/g versus 2.96 μg/g, respectively). Statistical analysis indicated a significant (P = 0.017) linear relationship between metallothionein and cadmium concentrations in

the placenta. Immunohistochemistry indicated a normal appearance of the placenta in the cadmium-treated group (Benitez et al., 2009).

2.2.4 Genotoxicity

Although cadmium is not a redox-active metal and therefore cannot bind directly to deoxyribonucleic acid (DNA), it has been shown to indirectly induce oxidative stress and thereby compromise the integrity of the genome. In addition to indirect DNA damage, cadmium also inhibits the mechanism necessary for DNA repair, although its precise mode of action has not been elucidated (reviewed in Giaginis, Gatzidou & Theocharis, 2006). Through this inhibition process, cadmium is able to enhance the mutagenicity induced by other DNA-damaging agents.

(a) In vitro

The excision repair of bulky DNA adducts induced by benzo(*a*)pyrene diolepoxide and of ultraviolet C–induced photolesions in two human cell lines was inhibited by non-cytotoxic concentrations of cadmium chloride or particulate cadmium oxide. Inhibition was associated with the dose-dependent uptake of Cd^{2+} into the nucleus. The mechanism of inhibition appeared to be via the disruption of the assembly and disassembly of nucleotide excision repair proteins (Schwerdtle et al., 2010).

(b) In vivo

In a micronucleus assay, cadmium chloride (15 mg/kg bw) in distilled water was administered to male Wistar rats (four per group) as a single dose or a daily gavage dose for 60 days. Negative and positive control groups received isotonic saline and mitomycin C at 2 mg/kg bw, respectively. Bone marrow and peripheral blood were sampled from the single- and repeated-dose groups, respectively, the day after the last dose. In the repeated-dose group, there was a significant increase in micronuclei in peripheral blood (5.5 versus 2.5 per 2000 polychromatic erythrocytes in the negative control; $P < 0.01$). A similar magnitude of increase occurred in bone marrow from the single-dose group (4.25 versus 2.25 per 2000 polychromatic erythrocytes in the negative control; $P < 0.001$), concomitant with cytotoxicity. These increases were approximately 4- to 5-fold lower than those of the positive control group. Blood cadmium levels were significantly higher ($P < 0.001$) in the treated groups relative to the control (approximately 2-fold higher) (Çelik et al., 2009).

2.3 Observations in humans

2.3.1 Absorption

Based on studies reviewed previously by the Committee, the gastrointestinal absorption of cadmium is influenced by diet and nutritional status, with iron status being particularly important. On average, a few per cent of the total oral exposure to cadmium is absorbed, but individual values range from less than 1% up to 5% in males and 10% in females. For women with a low iron status, gastrointestinal absorption estimates of up to 10% seem to be in concordance with toxicokinetic modelling of long-term dietary exposures and observed urinary cadmium levels

(Amzal et al., 2009). A recent study by Kippler et al. (2007) appears to confirm the important influence of iron status on cadmium absorption in women. They found that among 890 pregnant Bangladeshi women, urinary cadmium levels were higher for those with low serum ferritin and adequate plasma zinc than for those with adequate iron and zinc status ($P = 0.03$).

The cadmium content in the kidneys of 109 living donors (aged 24–70 years; median 51 years) in Sweden was determined from kidney biopsies. The average kidney cadmium concentration was 15 µg/g wet weight (median 12.9 µg/g wet weight). The concentrations of cadmium in the kidneys of non-smokers were similar to those observed in the 1970s, suggesting that exposure via the diet had changed little over the previous 40 years. Multiple linear regression analysis revealed that the kidney cadmium concentration increased by 3.9 µg/g ($P < 0.001$) for each 10-year increase in age and by 3.7 µg/g ($P < 0.001$) for each 10 pack-years of smoking. The cadmium concentrations increased by 4.5 µg/g ($P = 0.03$) for women with low iron stores (serum ferritin below 30 µg/l) and by 1.3 µg/g ($P = 0.03$) for a 10 kg reduction in body weight, suggesting that a low iron status is at least as important as 10 pack-years of smoking or a 10-year increase in age and much more important than a 10 kg reduction in body weight for cadmium accumulation in the kidney (Barregård et al., 2010).

2.3.2 Renal effects

Although there is good evidence to indicate a relationship between the urinary excretion of cadmium following renal damage and various renal biomarkers (e.g. β2MG, retinol binding protein [RBP], α_1-microglobulin [α1MG] and *N*-acetyl-β-D-glucosaminidase), especially from occupational exposure, the health significance of these nonspecific biomarkers in relation to renal damage in the general population remains uncertain. Assuming that the associations between the excretion of renal biomarkers and cadmium exposure observed in population-based studies reflect a causal relationship, then these associations imply potentially adverse effects, rendering the kidneys more susceptible to other stressors. However, these effects might also reflect only an early renal response to cadmium, which may be purely adaptive or reversible in nature. Another explanation is that the observed association between low molecular weight proteins and cadmium in urine might simply be a result of a co-excretion of markers of exposure and effect.

Several studies monitoring populations following a reduction in cadmium exposure have attempted to address the question of the reversibility of early renal changes. A modest increase in urinary excretion of β2MG or RBP (Table 2) in the range of 300–1000 µg/g creatinine, as found at the early stage, is unlikely to compromise renal function. Such a small increase is usually reversible after industrial cadmium exposure has ceased. Low molecular weight proteinuria in itself does not appear to give rise to any subjective symptoms or objective disease and is, in its early stage, not accompanied by any histological changes. By contrast, when the urinary excretion of these proteins is increased by more than an order of magnitude (10 000 µg/g creatinine), tubular dysfunction caused by cadmium becomes irreversible and may be associated with a lower glomerular filtration rate (GFR) and an accelerated decline of the GFR with ageing.

Table 2. Interpretation of elevated urinary β2MG and RBP concentrations induced by occupational or environmental exposure to cadmium

β2MG or RBP concentration in urine (μg/g creatinine)	Significance
<300	Normal value
300–1000	Incipient cadmium tubulopathy (possibility of some reversal after removal of exposure if urinary cadmium is not too high, i.e. below 20 μg/g creatinine)
>1000–10 000	Irreversible tubular proteinuria that may accelerate the decline of GFR with age. At this stage, GFR is normal or slightly impaired.
>10 000	Overt cadmium nephropathy usually associated with a decreased GFR.

Source: Adapted from Bernard (2008)

In a large cross-sectional study representative of adults in the USA (National Health and Nutrition Examination Survey [NHANES], n = 14 778), both cadmium and lead concentrations in blood were assessed in relation to chronic kidney disease (i.e. urinary albumin excretion and an estimate of GFR). Increased blood cadmium and lead levels were strong independent risk factors for the prevalence of albuminuria, reduced GFR and both outcomes together. The geometric mean cadmium and lead concentrations in blood were 0.41 μg/l and 16 μg/l, respectively. After adjustment for survey year, sociodemographic factors, chronic kidney disease risk factors and blood lead concentration, the odds ratios (ORs) for albuminuria (≥30 mg/g creatinine), reduced GFR (<60 ml/min per 1.73 m^2) and both albuminuria and reduced GFR were 1.92 (95% CI 1.53–2.43), 1.32 (95% CI 1.04–1.68) and 2.91 (95% CI 1.76–4.81), respectively, comparing the highest with the lowest blood cadmium quartiles. The ORs comparing participants in the highest with the lowest quartiles of both cadmium and lead concentrations were 2.34 (95% CI 1.72–3.18) for albuminuria, 1.98 (95% CI 1.27–3.10) for reduced GFR and 4.10 (95% CI 1.58–10.65) for both outcomes. Among the subgroup of persons who reported never having smoked, the OR for albuminuria was 1.43 (95% CI 1.12–1.84), comparing the 75th percentile with the 25th percentile, but was not significant for reduced GFR. The major advantages with the present study were that the kidney effects represent clinically defined end-points and that for albuminuria, the exposure and effects markers were not assessed in the same medium (i.e. cadmium in blood versus albumin in urine). Thus, a possible co-excretion of cadmium and proteins is of no concern. The main limitation with the study is its cross-sectional design. As the effect markers used in the present study are related to glomerular damage only, the results may be of greater concern. According to the authors, their analysis may underestimate the consequences of the exposure due to the known limitations of albuminuria and creatinine-based GFR as markers of kidney damage, and the OR observed in models with both outcomes may reflect, in part, improved specificity in outcome assessment (Navas-Acien et al., 2009).

2.3.3 Osteoporosis and fractures

It has been established that prolonged exposure to cadmium affects the metabolism of calcium, leading to osteomalacia subsequent to proximal tubular dysfunction in the damaged kidneys; in the most severe cases, patients develop itai-itai disease, with osteomalacia as well as osteoporosis. A possible link between osteoporosis and exposure to cadmium at concentrations considerably lower than those found in itai-itai disease has been evaluated starting in 1999.

Except for fracture incidence, considered the most adverse end-point with regard to bone, reduced bone mineral density is frequently used as a biomarker of an adverse effect on bone. Osteoporosis is defined as a *t*-score below 2.5 (i.e. 2.5 standard deviations below the mean bone mineral density for young adults) (WHO, 1994). A *z*-score below –1 is sometimes used to define low bone mineral density (i.e. 1 standard deviation below a sex- and age-standardized mean bone mineral density) (Kanis et al., 1997). Based on a meta-analysis, a 1 standard deviation reduction in bone mineral density was reported to result in a relative risk (RR) of 1.5 (95% CI 1.4–1.6) for a fracture at any site. However, a 1 standard deviation reduction in spinal bone density was reported to result in an RR of 2.3 (95% CI 1.9–2.8) for vertebral fractures, and for hip bone density, another measurement for predicting vertebral fractures, the RR was 2.6 (95% CI 2.0–3.5) (Marshall, Johnell & Wedel, 1996).

Several epidemiological studies have reported on the association between cadmium and bone mineral density. So far, two studies have considered fracture incidence, whereas the other studies have included markers of bone metabolism.

In the OSCAR (**Os**teoporosis—**Ca**dmium as a **R**isk Factor) study, both bone mineral density and risk of fractures were assessed in relation to urinary and blood cadmium levels. The OSCAR study involved all people aged 16–80 years who had lived for at least 5 years during the period 1910–1992 in the proximity of a nickel–cadmium battery plant in southern Sweden. An additional group of age- and sex-matched people was randomly selected from a general medical practice register in a nearby area and was included in this “environmentally exposed” group. A group of workers at the battery plant was also enrolled. The overall participation rate was 60%, resulting in a total sample size of 1021. The mean (10th–90th percentiles) urinary concentration of cadmium was 0.82 (0.18–1.8) mg/g creatinine in men and 0.66 (0.21–1.3) mg/g creatinine in women. Bone mineral density was measured at a distal site on an individual’s non-dominant forearm while in the supine position. The degree of osteoporosis was expressed as an individual’s *z*-score. The risk of the *z*-score being less than −1 was assessed in relation to both urinary cadmium and blood cadmium concentrations. Inverse relationships were found between cadmium and both tubular proteinuria and bone mineral density. It was particularly apparent in people over 60 years of age. There was also a dose–response relationship between urinary cadmium concentration and *z*-score being less than −1. The ORs for men were 2.2 (95% CI 1.0–4.8) for the group having a urinary cadmium concentration of 0.5–3 nmol/mmol creatinine and 5.3 (2.0–14) for the group with the highest urinary cadmium concentrations (≥3 nmol/mmol creatinine) compared with the group with the lowest urinary cadmium concentrations

(<0.5 nmol/mmol creatinine). For women, the OR was 1.8 (95% CI 0.65–5.3) in the group with urinary cadmium concentrations of 0.5–3 nmol/mmol creatinine (Alfvén et al., 2000). For the analysis with blood cadmium levels, in a multiple linear regression, there was a negative association between blood cadmium concentration and bone mineral density for both men and women in the older age group (>60 years of age)—significant for women and close to significant for men. In the whole group (all ages), no significant correlations could be found among blood cadmium concentration, blood lead concentration and bone mineral density. Smoking did not appear to affect the outcome. The dose–response relationships in relation to low bone mineral density (*z*-score less than 1) showed ORs for three blood cadmium groups for people over 60 years of age, adjusted for weight and smoking (*z*-score already includes adjustment for age and sex). Compared with the group with blood cadmium concentrations below 5 nmol/l, ORs were 2.0 (95% CI 1.1–3.9) for the group with blood cadmium concentrations greater than or equal to 5 nmol/l and less than 10 nmol/l (mean 7.2 nmol/l) and 2.0 (95% CI 1.4–5.8) for the group with blood cadmium concentrations greater than or equal to 10 nmol/l (mean 21 nmol/l) (Alfvén, Järup & Elinder, 2002).

Fracture incidence was also assessed retrospectively in the Swedish OSCAR study. For fractures occurring after the age of 50 years ($n = 558$, 32 forearm fractures), the fracture hazard ratio, adjusted for sex and other relevant covariates, increased by 18% (95% CI 1.0–38%) per unit urinary cadmium (1 nmol/mmol creatinine). When subjects were grouped in exposure categories, the hazard ratio reached 3.5 (90% CI 1.1–11) in the group of subjects with urinary cadmium concentrations between 2 and 4 nmol/mmol creatinine and 8.8 (90% CI 2.6–30) in the group of subjects with urinary cadmium concentrations greater than or equal to 4 nmol/mmol creatinine. Associations between cadmium and fracture risk were absent before the age of 50 (Alfvén et al., 2004).

In Belgium, the exposure to cadmium in relation to fracture incidence was assessed in populations living close to zinc smelters and in control areas (Staessen et al., 1999). Urinary cadmium excretion in the 225 residents of the six districts near the smelters (mean 9.7 nmol/day; 10th–90th percentiles 6.9–24.1 nmol/day) was 22.8% higher ($P = 0.001$) than in the 281 inhabitants of the four low-exposure districts (mean 7.9 nmol/day; 10th–90th percentiles 3.4–16.3 nmol/day). These levels correspond to approximately 0.58 and 0.83 µg/g creatinine in men and women, respectively. The approximate 90th percentile urinary cadmium excretion corresponds to 2 µg/g creatinine. In their prospective cohort, including 506 subjects, Staessen et al. (1999) observed RRs associated with doubled urinary cadmium concentrations of 1.73 (95% CI 1.16–2.57; $P = 0.007$) for fractures in women and 1.60 (95% CI 0.94–2.72; $P = 0.08$) for height loss in men. Similar risk estimates were observed if cadmium concentrations in soil, leek and celery sampled in the relevant district of residence were used as a proxy of cadmium exposure instead of urinary cadmium concentration.

More recent Swedish and Belgian data confirmed the adverse effects of low-level cadmium exposure on bone mineral density. The median urinary cadmium concentration was 0.67 (5th–95th percentiles 0.31–1.6) μg/g creatinine in the Swedish study, and the median blood cadmium concentration was 0.38 (5th–95th percentiles 0.16–1.8) μg/l (Åkesson et al., 2006); the median blood cadmium concentration was 8 nmol/l (0.90 μg/l) in the Belgian study (Schutte et al., 2008a). Both studies suggest direct effects of cadmium on bone resorption, which seemed to be intensified after menopause. Even in the absence of cadmium-induced renal tubular dysfunction, low-level environmental exposure to cadmium seems to mobilize bone minerals from the skeletal tissue, indicated by increased calciuria and reactive changes in calciotropic hormones. Because cadmium was associated with lower levels of parathyroid hormone in both studies, the cadmium-associated calciuria was most likely a result of increased bone resorption, rather than decreased tubular reabsorption. If the calciuria was due to kidney damage, an increase in parathyroid hormone levels would then be a more likely scenario. An assessment of the benchmark dose (BMD) of cadmium in relation to bone mineral density was recently performed in the study on Swedish women mentioned above (Åkesson et al., 2006).

A large study in the USA using NHANES data reported an increased risk of osteoporosis in the hip in 3207 women aged 50 years and older; the ORs were 1.43 (95% CI 1.03–2.0) at urinary cadmium levels between 0.50 and 1.0 μg/g creatinine and 1.40 (95% CI 0.97–2.03) for urinary cadmium concentrations greater than 1.0 μg/g creatinine compared with the reference (<0.50 μg/g creatinine) (Gallagher et al., 2008). Only 15% of the women had urinary cadmium concentrations above 1.0 μg/g creatinine. Osteoporosis was defined according to *t*-score less than −2.5 (in this case, <0.56 g/cm^2), and this study was the first to assess bone mineral density at a site on the skeleton with high relevance to a fracture with great public health concern (i.e. hip fracture). This multivariate-adjusted model included adjustment for age, race, income, ever-smoking and underweight. Dose–response relationships were reported between the risk of osteoporosis and urinary cadmium as a continuous variable expressed in microgram per gram creatinine (OR 1.15; 95% CI 1.00–1.33).

In a recent publication, an association between urinary cadmium levels and bone mineral density was confirmed in occupationally exposed individuals (Nawrot et al., 2010). In 83 male (ex-)workers (mean age 45 years; range 24–64 years) of a radiator factory using cadmium-containing solder, bone mineral density in distal forearm, hip and lumbar spine (by dual-energy photon absorptiometry) and urinary calcium excretion were assessed. The geometric mean urinary cadmium concentration was 1.02 μg/g creatinine (5th–95th percentiles 0.17–5.51 μg/g creatinine). Bone mineral density was negatively correlated with urinary excretion of cadmium: the partial correlation coefficients (r) adjusted for age, body mass index (BMI) and current smoking were −0.30 (P = 0.008) for bone mineral density in the forearm, −0.27 (P = 0.017) in the hip and −0.17 (P = 0.15) in the spine. Urinary calcium levels correlated positively (r = 0.23; P = 0.044) with the urinary cadmium excretion. Adjusted for the same covariates, the risk of osteoporosis (defined as a *t*-score below −2.5 in at least one measured bone site) increased dose dependently.

Compared with the lowest tertile of urinary cadmium concentration, the risks were 4.8- and 9.9-fold higher in the middle and highest tertiles, respectively. Only four (5%) men had evidence of renal tubular dysfunction (β2MG concentration above 300 µg/g creatinine). It was concluded that even in the absence of renal tubular dysfunction, occupational exposure of men to cadmium is associated with lower bone mineral density, a higher risk of osteoporosis and higher urinary calcium excretion, suggesting a direct osteotoxic effect of cadmium (Nawrot et al., 2010).

The long-term effects of cadmium on forearm bone mineral density after the cessation of the ingestion of cadmium-polluted rice were investigated in 458 persons (294 women, 164 men) from three cadmium exposure areas (low, moderate and heavy) in China. Those living in the moderate and heavy exposure areas ceased ingesting cadmium-polluted rice (0.51 mg/kg and 3.7 mg/kg, respectively) in 1996 (10 years prior to the present analysis). The bone mineral density was measured by dual-energy X-ray absorptiometry at the proximal radius and ulna. The cadmium concentrations in urine and blood in 1998 were used as cadmium exposure markers. The values of the absolute decrease and per cent decrease in bone mineral density from 1998 to 2006 increased with increasing urinary and blood cadmium levels and were significant at urinary cadmium concentrations above 5 µg/g creatinine and at blood cadmium concentrations above 10 µg/l compared with the low exposure groups (urinary cadmium <2 µg/g creatinine and blood cadmium <2 µg/l) in all subjects (after stratification by sex, these differences were significant in the women only; $P < 0.001$). Analysis of the *z*-score revealed that the prevalence of osteoporosis in 2006 was higher than that in 1998 and increased along with the level of urinary and blood cadmium in both men and women, especially for those women with higher blood cadmium (blood cadmium >5 µg/l, OR = 3.45 [0.95–13.6]; blood cadmium >10 µg/l, OR = 4.51 [1.57–13.54]) and urinary cadmium (urinary cadmium >10 µg/g creatinine, OR = 4.74 [1.82–12.81]). The authors concluded that decreasing dietary cadmium exposure at the population level is not associated with bone recovery at the individual level (Chen et al., 2009).

Two other studies have failed to establish any association between urinary cadmium levels and bone mineral density. In 196 men and 184 women (Swedish fishermen and their wives), there was no significant association between urinary cadmium concentration and forearm bone mineral density (Wallin et al., 2005). In a study comprising 170 women and 100 men, urinary and blood cadmium concentrations and the markers of renal tubular dysfunction and forearm bone mineral density were measured. The results of the multivariate analysis did not indicate an association between exposure to cadmium and a reduction in bone density. The authors concluded that the excretion of low molecular weight proteins occurred at a lower level of cadmium exposure than that associated with potential loss of bone mass (Trzcinka-Ochocka et al., 2010).

A third study included 908 Swedish women with data on single photon absorptiometry in the non-dominant forearm. Cadmium level in blood was negatively associated with bone mineral density and parathyroid hormone level and positively associated with the biochemical marker of bone resorption (serum crosslinked C-telopeptide of type I collagen, or CTX). However, this association disappeared after adjustment for smoking, and it was concluded that no convincing

associations were observed between cadmium concentration in blood and bone mineral density (Rignell-Hydbom et al., 2009).

2.3.4 Cardiovascular disease

A cross-sectional analysis of data from the NHANES in the USA found an association between urinary cadmium concentration and myocardial infarction (Everett & Frithsen, 2008) and between blood cadmium concentration and risk of reported stroke and heart failure (Peters et al., 2010). In subjects with environmental cadmium exposure in Belgium, the urinary cadmium excretion was correlated with changes in some physiological indicators of cardiovascular function: pulse wave velocity, arterial pulse pressures and arterial compliance and distensibility (Schutte et al., 2008b). The pathogenesis of these cadmium-associated abnormalities is unclear at present. In a study conducted in the Republic of Korea, blood cadmium level was associated with increased risk of hypertension (Eum, Lee & Paek, 2008), and cadmium levels in blood, but not in urine, were associated with a modest elevation in blood pressure levels in the NHANES data (Tellez-Plaza et al., 2008).

2.3.5 Cancer

Cadmium is classified as a cancer-causing agent in humans based on an elevated incidence of lung cancer and mortality data derived from occupational groups with evidence of elevated exposure to cadmium. Occupational exposures have historically been through inhalation (IARC, 1993). The available evidence was considered sufficient for lung cancer, but limited for kidney, liver and prostate cancer.

The previous evidence with respect to prostate cancer has not been regarded as convincing (Verougstraete, Lison & Hotz, 2003; Sahmoun et al., 2005), but the available human studies have limited ability to detect an effect (Huff et al., 2007). A recent case–control study (40 cases and 58 hospital-based controls from two provinces in southern and northern Italy), which compared newly diagnosed cases of prostate cancer with the cadmium levels in their toenails, showed an apparent relationship for an increased prostate cancer risk. An excess prostate cancer risk in subjects in the third and fourth (highest) quartiles of toenail cadmium concentration (ORs of 1.3 and 4.7, respectively), compared with subjects in the bottom quartile, was observed. Results were basically unchanged when limiting the analysis to each province or entering toenail cadmium concentrations as continuous values in the regression model ($P = 0.004$). Despite the limited statistical stability of the point estimates, these findings appear to support the hypothesis that cadmium exposure increases prostate cancer risk (Vinceti et al., 2007).

A prospective cohort study from Belgium assessed the association between environmental exposure to cadmium and cancer incidence. This study was a prolongation of the Flemish part of the CadmiBel study, including six districts with high cadmium exposure close to zinc smelters and four districts with low exposure. In total, 994 subjects were included at baseline. Occupationally exposed people were not excluded, but a sensitivity analysis was performed based on environmentally exposed people alone. The population-attributable risk of lung cancer was

67% (95% CI 33–101) in a high exposure area, compared with 73% (95% CI 38–108) for smoking. For lung cancer (*n* = 19, of which 18 occurred in the high exposure area), the adjusted hazard ratio was 1.70 (95% CI 1.13–2.57; *P* = 0.011) for a doubling of the 24 h urinary cadmium excretion, 4.17 (95% CI 1.21–14.4; *P* = 0.024) for residence in the high exposure area compared with the low exposure area and 1.57 (95% CI 1.11– 2.24; *P* = 0.012) for a doubling of cadmium concentration in soil (Nawrot et al., 2006). Overall cancer incidence (*n* = 70) was also increased in the high exposure group, but a clear excess was seen only with regard to lung. The median urinary cadmium excretion in this study was 0.8 µg/g creatinine, and the 25th–75th percentile range was about 0.5–1.4 µg/g creatinine (EFSA, 2009a). The exact relevance to dietary cadmium exposure is not clear.

A Belgian case–control study of bladder cancer (172 cases and 359 population controls) showed an OR of 5.7 (95% CI 3.3–9.9) for bladder cancer, comparing the highest tertile of blood cadmium with the lowest after adjustments for sex, age, smoking status (current/non-current), number of cigarettes smoked per day, number of years of smoking and occupational exposure to polycyclic aromatic hydrocarbons or aromatic amines (Kellen et al., 2007).

More recently, a case–control study (246 cases and 254 controls) in the USA showed that women in the highest quartile of creatinine-adjusted urinary cadmium levels had twice the breast cancer risk of those in the lowest quartile after adjustment for established risk factors, and there was a statistically significant increase in risk with increasing cadmium level (McElroy et al., 2006).

The significance of the estrogen-mimicking effects, such as the well-characterized estrogenic responses of the endometrial lining (hypertrophy and hyperplasia), observed in experimental animals exposed to environmentally relevant doses of cadmium (Johnson et al., 2003) was further explored in humans. In a large population-based prospective cohort among Swedish postmenopausal women (*n* = 32 210), the association between dietary cadmium exposure and endometrial cancer incidence was assessed (Åkesson, Julin & Wolk, 2008). This is the first study exploring health effects in relation to dietary cadmium exposure, in contrast to smaller studies in which cadmium concentration has been monitored in urine. Thus, based on the construction of a food cadmium database in the cohort, a large study population was utilized, and the incidence was assessed prospectively. This design, on one hand, reduces the selection bias that often occurs in case–control studies, but it is, on the other hand, dependent on the assumption that estimated dietary cadmium exposure is a valid reflection of the internal dose of cadmium. The average estimated dietary cadmium exposure was 15 µg/day (1.5 µg/kg bw per week). During 16 years of follow-up, 378 cases of endometrial adenocarcinoma were ascertained through computerized linkage to the Swedish Cancer Registry, with virtually no loss to follow-up. The highest versus lowest tertile of cadmium exposure was associated with risk of endometrial cancer (RR 1.39, 95% CI 1.04–1.85; *P* for trend = 0.02). To reduce the influence of endogenous estrogen exposure, analyses were stratified by BMI and by postmenopausal hormone use. Analyses were also stratified by smoking status, because an anti-estrogenic effect of cigarette smoking on circulating estrogen concentrations has been shown as a result of increased metabolic clearance, a reduction in relative

body weight and an earlier age at menopause (Terry et al., 2002). Among never-smoking, non-overweight women, the RR was 1.86 (95% CI 1.13–3.08; *P* for trend = 0.009). A 2.9-fold increased risk (95% CI 1.05–7.79) was observed with long-term cadmium exposure consistently above the median exposure in 1987 and in 1997 in never-smoking women with low available estrogen (non-overweight and non-users of postmenopausal hormones). Although the data support the hypothesis that cadmium may exert estrogenic effects and possibly increase the risk of hormone-related cancers, this needs to be confirmed by other studies.

The association between cancer mortality and cadmium of dietary origin was assessed in Japanese cohorts. In the Kakehashi cohort with high baseline urinary cadmium concentrations (median urinary cadmium concentrations for men and women were 7.0 and 12.1 μg/g creatinine, respectively), a 2.5-fold increase in cancer mortality was observed among women with permanent tubular impairment (Nishijo et al., 2006). Similarly, Arisawa et al. (2007a) observed a 2.58-fold concurrent increased risk of cancer mortality among those with tubular impairment in the Nagasaki cohort I.

2.3.6 Reproductive toxicity

Maternal blood cadmium concentration is not highly correlated with blood cadmium concentration in the newborn, but it is correlated with cord blood cadmium concentration, with correlation coefficients of 0.5–0.6 (Galicia-Garcia et al., 1997; Salpietro et al., 2002). Limited evidence suggests that neonatal outcomes, such as reduced birth weight (Galicia-Garcia et al., 1997; Nishijo et al., 2002; Salpietro et al., 2002) and reduced length of gestation (Nishijo et al., 2002), are related to indices of prenatal exposure to cadmium (maternal urinary cadmium level, maternal blood cadmium level). The fact that, in the study by Nishijo et al. (2002), urinary cadmium concentration was not significantly associated with infant height and weight after adjustment was made for gestational age suggested that fetuses with higher prenatal exposures to cadmium were of appropriate gestational age for date, but tended to be born earlier. In this study, conducted among women living in the Jinzu River basin in Toyama, the area in which itai-itai disease was most common, most adverse fetal outcomes were no longer significantly associated with urinary cadmium concentration after adjustment was made for maternal age. Because the study area was known to have been contaminated with cadmium over a long period, maternal age is likely to have been a marker for duration of exposure and thus for body burden of cadmium. Thus, controlling for maternal age might represent overcontrol, and alternative analytical approaches, such as stratification, might have been preferable.

Concentrations of cadmium were measured in the placenta, cord blood and urine of 44 non-smoking, rural Bangladeshi women. The concentrations of magnesium, calcium, manganese, copper, zinc, arsenic and selenium in the placenta and cord blood and the level of metallothionein in the placenta were also measured. Concentrations of cadmium in urine, placenta and cord blood were variable, with means (range) of 1.4 μg/l (0.29–10 μg/l), 130 mg/kg (40–492 mg/kg) and 0.16 μg/kg (0.074–0.32 μg/kg), respectively. The concentration of cadmium in the placenta was positively associated with that in cord blood ($r_s = 0.62$; $P < 0.001$),

but negatively associated with zinc concentration in cord blood (r_s = −0.30; P = 0.05). Placenta metallothionein protein expression was positively associated with cadmium (P < 0.01) and arsenic (P = 0.04) concentrations in placenta, but not with zinc or copper concentration in placenta. The authors concluded that the cadmium concentrations in placenta were clearly elevated, which seemed to impair zinc transfer to the fetus. Induction of metallothionein explained the placental accumulation of cadmium, but not the impairment of zinc transport (Kippler et al., 2010).

2.3.7 Mortality

(a) Japanese data

Recent studies from Japan found that the mortality risk was significantly increased among subjects with a urinary cadmium concentration of more than 3 µg/g creatinine in proportion to the increases in the urinary cadmium concentration after adjustment for age, especially in women (Nakagawa et al., 2006). In a recent study on mortality of targeted participants in the 1974–1975 health impact survey in the Kakehashi River basin, Japan, standardized mortality ratios were assessed instead of proportional mortality ratios. An increased mortality risk from cerebral infarction in men was found in the category with a urinary β2MG concentration of 300–1000 µg/g creatinine during observation for 15 years. Therefore, the increase in mortality from cerebral infarction may contribute to the increase in mortality for men exposed to cadmium. Whereas the increase in mortality from cerebral infarction did not reach significance in women with urinary β2MG concentrations of 300–1000 µg/g creatinine (hazard ratio 1.88, 95% CI 0.82–4.29), the mortality from heart failure was significantly increased in this subgroup of women (hazard ratio 1.94, 95% CI 1.08–3.48) (Nishijo et al., 2006).

In 1992, Iwata et al. reported that individuals who resided in the cadmium-polluted areas of Nagasaki and Akita prefectures in Japan and had renal tubular dysfunction and glomerular dysfunction had a reduced life expectancy. In a follow-up study 23 years later, Arisawa et al. (2007b) compared the same cohort (cohort I) with a second younger cohort of adults (≥40 years) from the same area. The daily cadmium exposure from food had decreased from over 200 µg/day in 1967 to less than 100 µg/day after 1980–1983 due to the restoration of cadmium-polluted paddy fields. In the older cohort I, the mortality rate among those with urinary β2MG concentration greater than or equal to 1000 µg/g creatinine was 1.41 (95% CI 1.07–1.83) times higher than the regional reference rate. After adjusting for age and other variables, urinary *N*-acetyl-β-D-glucosaminidase activity in men and serum creatinine level, β2MG clearance and urinary β2MG concentration in women were significantly associated with increased mortality. However, in cohort II, urinary β2MG or total protein level was not significantly associated with survival. The authors concluded that these findings indicated that cadmium-induced renal dysfunction was a significant predictor of mortality, but that such an association is disappearing, probably because of the selective loss of advanced cases and reduced exposure and body burden.

(b) Data from Europe and the USA

A recent European prospective cohort study (*n* = 1107 subjects at baseline, of the original 1419 subjects invited to participate between 1985 and 1989) assessed the long-term changes in body burden of cadmium and the incidence of mortality simultaneously. The study was a prolongation of the Flemish part of the CadmiBel study, including six districts with high cadmium exposure close to zinc smelters (cadmium concentrations above 3 mg/kg soil) and four districts with low exposure more than 10 km away from the smelters (cadmium concentrations below 1 mg/kg soil). The median urinary cadmium concentrations at baseline were 1.03 μg/g creatinine and 0.74 μg/g creatinine in high and low exposure areas, respectively. Individuals reporting possible exposure to cadmium at work were excluded from the baseline population of 1107 subjects, and some subjects did not provide blood or urine samples, leaving 956 persons to be followed up (50% from each of low and high exposure areas). Except for blood cadmium level and 24 h urinary cadmium excretion, blood pressure, serum creatinine level, high-density lipoprotein concentration, total cholesterol level, serum γ-glutamyltransferase activity (as index of alcohol intake), urinary creatinine concentration and RBP level were assessed. Among the group with data on urinary cadmium concentration, 208 deaths occurred during an average 20.3 years of follow-up. Multivariate-adjusted hazard ratios (adjusted for age, sex, BMI, smoking, serum γ-glutamyltransferase activity and socioeconomic status) for all-cause and non-cardiovascular mortality and the risk of death from all cancers and lung cancer increased with high urinary cadmium excretion. The risks ($P \leq 0.04$) associated with a doubling of baseline urinary cadmium concentration were 20% (95% CI 4–39%), 44% (95% CI 16–79%) and 43% (95% CI 8–89%) for total mortality, non-cardiovascular mortality and total cancer mortality, and those associated with a doubling of blood cadmium concentration were 25% (95% CI 4–50%) for total mortality and 33% (95% CI 1–75%) for non-cardiovascular mortality. The increase in risk corresponds to a difference in urinary cadmium concentration between 0.68 μg/g creatinine (the mean in the analysed population) and 1.36 μg/g creatinine. The authors concluded that the increased mortality was directly related to the toxic effects of cadmium, but not directly related to renal dysfunction, as measured by urinary RBP level and serum creatinine level; and that even if zinc smelters close, historical environmental contamination remains a persistent source of exposure, and this exposure increases mortality in a continuous fashion (Nawrot et al., 2008). This study provides the strong advantage of being a prospective cohort with longitudinal exposure assessment. The relevance to dietary cadmium exposure alone cannot be estimated.

Mortality was also assessed in a prospective cohort based on the NHANES (baseline 1988–1994) data, including 13 956 people followed through December 2000. Multivariate models included adjustments for age, race/ethnicity, menopausal status, urban/rural residence, cigarette smoking, alcohol consumption, education, physical activity, income, serum C-reactive protein, total cholesterol, diabetes, blood pressure, use of antihypertensive drugs and GFR. The hazard ratios (95% CI) for all-cause mortality, cancer mortality, cardiovascular disease mortality and coronary heart disease mortality associated with a 2-fold higher creatinine-

corrected urinary cadmium concentration were 1.28 (1.15–1.43), 1.55 (1.21–1.98), 1.21 (1.07–1.36) and 1.36 (1.11–1.66), respectively, for men and 1.06 (0.96–1.16), 1.07 (0.85–1.35), 0.93 (0.84–1.04) and 0.82 (0.76–0.89), respectively, for women. A 2-fold increase in urinary cadmium corresponds approximately to the difference between the median (0.32 μg/g creatinine) and the 75th percentile (0.61 μg/g creatinine). Thus, environmental cadmium exposure was associated with an increased risk of all-cause, cancer, cardiovascular disease and coronary heart disease mortality among men, but not among women (Menke et al., 2009).

A recent study that monitored glomerular dysfunction in 50 subjects who had ingested household rice for 10 years after cadmium-polluted soil in rice paddies was replaced revealed very little improvement based on measured urinary biomarkers (β2MG, RBP, total protein, amino acid nitrogen and glucose levels), with the exception of urinary amino acid nitrogen level in men. The urinary concentration of these biomarkers continued to increase in both sexes over time, with statistically significant differences in RBP and total protein levels in both sexes and glucose level in men. Therefore, although cadmium concentrations in rice and urine were shown to be less after soil replacement, cadmium-induced renal tubular injury (i.e. where β2MG concentration is greater than or equal to 1000 μg/g creatinine) seemed to be irreversible (Kobayashi et al., 2008).

In a study reported in 2009 that investigated the effects of environmental co-exposure to arsenic and cadmium in 290 adults (86 males, 204 females) in the Republic of Korea, biomarkers of kidney toxicity (the concentrations of *N*-acetyl-β-D-glucosaminidase and β2MG in urine) and biomarkers of oxidative stress (urinary malondialdehyde and 8-hydroxy-2′-deoxyguanosine) were determined. The concentrations of these biomarkers were matched to the urinary concentrations of arsenic and cadmium. Oxidative stress biomarkers, such as urinary malondialdehyde and 8-hydroxy-2′-deoxyguanosine, were positively correlated with both cadmium and arsenic levels in urine. However, the correlation was more pronounced with co-exposure than with each metal separately. The urinary concentration of *N*-acetyl-β-D-glucosaminidase but not β2MG was positively correlated with urinary levels of cadmium and arsenic and indices of oxidative stress. The authors concluded that these data indicated that tubular damage in the kidneys was probably related to oxidative stress and that the effect of co-exposure to arsenic and cadmium was more pronounced than exposure to each individual metal (Huang et al., 2009).

3. ANALYTICAL METHODS

3.1 Chemistry

Cadmium is a soft, ductile and silvery-white heavy metal with the atomic number 48, atomic weight 112.411 and density 8.65 g/cm^3 (at 25 °C). It occurs mostly in the valence state of +2. Cadmium forms a number of inorganic salts, and the salts exhibit properties similar to those of the corresponding zinc compounds. The halides and the nitrate of Cd^{2+} are very soluble in water, whereas the hydroxide is insoluble. Cadmium oxide and cadmium carbonate might, however, be soluble at gastric pH.

Cadmium occurs naturally in Earth's crust as part of cadmium-rich geological materials, such as greenockite (cadmium sulfide). Cadmium is also found in ores containing other elements, mainly associated with zinc, and is recovered as a by-product of zinc mining. Approximately 3 kg of cadmium is produced for each tonne of zinc. Cadmium is primarily used for metal plating and coating operations, including transportation equipment, machinery and baking enamels, photography and television phosphors. It is also used in nickel–cadmium and solar batteries and in pigments.

Cadmium occurs in the environment in its inorganic form as a result of volcanic emissions and exfoliation of rocks and minerals (Pacyna & Pacyna, 2001). It is released into the environment via the smelting of other metals, burning of fossil fuels, incineration of waste materials and use of phosphate and sewage sludge fertilizers. An increase in cadmium levels in soil results in an increase in the uptake of cadmium by plants. Although cadmium may bind to proteins and other organic molecules and form salts with organic acids, these compounds are regarded as inorganic with respect to cadmium (IPCS, 1992a). Organic cadmium compounds (compounds in which cadmium binds covalently to carbon) are normally not identified in nature (IPCS, 1992b). Although studies in marine polar regions indicate microbial formation of monomethyl cadmium ($CdCH_3^+$), the significance of these findings is currently not known (Pongratz & Heumann, 1999; Fairbrother et al., 2007). In the aquatic environment at low salinity, cadmium is present as the free Cd^{2+} ion with cadmium hydroxide and organic complexes at levels dependent on pH and amounts of soluble organic material. As salinity increases, the degree of complexation with chloride increases; in 100% seawater, the cadmium exists almost solely as cadmium chloride and $CdCl^+$ complexes (Simpson, 1981). Cadmium is most readily absorbed by aquatic organisms in its free form, Cd^{2+}, and increased salinity has been found to reduce its bioaccumulation (IPCS, 1992a). Shellfish, crustaceans and fungi are natural accumulators of cadmium.

3.2 Description of analytical methods

Analytical methods for the determination of cadmium in foods, water and biological materials are well established. The detection techniques include flame atomic absorption spectrometry (FAAS), electrothermal (graphite furnace and Zeeman furnace) atomic absorption spectrometry (ETAAS), beam injection flame furnace atomic absorption spectrometry (BIFF-AAS), thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS), hydride generation atomic fluorescence spectrometry (HG-AFS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).

The sensitivity of FAAS can be improved by increasing the efficiency of aerosol generation/transport and the residence time of free atoms in the absorption volume. In the TS-FF-AAS technique, a nickel tube is placed above an air/acetylene flame, and the sample is directly introduced through a ceramic capillary connected to the nickel tube positioned on the burner of the atomic absorption spectrometer (Da-Col, Domene & Pereira-Filho, 2009). This procedure, unlike FAAS, allows

introduction of total sample and long residence time of gaseous atoms in the spectral zone, which offers good sensitivity (Pereira-Filho, Berndt & Arruda*,* 2002).

Owing to the high sensitivity (limit of detection [LOD] approximately 100 times lower than that of FAAS) and selectivity, ETAAS has been widely used for the determination of cadmium. However, the response of ETAAS is often perturbed by multiple physical or chemical reactions in the atomizer, and the LODs are not always adequate for trace analysis (Sardans, Montes & Peñuelas, 2010). This technique requires the use of modifiers to stabilize cadmium, allowing its quantification without matrix effect. Various modifiers used include palladium(II) nitrate/palladium (Daftsis & Zachariadis, 2007), palladium(II) chloride/palladium and ascorbic acid (Licata et al., 2004) and ammonium phosphate, Triton X-100 and monoammonium dihydrogen phosphate (Viñas, Pardo-Martínez & Hernández-Córdoba*,* 2000). Many improvements have enhanced the performance of ETAAS, including background correction systems, especially the Zeeman background correction; advances in atomizer designs; development of in situ trapping methods; improvements in the light source and detector; and use of appropriate modifiers. Use of transversally heated atomizers with platforms further improved the sensitivity. In recent years, the high-resolution continuum source electrothermal atomic absorption spectrometer (HR-CS-ETAAS) allows the direct analysis of solids with low LODs.

ICP-OES and ICP-MS methods offer simultaneous determination of several elements and are used for the determination of cadmium in foods, water and biological samples. ICP-OES instruments are available in two configurations: radial and axial. The benefit of the axial design is that more photons are seen by the detector; as a result, it offers 5–10 times lower LODs compared with the radial configuration. The fundamental difference between ICP-OES and ICP-MS is that the plasma is used in the latter not to generate photons of light, but to generate ions. The ions produced in the plasma are transported and separated according to their atomic mass to charge ratio by means of a mass spectrometer. The generation of large numbers of positively charged ions allows ICP-MS to achieve LODs better than those attainable using ETAAS. Additionally, ICP-MS offers high specificity through spectral interpretation and isotopic information (Nardi et al., 2009). However, polyatomic interferences resulting from the combination of matrix ions with argon may affect this technique. Some of the interferences can be controlled or eliminated by using different sample preparation techniques, such as ashing or microwave digestion prior to determination, whereas others have to be controlled using a mathematical approach (Rocha et al., 2009). The recent use of the dynamic reaction cell technology combined with ICP-MS (DRC-ICP-MS) has allowed the removal of the interferences with a minimum loss of sensitivity. This technology may be considered an interesting alternative to the above-mentioned spectrometric techniques, because it offers various possibilities for the element's determination in different matrices (D'Llio et al., 2008).

Alternative methods, such as stripping voltammetry (Melucci, Torsi & Locatelli*,* 2007; Jannat, et al., 2009), have limitations for the determination of cadmium.

3.2.1 Determination of cadmium in foods

ETAAS and ICP-MS have been largely used for the determination of cadmium in foods and water. FAAS has been used to a lesser extent, owing to its low sensitivity. ICP-MS has been the method of choice, as it offers lower LODs and wide dynamic range and allows simultaneous determination of several elements. Additionally, ICP-MS offers high specificity through spectral interpretation and isotopic information.

Sample preparation for the analysis of cadmium depends on the type of food matrix as well as the quantification method. The two most common techniques used are wet digestion using acids and dry ashing following leaching. The wet digestion technique, which uses a combination of highly pure acids (nitric, hydrochloric, sulfuric) along with oxidants such as hydrogen peroxide to assist digestion, has been most commonly employed in sample preparation. Microwave-assisted acid digestion has been extensively used. This technique allows the use of large sample masses (1–2 g) under controlled temperature and pressure, reducing contamination and avoiding losses of the element during mineralization. The dry ashing and leaching technique has been used to a limited extent, as calcinations at temperatures above 400 °C may induce losses of cadmium. Another technique, which is less commonly used, is the slurry sampling technique. It has some advantages over the microwave-assisted acid digestion technique, such as low sample preparation time, safety and cost. However, this technique requires the optimization of particle size, slurry concentration and homogeneity. This technique may be used in combination with ETAAS, as the ashing step is carried out in the graphite furnace itself.

The performances of some analytical methods for the determination of cadmium contamination levels in different foods are presented in Table 3.

(a) Quality assurance for the determination of cadmium in foods

In general, most countries used validated analytical methods and followed good quality assurance programmes to demonstrate the accuracy and reliability of the data. Certified standards and certified reference materials (CRMs) available from institutions such as the Institute for Reference Materials and Measurements (European Commission), the National Institute of Standards and Technology (USA), the Federal Institute for Materials Research and Testing (Germany) and the LGC (formerly the Laboratory of the Government Chemist; United Kingdom) have been used during method validation and accuracy studies. Many laboratories have also participated in the proficiency testing programmes and achieved good *z*-scores in different participation rounds. Samples from proficiency testing programmes have also been used in method validation as well as internal quality control.

Table 3. Analytical methods for the determination of cadmium in foods

Commodity	*n*	Country (year)	Sample preparation method	Determination method	LOD/LOQ	*n* <LOD or <LOQ	Mean concentration (range)	Reference
Milk	42	Brazil (2004)	Acid digestion (HNO_3 + HCl)	ETAAS	0.13 ng/ml (LOQ)	42	<LOQ	Soares et al. (2010)
Tomato, pepper, onion	9	Mediterranean countries	Microwave-assisted digestion (HNO_3 + H_2O_2)	ETAAS	0.05/0.15 ng/g	0	(10.7–19.9 ng/g)	Bakkali et al. (2009)
Anchovy, spinach, cabbage, onion, dill, parsley, lettuce, tea, rice, salami, chicken	23	Turkey	Coprecipitation with MBT	ETAAS	0.02 ng/g (LOD)	2	(2.67–510 ng/g)	Oymak et al. (2009)
Vegetables (100 varieties)	416	China	Acid digestion (HNO_3 + $HClO_4$ + H_2SO_4)	ETAAS	1 ng/g (LOD)	—	10 ng/g (ND–101 ng/g)	Song et al. (2009)
Konjac flour	7	China	Enzymatic hydrolysis and slurry preparation	ETAAS	3 ng/g (LOD)	0	(52.6–130.7 ng/g)	Chen et al. (2008)
Cabbage, wheat, potato, eggs, instant milk, mussels, marine fish, river fish, baby milk formula, baby foods	14	Slovenia	Microwave-assisted digestion (HNO_3 + HF)	ETAAS	0.03 µg/g (LOD)	13	(<0.03–0.57 µg/g)	Milacic & Kralj (2003)

Table 3 (contd)

Commodity	*n*	Country (year)	Sample preparation method	Determination method	LOD/LOQ	*n* <LOD or <LOQ	Mean concentration (range)	Reference
Spinach, palmito, crab, shrimps, mussel, sardine, squid	7	Brazil	Cryogenic grinding + slurry preparation	ETAAS	3.3 ng/g (LOD)	1	(ND–22.3 ng/g)	Santos et al. (2002)
Seafood (CRM)	0	—	Solid sampling	SS-ZAAS	0.0013 ng/g (LOD)	—	—	Detcheva & Grobecker (2006)
Seaweed: *Porphyra*, *Laminaria*	4	France, Japan, Republic of Korea and Spain (2004)	Microwave-assisted digestion ($HNO_3 + H_2O_2$)	ICP-MS	0.28 ng/g (LOD)	0	(0.31–3.18 ng/g)	Rocha et al. (2009)
Rice, wheat, beans, egg, meat, fish, bread, sugar, cheese, milk powder, butter, vegetables, pear, Brazil nut, coffee, chocolate, biscuits, pasta	18	Brazil	Microwave-assisted digestion ($HNO_3 + H_2O_2$)	ICP-MS	0.2 ng/g (LOD)	1	(ND–9.1 ng/g)	Nardi et al. (2009)
Semolina	3	Italy	Microwave-assisted digestion ($HNO_3 + H_2O_2$)	ICP-MS	6.1 ng/l (LOD)	0	(15.9–26.4 ng/g)	Cubadda & Raggi (2005)
Milk and infant formula	8	Italy	Microwave-assisted digestion ($HNO_3 + H_2O_2$)	DRC-ICP-MS	0.08/0.24 ng/g	9	(ND–3.7 ng/g)	D'Llio et al. (2008)

Table 3 (contd)

Commodity	*n*	Country (year)	Sample preparation method	Determination method	LOD/LOQ	*n* <LOD or <LOQ	Mean concentration (range)	Reference
Guaraná, cabbage	2	Brazil	Acid digestion + solid-phase extraction (minicolumn of Amberlite XAD-4 modified with DHB)	ICP-OES	0.02/0.07 ng/ml	0	(0.1–0.25 µg/g)	Bezerra et al. (2007)
Coffee, fish, black tea, green tea	4	Turkey	Coprecipitation with zirconium(IV) hydroxide	FAAS	0.27 ng/ml (LOD)	4	ND	Citak, Tuzen & Soylak (2009)
Food supplement	1	Brazil	Microwave-assisted digestion (HNO_3 + H_2O_2)	TS-FF-AAS	0.6/2.0 ng/ml	—	—	Da-Col, Domene & Pereira-Filho (2009)
Infant formula	1	Islamic Republic of Iran	Acid digestion (HNO_3) + dry ashing	DPASV	5 ng/g (LOD)	0	0.359 mg/kg	Jannat et al. (2009)

CRM, certified reference material; DHB, dihydroxybenzoic acid; DPASV, differential pulse anodic stripping voltammetry; LOQ, limit of quantification; MBT, 2-mercaptobenzothiazole; *n*, number of samples analysed; ND, not detected; SS-ZAAS, solid sampling Zeeman atomic absorption spectrometry

A proficiency testing programme for determining cadmium in seawater shrimp under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation was discussed by Kong, Chan & Wong (2008). The performance of an interlaboratory collaborative study for the determination of cadmium by ICP-MS after pressure digestion including microwave heating was reported. Thirteen laboratories participated, and the method was tested on a total of seven foodstuffs: carrot purée, fish muscle, mushroom, wheat flour, simulated diet, scampi and mussel powder. The elemental concentration of cadmium (dry matter) ranged from 0.28 to 1.70 mg/kg. The study indicated that the ICP-MS method is satisfactory for the determination of cadmium in foods (Julshamn et al., 2007).

3.2.2 Determination of cadmium in blood and biological materials

Determination of cadmium in biological materials such as blood, urine and tissues poses problems, mainly due to its presence in low concentrations as well as the complexity of the sample matrix. ETAAS has been widely used for the determination of cadmium in blood and clinical analyses. Ashing and atomization of the sample in the presence of chemical modifiers and use of Zeeman background correction were studied (Sardans, Montes & Peñuelas, 2010). A simultaneous atomic absorption spectrometry (SIMAAS) method for the determination of cadmium was proposed by Kummrow et al. (2008). The method requires a sample volume of 200 µl and presented an LOD of 0.026 ng/ml for cadmium.

ICP-MS has proven to be a superior and attractive alternative method to ETAAS, owing to its low LODs and its simple sample pretreatment. Whole blood samples could be analysed directly after dilution or decomposition of the organic matrix by ICP-MS (Heitland & Köster, 2006). However, the direct analysis of whole blood after dilution can cause clogging of the sample introduction devices and signal instability in the ICP-MS. Several digestion procedures have been reported, including high-pressure ashing.

The performances of some analytical methods for the determination of cadmium in blood and other biological materials are presented in Table 4.

Cadmium exposure in children and their mothers living in the vicinity of industrial sources (city of Duisburg and a rural area of North Rhine Westphalia, Germany) was assessed by a cross-sectional study performed in 2000. In total, 238 children (mean age 6.4 years, range 5.5–7.7 years; 49% males, 51% females) and 213 mothers (mean age 36 years, range 23–48 years) were included in the study. Mean cadmium levels (children/mothers) in the blood from the industrialized area were higher (0.21/0.61 ng/ml) than those from the rural area (0.19/0.44 ng/ml). Mean cadmium levels (children/mothers) in the urine from the industrialized area were higher (0.13/0.43 ng/ml) than those from the rural area (0.11/0.30 ng/ml) (Wilhelm et al., 2005).

The Centre for Environment and Health in Flanders, in the northern part of Belgium, started a biomonitoring programme on adolescents in 2003. In total, 1679 adolescents from nine areas with different patterns of pollution were selected to participate in this study. Possible confounding effects of lifestyle and personal characteristics were taken into account. A median blood cadmium level of 0.39 ng/ml was reported (Schroijen et al., 2008).

Table 4. Analytical methods for the determination of cadmium in blood and urine

Country (year)	*n*	Analytical sample size	Sample preparation	Technique	LOD/LOQ	Mean concentration (range)	*n* <LOQ or <LOQ	Reference
Spain (2010)	0 (method validation only, no samples analysed)	—	Microwave digestion	ETAAS	0.03/0.09 ng/ml	—	—	Olmedo et al. (2010)
Brazil (2007)	40	200 µl	Protein precipitation and dilution	ETAAS	0.026 ng/ml (LOD)	0.32 ng/ml (0.13–0.71 ng/ml)	—	Kummrow et al. (2008)
—	—	5 ml	Wet digestion (HNO_3)	ETAAS	1–11 ng/g (LOD) for different fractions	(ND–4 ng/g)	—	Daftsis & Zachariadis (2007)
Germany (2002–2003)	430 (children, age about 10 years)	5 ml	—	ZF-AAS	0.15 ng/ml (LOQ)	0.29 ng/ml (<0.15–3.1 ng/ml)	43	Link et al. (2007)
—	CRM	2 ml	Microwave-assisted digestion	ETAAS with Pd modifier	0.021/0.057 ng/ml	—	—	Viitak & Volynsky (2006)
Germany (2000)	Children/mothers Blood: 238/213 Urine: 149/129	—	—	ETAAS	0.03 ng/ml (LOQ) for blood and urine	Children/mothers Blood 0.21/0.57 ng/ml Urine 0.12/0.39 ng/ml	Children/ mothers Blood 57/6 Urine 13/0	Wilhelm et al. (2005)

Table 4 (contd)

Country (year)	*n*	Analytical sample size	Sample preparation	Technique	LOD/LOQ	Mean concentration (range)	*n* <LOQ or <LOQ	Reference
Egypt	Blood and urine: 93 Hair: 93 Nails: 68	1 ml (blood and urine) 500 mg (hair and nail)	Oxidation with $KMnO_4$	ETAAS	Blood 0.23/0.59 ng/ml Urine 0.32/0.68 ng/ml Hair 0.015/0.031 µg/g Nails 0.018/0.037 µg/g	Blood 2.07 ng/ml Urine 1.93 ng/ml Hair 0.35 µg/g Nails 1.35 µg/g	—	Mortada et al. (2002)
Italy (2004)	110	10 ml	Wet digestion (HNO_3)	ICP-MS	—	0.99 ng/ml	—	Alimonti et al. (2005)
Sweden	31	1 ml	Microwave digestion	ICP-MS	0.03 ng/ml (LOD)	0.116 ng/ml (<0.03–0.317 ng/ml)	6	Rodushkin, Ödman & Branth (1999)
Belgium (2003)	1679 adolescents	500 µl	Wet digestion ($HNO_3 + H_2O_2$)	HR-ICP-MS	0.09 ng/ml (LOD)	Median 0.39 (0.045–1.26 ng/ml)	—	Schroijen et al. (2008)
Germany (2005)	130	500 µl	Dilution (Triton X-100 + NH_4OH)	DRC-ICP-MS	0.1 ng/ml (LOQ)	0.57 ng/ml (0.1–4.1 ng/ml)	—	Heitland & Köster (2006)
—	—	1 ml	Microwave-assisted acid digestion	SF-ICP-MS	Blood: 0.03 ng/ml (LOD) Urine: 0.007 ng/ml (LOD)	—	—	Bocca et al. (2005)

HR-ICP-MS, high-resolution inductively coupled plasma mass spectrometry; LOQ, limit of quantification; *n*, number of samples analysed; ND, not detected; SF-ICP-MS, sector field inductively coupled plasma mass spectrometry; ZF-AAS, Zeeman furnace atomic absorption spectrometry

Heitland & Köster (2006) evaluated 37 trace metals, including cadmium, in blood of 130 people (ranging in age from 18 to 70 years) living in northern Germany who were assumed to be unexposed to cadmium from environmental sources. Samples were collected in 2005, and trace metals in blood were determined by DRC-ICP-MS. External quality assurance was performed by participation in three national and international quality assessment schemes to ensure the accuracy and reliability of the data. The mean cadmium level in blood was found to be 0.57 ng/ml.

4. SAMPLING PROTOCOLS

Guidelines on sampling of various foods are described in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (FAO/WHO, 2004).

5. PREVENTION AND CONTROL

Cadmium contamination of food arises mainly from the uptake of cadmium from soil by plants and grass, resulting in increased cadmium levels in food and feeds (UNEP, 2006). Background cadmium levels in surface soils range from 0.01 to 2.7 mg/kg (Kabata-Pendias, 2001). Cadmium levels in soil are given in the reports of the United Nations Environment Programme (e.g. UNEP, 2006, 2008). However, cadmium is much less mobile in soils than in air and water. The major factors governing cadmium mobility in soils are speciation, pH, soluble organic matter content, hydrous metal oxide content, clay content and type, presence of organic and inorganic ligands, and competition from other metal ions. Cadmium in soil tends to be more available when the soil pH is low (OECD, 1994). Elevated concentrations of cadmium in soils (compared with background values) have also been reported following the application of sewage sludge and farmyard manure, which contain variable and occasionally excessive cadmium concentrations (Steineck et al., 1999; Eriksson, 2000; Bergkvist et al., 2003). Fertilizers also play a role in the cadmium content of plants. The European Union suggested a cadmium limit of 46 mg/kg phosphorus in phosphate fertilizers (European Commission, 2001).

Analyses showed that cadmium levels in fruits and vegetables could be up to 9-fold higher than, and in meat and offal twice as high as, in non-contaminated areas. Cadmium levels appear to be higher in samples produced conventionally than in the corresponding organic products. The observed differences varied from 17% in green beans to 90% in lettuce. This observation could be explained by the cadmium impurities in phosphate fertilizers used in conventional production systems (EFSA, 2009a).

Washing of fruits and vegetables and peeling of roots and tubers can reduce cadmium contamination to some extent. There have been worldwide efforts to reduce cadmium exposure, including implementation of MLs for cadmium in foods, food additives and water. Other prevention and control measures include controlling cadmium levels in fertilizers and feeds and following good agricultural and manufacturing practices.

6. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

6.1 National and regional occurrence data

The Committee reviewed new cadmium occurrence data submitted by the European Food Safety Authority (EFSA) covering 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Estonia, France, Germany, Greece, Iceland, Ireland, Italy, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom); France also submitted data independently of EFSA. Occurrence data were submitted by 10 other countries (Australia, Brazil, Canada, Chile, China, Ghana, Japan, Singapore, the USA and Viet Nam). The food industry submitted data on cadmium levels in products that are distributed and used worldwide.

The majority of data submitted were individual analytical results for a wide range of foods. Aggregated data were also submitted, with the mean and maximum values reported. Most countries analysed samples using validated analytical methods and followed good internal and external quality control programmes to ensure the accuracy and reliability of the data.

As the Committee last conducted a complete assessment on cadmium in 2003, only data from 2003 to the present were included in the present assessment. In addition, data for certain foods that could not be linked to a specific commodity (e.g. multi-ingredient foods, infant formula, dietary supplements) were excluded from the assessment.

The total number of samples represented by the data submissions was 155 496, with 84.4% coming from Europe, 5.2% from North America, 1.5% from Asia, 1.4% from Latin America, 0.3% from the Pacific region and 0.1% from Africa (Table 5). The data submitted by industry accounted for 7.0% of the data.

In order to summarize and compare all submitted data, it was necessary to group the data by food category. As there were no common food codings or food descriptors used among all data sets, the following food categories were used to summarize the data and have been used throughout this section when presenting and discussing the data:

- Wheat (including breads)
- Rice
- Oats
- Baked goods
- Cereals/grains, other
- Roots & tubers
- Pulses & legumes
- Fruits
- Dried fruit
- Fruit juices
- Vegetables

Table 5. Distribution of new cadmium occurrence data by region

Region	Country	Total no. of samples	Samples with no detectable levels of cadmium (ND)[a]		% of samples by region
			No. of samples	% of total	
Asia	China	1 491	569	38.2	1.5
	Japan	67	0	0.0	
	Singapore	482	208	43.2	
	Viet Nam	317	0	0.0	
	Subtotal	*2 357*	*777*	*33.0*	
Europe	Combined data from 19 European countries[b]	131 167	44 133	33.6	84.4
Latin America	Brazil	2 241	2 067	92.2	1.4
	Chile	9	0	0.0	
	Subtotal	*2 250*	*2 067*	*91.9*	
North America	Canada	706	0	0.0	5.2
	USA	7 411	3 064	41.3	
	Subtotal	*8 117*	*3 064*	*37.7*	
Pacific region	Australia	532	190	35.7	0.3
Africa	Ghana	144	132	91.7	0.1
Food industry	—	10 929	4 418	40.4	7.0
	Total	*155 496*	*54 781*	*35.2*	*100*

ND, non-detects
[a] Samples with results below the LOD, LOQ or level of reporting (LOR).
[b] Submitted by EFSA.

- Vegetables, dried
- Meat & poultry muscle, not further specified (NFS)
- Meat & poultry offal, NFS
- Meat muscle
- Meat kidney
- Meat liver
- Meat offal, NFS
- Poultry muscle
- Poultry liver
- Poultry kidney
- Poultry offal, NFS
- Eggs

- Fish & seafood, NFS
- Finfish
- Shellfish/molluscs
- Dairy products
- Nuts & oilseeds
- Vegetable oils & fats
- Animal fats
- Coffee, tea & cocoa
- Sugar, honey & sweets
- Spices
- Alcoholic beverages
- Drinking-water (bottled & tap)

It was not always possible to determine the form (e.g. fresh versus dried) of the foods that were analysed, so in some cases both fresh and dried products may have been included in the same food category. Additionally, specific foods within a food category are known to contain higher levels of cadmium than most other foods in the category (e.g. horse meat compared with other meat muscle). Both cases could account for an unusually high maximum concentration of cadmium reported within a food category when compared with the mean concentration.

The data from each country or region that were included in the assessment are described below and are summarized in Table 6. Unless otherwise specified, mean values were calculated, assuming a value of zero for samples with results below the LOD, level of quantification (LOQ) or level of reporting (LOR) (i.e. non-detects or ND). In the case of aggregated data, weighted means were calculated based on sample size.

6.1.1 Australia

Food Standards Australia New Zealand submitted data collected in the 23rd Australian Total Diet Study (TDS) conducted in 2008–2009. Individual analytical results for a total of 532 samples covering all food categories were used in the assessment. The LOR for Australian TDS samples was 0.005 mg/kg. The highest mean cadmium levels were found in roots and tubers (0.033 mg/kg) and nuts and oilseeds (0.019 mg/kg).

6.1.2 Brazil

Brazil provided data on cadmium levels in kidney and muscle of meat and poultry samples collected from 2003 to 2009. Aggregated results representing 2241 samples were provided for meat muscle, meat kidney, poultry muscle and poultry kidney; weighted means were calculated for each of the four categories. The LOD was 0.005 mg/kg, and the LOQ was 0.01 mg/kg. Mean cadmium concentrations were higher in kidney (0.012–0.025 mg/kg) than in muscle (0.003 mg/kg), although the maximum cadmium level (0.286 mg/kg) was found in one sample of meat muscle.

Table 6. Cadmium occurrence data by data source

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
Australia					
	Wheat (including breads)	16	2	0.009	0.025
	Rice	4	2	0.004	0.009
	Oats	4	0	0.004	0.005
	Baked goods	20	3	0.012	0.032
	Cereals/grains, other	24	1	0.013	0.044
	Roots & tubers	12	0	0.033	0.087
	Pulses & legumes	4	0	0.004	0.006
	Fruits	80	48	0.006	0.051
	Dried fruit	12	1	0.009	0.030
	Fruit juices	12	2	0.001	0.002
	Vegetables	118	12	0.006	0.046
	Meat muscle	44	19	0.002	0.010
	Poultry muscle	20	18	0.000 2	0.003
	Poultry liver	4	0	0.006	0.010
	Eggs	10	8	0.000 3	0.002
	Finfish	18	1	0.006	0.015
	Shellfish/molluscs	8	0	0.014	0.070
	Dairy products	42	30	0.004	0.105
	Nuts & oilseeds	8	1	0.019	0.037
	Animal fats	4	4	ND	ND
	Vegetable oils & fats	8	8	ND	ND
	Coffee, tea & cocoa	12	10	0.000 1	0.001
	Sugar, honey & sweets	8	7	0.000 1	0.001
	Spices	8	0	0.006	0.009
	Alcoholic beverages	20	5	0.002	0.007
	Drinking-water (bottled & tap)	12	8	0.000 09	0.000 5
	Total number	532	190		

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
Brazil					
	Meat muscle	476	438	0.003	0.286
	Meat kidney	118	85	0.025	0.271
	Poultry muscle	1 503	1 418	0.003	0.228
	Poultry kidney	144	126	0.012	0.285
	Total number	2 241	2 067		
Canada					
	Shellfish/ molluscs	706	0	4.820	94.65
Chile					
	Shellfish/ molluscs	9	0	0.949	1.364
China (TDS)					
	Cereals/grains	12	0	0.010	0.068
	Roots & tubers	12	0	0.006	0.024
	Pulses & legumes	12	0	0.016	0.100
	Fruits	12	1	0.001	0.007
	Vegetables	12	0	0.010	0.033
	Meat & poultry, NFS	12	0	0.042	0.249
	Eggs	12	0	0.002	0.011
	Fish & seafood, NFS	12	1	0.077	0.414
	Dairy products	12	12	ND	ND
	Sugar	12	12	ND	ND
	Alcoholic beverages	12	10	0.000 4	0.000 2
	Beverages & water	12	12	ND	ND
	Total number	144	48		

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
China (monitoring data)					
	Fruits	1 109	485	0.006	0.270
	Vegetables	223	36	0.019	0.210
	Molluscs	15 mean values	NA	0.599 (average of means)	NA
	Total number	1 347	521		
EFSA (covering 19 European countries)[a]					
	Cereals and cereal products	12 179	1 705	0.023	0.220
	Starchy roots or potatoes	2 135	320	0.021	0.142
	Vegetables, nuts and pulses	16 335	3 430	0.067	2.709
	Fruits	4 300	2 408	0.004	0.050
	Fruit & vegetable juices, fruit juice drinks (excluding bottled water)	2 920	1 531	0.003	0.090
	Meat and meat products and substitutes (including poultry)	20 142	11 280	0.017	8.746
	Edible offal & offal products (meat and poultry)	16 049	1 765	0.206	34.50
	Eggs	667	320	0.003	0.018
	Seafood & seafood products	5 780	1 040	0.215	4.525
	Fish & fish products	10 172	4 781	0.023	0.660
	Milk- & dairy-based products	7 305	3 433	0.005	0.097
	Fats (vegetable & animal)	1 064	149	0.006	0.104
	Coffee, tea & cocoa	2 115	254	0.074	2.075
	Sugar & sugar products including chocolate	3 810	1 410	0.031	0.470
	Spices	1 336	214	0.062	0.612
	Alcoholic beverages	3 410	1 944	0.002	0.250
	Water, tap	19 000	6 460	0.000 4	0.010
	Bottled water	2 448	1 689	0.000 4	0.003 0
	Total number	131 167	44 133		

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
France					
	Wheat (including breads)	36	2	0.051	0.170
	Rice	30	18	0.010	0.043
	Cereals/grains, other	62	10	0.085	1.260
	Roots & tubers	74	10	0.029	0.560
	Pulses & legumes	28	23	0.008	0.115
	Fruits	1 178	1 047	0.005	3.000
	Dried fruit	27	16	0.003	0.019
	Fruit juices	8	8	ND	ND
	Vegetables	1 279	181	0.203	6.130
	Vegetables, dried	5	0	1.952	7.500
	Meat muscle	6 875	5 245	0.061	40.02
	Meat liver	2 960	328	0.099	40.02
	Meat offal, NFS	1 324	1 035	1.086	47.66
	Poultry muscle	1 149	122	0.005	2.620
	Poultry offal	1 070	255	0.069	10.13
	Finfish	834	681	0.010	1.655
	Shellfish	61	14	0.390	12.27
	Molluscs	357	15	0.467	6.480
	Dairy products	3	3	ND	ND
	Nuts & oilseeds	168	57	0.083	0.740
	Vegetable oils & fats	16	13	0.007	0.055
	Coffee, tea & cocoa	107	6	0.492	5.239
	Sugar, honey & sweets	3	2	0.002	0.005
	Spices	141	18	0.046	0.500
	Alcoholic beverages	104	76	0.0002	0.002
	Drinking-water (bottled & tap)	40	40	ND	ND
	Total number	17 939	9 225		
Ghana					
	Finfish	144	132	0.000 02	0.000 2

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
Japan					
	Shellfish/molluscs	67	0	0.346	1.400
Singapore					
	Baked goods	8	8	ND	ND
	Cereals/grains, other	2	2	ND	ND
	Fruit juices	2	2	ND	ND
	Vegetables	28	24	0.015	0.130
	Vegetables, dried	228	35	0.986	14.87
	Shellfish/molluscs	17	1	0.288	0.790
	Finfish	1	1	ND	ND
	Nuts & oilseeds	9	2	0.086	0.430
	Vegetable oils & fats	27	27	ND	ND
	Coffee, tea & cocoa	74	30	0.149	0.490
	Sugar, honey & sweets	58	58	ND	ND
	Spices	27	17	0.024	0.130
	Alcoholic beverages	1	1	ND	ND
	Total number	482	208		
USA (USFDA TDS)					
	Wheat (including breads)	160	1	0.021	0.047
	Rice	20	0	0.007	0.014
	Oats	20	1	0.003	0.004
	Cereals/grains, other	40	20	0.008	0.029
	Roots & tubers	100	2	0.015	0.058
	Pulses & legumes	60	4	0.003	0.010
	Fruits	360	167	0.003	0.054
	Dried fruit	20	16	0.001	0.006
	Fruit juices	166	106	0.001	0.008
	Vegetables	640	69	0.018	0.420
	Meat muscle	200	183	0.000 3	0.011
	Meat liver	20	0	0.060	0.171

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
	Poultry muscle	60	52	0.000 4	0.006
	Eggs	40	38	0.000 1	0.003
	Finfish	60	32	0.005	0.029
	Shellfish/molluscs	20	6	0.005	0.026
	Dairy products	140	134	0.000 2	0.005
	Nuts & oilseeds	80	12	0.131	0.874
	Animal fats	20	17	0.001	0.006
	Vegetable oils & fats	40	39	0.000 1	0.004
	Coffee, tea & cocoa	20	19	0.000 1	0.001
	Sugar, honey & sweets	20	19	0.002	0.042
	Total number	2 306	937		
USA (USFDA monitoring data)					
	Rice	6	0	0.021	0.047
	Baked goods	27	2	0.020	0.040
	Cereals/grains, other	9	3	0.025	0.058
	Roots & tubers	1	1	ND	ND
	Pulses & legumes	5	3	0.000 8	0.003
	Fruits	84	57	0.002	0.023
	Dried fruit	47	31	0.004	0.070
	Fruit juice	36	28	0.001	0.011
	Vegetables	37	18	0.026	0.360
	Vegetables, dried	2	0	0.085	0.130
	Finfish	124	90	0.006	0.139
	Shellfish	354	8	0.429	3.420
	Molluscs	90	9	0.091	0.799
	Dairy products	117	109	0.000 3	0.009
	Nuts & oilseeds	7	0	0.117	0.508
	Spices	5	0	0.228	0.251
	Total number	951	359		

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
USA (USDA)					
	Meat muscle	958	941	0.001	0.277
	Meat kidney	961	1	0.498	9.054
	Meat liver	307	0	0.073	0.415
	Poultry muscle	852	823	0.002	0.837
	Poultry kidney	876	3	0.464	11.40
	Poultry liver	200	0	0.176	1.820
	Total number	4 154	1 768		
Viet Nam					
	Molluscs	317	0	0.487	0.980
Industry (ingredients used worldwide)					
	Wheat (including breads)	1 327	146	0.035	0.390
	Rice	2 265	426	0.023	0.510
	Cereals/grains, other	371	179	0.010	0.120
	Oats	187	34	0.016	0.096
	Roots & tubers	59	13	0.035	0.160
	Pulses & legumes	88	38	0.030	0.220
	Fruits	369	263	0.007	0.380
	Fruit juices	796	713	0.002	0.085
	Vegetables	790	118	0.095	1.800
	Vegetables, dried	118	16	0.330	2.400
	Meat muscle	37	30	0.002	0.024
	Poultry muscle	65	51	0.010	0.150
	Eggs	7	5	0.007	0.030
	Molluscs	7	0	4.213	22
	Shellfish	13	3	0.648	6.000
	Dairy products	1 592	1 403	0.002	0.763
	Nuts & oilseeds	246	68	0.038	0.590

Table 6 (contd)

Data source	Food category	No. of samples	No. of non-detects	Mean concentration (mg/kg) (ND = 0)[a]	Maximum concentration (mg/kg)
	Vegetable oils & fats	447	409	0.002	0.031
	Coffee, tea & cocoa	1 284	196	1.750	1327
	Spices	861	307	0.106	1.400
	Total number	10 929	4 418		

NA, not available; ND, non-detects

[a] In calculation of mean cadmium concentration, cadmium concentration set at 0 mg/kg for non-detects, except for EFSA results, in which cadmium concentration set at LOD/2 or LOQ/2 for non-detects.

6.1.3 Canada

Aggregated data on cadmium levels in wild and farmed molluscs (scallops) were submitted by Canada. A weighted mean cadmium level of 4.82 mg/kg was calculated from the 43 aggregated results representing 706 samples collected in 2003–2004. The maximum cadmium level was 94.65 mg/kg. Neither the LOD nor the LOQ was specified.

6.1.4 Chile

Chile submitted one aggregated mean value representing nine samples of mussels collected in 2008. The mean cadmium level was found to be 0.949 mg/kg, and the maximum value was 1.364 mg/kg. The LOQ was 0.01 mg/kg.

6.1.5 China

Data from three different sources were provided by China.

Results from the 2007 Chinese TDS included cadmium levels in 12 food group composites collected from 12 provinces; the national average cadmium levels and maximum values reported are provided in Table 6. The highest mean cadmium levels were found in fish and seafood (0.077 mg/kg) and in meat and poultry (0.042). The highest maximum values were reported for fish and seafood (0.414 mg/kg) and meat and poultry (0.249 mg/kg). The LOD was 0.0005 mg/kg.

Individual results were also submitted by China for 1332 samples of fruits and vegetables collected in 2004–2005. Mean cadmium levels were 0.006 mg/kg in fruits and 0.019 mg/kg in vegetables. The highest cadmium level (0.270 mg/kg) was found in one sample of fruit. Neither the LOD nor the LOQ was specified.

Aggregated data were submitted on 15 samples of molluscs collected in China in 2003–2007; as sample sizes were not provided for all results, only the average of the aggregated means (0.599 mg/kg) is presented in Table 6. The LODs and LOQs were not reported.

6.1.6 Europe

EFSA recently completed an assessment on cadmium in the food-chain, including the risks to humans from dietary exposure to cadmium (EFSA, 2009a). The work was carried out by the Scientific Panel on Contaminants in the Food Chain at the request of the European Commission. For the EFSA assessment, cadmium data on about 140 000 samples, including a wide range of foods covering the period from 2003 to 2007, were submitted by 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Estonia, France, Germany, Greece, Iceland, Ireland, Italy, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom). EFSA grouped the data into 15 major food categories and in some cases subcategories that were directly related to the consumption data (EFSA's Concise European Food Consumption Database) used to estimate dietary exposure (EFSA, 2008). The EFSA food categories that were included in this assessment, as reported in Table 6, were similar but not identical to the categorization scheme used for the current assessment by the Committee. Results for foods that did not match the food categories used for the current assessment (i.e. foods for special dietary use, supplements and meat- and fish-based preparations) were excluded; the total number of samples from EFSA as reflected in Table 6 is 131 167.

EFSA submitted to the Committee both the final report of its assessment and the individual cadmium occurrence data used in the assessment. As EFSA had undertaken a very detailed analysis and aggregation of the occurrence data for its assessment, the Committee agreed that the summary results from the EFSA report would be used in the present assessment rather than the individual occurrence data. In the EFSA report, two mean cadmium values were reported for each food category. The first value was calculated as the mean of combined occurrence data from all countries, assuming one half the LOD or LOQ for results below the LOD/LOQ; these values are reported in Table 6. It should be noted, however, that in its estimation of dietary exposure, EFSA weighted these mean values by applying the sampling adjustment factor that corrected for the unbalanced proportion of samples analysed in food categories in relation to their relative consumption amounts. The weighted means are not reported here but are available in the EFSA report. Highest mean cadmium levels were found in meat and poultry offal (0.206 mg/kg) and in seafood and seafood products (0.215 mg/kg). Individual foods with the highest cadmium concentrations included fungi (2.71 mg/kg), horse meat (8.75 mg/kg) and offal, not further specified (34.50 mg/kg).

6.1.7 France

Individual results for samples collected between 2003 and 2007 were submitted by the French Food Safety Agency. The samples included a wide range of foods collected by the General Directorate for Competition, Policy, Consumer Affairs and Fraud Control and the General Directorate for Food. Meat and poultry

products make up the majority of samples. LODs ranged from 0.0002 to 0.01 mg/kg. In all, results for 17 939 samples were included in the current assessment. The highest mean cadmium levels were found in meat offal (1.086 mg/kg) and dried vegetables (1.952 mg/kg). The maximum cadmium value of 47.60 mg/kg was found in meat offal.

The majority of cadmium occurrence data submitted by France were also included in the EFSA cadmium assessment and are reflected in the EFSA data summary. Although the data submitted by France are presented separately in Table 6, to avoid double-counting of data, they have not been included in the total count of sample results submitted for the present assessment (Table 5) or in the summary of data by food category (see Table 7 below).

6.1.8 Ghana

Data on cadmium levels in two species of marine finfish in Ghana were submitted for the present assessment. A total of 144 samples of fish were collected in 2008–2009. Of those, only 12 samples had detectable levels of cadmium (LOD = 0.002 mg/kg); the mean cadmium level overall was 0.000 02 mg/kg.

6.1.9 Japan

Aggregated data on cadmium levels in molluscs were submitted by the Environmental Science Research Laboratory in Japan. The data represented 67 samples of molluscs collected from 2004 to 2007. The weighted mean level was found to be 0.346 mg/kg, with a maximum cadmium level reported to be 1.4 mg/kg.

6.1.10 Singapore

Cadmium data on a range of foods were provided by Singapore. A total of 482 individual results were submitted for samples collected in 2008. The majority (228) of samples were dried vegetables; these foods also had the highest cadmium levels, with a mean of 0.986 mg/kg and a maximum value of 14.87 mg/kg. Other foods that contained relatively high cadmium levels were shellfish/molluscs (mean of 0.288 mg/kg) and coffee, tea and cocoa (mean of 0.149 mg/kg). The LOD was 0.04 mg/kg.

6.1.11 USA

Three different sources of individual data were provided by the USA. The United States Food and Drug Administration (USFDA) provided 2306 results from samples collected in its TDSs between 2004 and 2008. LODs ranged from 0.001 to 0.004 mg/kg. Nuts and oilseeds were found to have the highest cadmium levels (mean of 0.131 mg/kg and maximum of 0.874 mg/kg). Meat liver had a mean cadmium concentration of 0.06 mg/kg. All other foods had mean concentrations of 0.021 mg/kg or less.

Cadmium results from other USFDA monitoring programmes were also submitted; these included 951 results from samples collected from 2003 to 2006. Nine food categories were represented, although finfish and shellfish comprise the

majority of samples. LODs or LOQs were not reported. Nuts and oilseeds, spices and shellfish had the highest mean cadmium levels (0.117, 0.228 and 0.429 mg/kg, respectively).

The United States Department of Agriculture (USDA) submitted a total of 4154 results for three subcategories (muscle, liver and kidney) of meat and poultry tissue. The samples were collected from 2003 through 2008. Kidney and liver tissues were found to contain higher mean cadmium levels than muscle. Highest levels were found in meat kidney (mean of 0.498 mg/kg and maximum of 9.054 mg/kg) and poultry kidney (mean of 0.464 mg/kg and maximum of 11.40 mg/kg).

6.1.12 Viet Nam

Aggregated data on cadmium levels in oysters and clams from samples collected in 2007 were submitted by Viet Nam. The weighted mean of all samples was found to be 0.487 mg/kg. The maximum level found was 0.980 mg/kg. Neither the LOD nor the LOQ was specified.

6.1.13 Food industry

Aggregated data on raw materials used worldwide were submitted by the food industry. The samples were collected between 2002 and 2009; it was not possible to separate the data by sampling year, so results prior to 2003 are included in the data summaries. Overall, the data represented analytical results of 10 929 samples. Weighted mean cadmium levels were calculated for all food categories. Highest mean levels were found in molluscs (4.213 mg/kg) and in coffee, tea and cocoa (particularly the latter) (1.75 mg/kg). LODs and LOQs were not reported.

6.2 Cadmium occurrence data by food category

Cadmium occurrence data submitted for this meeting are summarized by food category in Table 7. For each food category, the total number of samples and the number of countries or region (i.e. Europe or industry) represented by the data are reported. As noted above, because the data for Europe that were submitted by EFSA included data from France, the data submitted separately by France have not been included in Table 7. The range of national or regional mean cadmium concentrations is reported, as well as the current Codex MLs that have been established for commodities within the food category.

For all food categories, calculations of mean concentrations included results below the LOD or LOQ (i.e. non-detects). EFSA assumed a value of one half the LOD or LOQ when calculating mean concentrations; mean cadmium levels for other countries were calculated assuming a concentration of 0 mg/kg for non-detects.

National mean concentrations of cadmium ranged between not detected (ND) and 0.04 mg/kg in most food categories. Higher national mean concentrations, ranging from 0.1 to 4.8 mg/kg, were reported for vegetables (including dried); meat and poultry offal; shellfish/molluscs; nuts and oilseeds; coffee, tea and cocoa; and spices.

Table 7. Cadmium occurrence data summarized by food category

Food category	Total no. of samples	No. of countries/region contributing data	Range of national or regional mean cadmium concentrations (mg/kg)	Codex MLs (mg/kg)
Wheat (including breads)	1 503	3	0.009–0.04	0.2
Rice	2 295	3	0.004–0.02	0.4
Oats	211	3	0.003–0.02	0.1
Baked goods	55	3	ND–0.02	—
Cereals/grains, other	12 637	6	ND–0.02	0.1
Roots & tubers	2 319	5	0.006–0.04	0.1
Pulses & legumes	169	4	0.003–0.03	0.1 (pulses)
Fruits	6 314	5	0.001–0.007	—
Fruit juices	3 932	5	ND–0.003	—
Dried fruit	79	2	0.003–0.009	—
Vegetables	18 183	6	0.006–0.1	0.05–0.2[a]
Dried vegetables	348	3	0.09–1.0	—
Meat and poultry, NFS	20 154	2	0.008–0.04	—
Meat and poultry offal, NFS	16 049	1	0.1	—
Meat muscle	1 715	4	0.001–0.003	—
Meat offal	1 406	3	0.03–0.5	—
Poultry muscle	2 500	4	0.0002–0.01	—
Poultry offal	1 224	3	0.006–0.5	—
Eggs	736	5	0.0001–0.007	—
Finfish	10 531	6	ND–0.008	—
Shellfish/molluscs	7 403	10	0.01–4.8	2[b]
Dairy products	9 208	5	ND–0.004	—
Nuts & oilseeds	350	4	0.02–0.1	—
Animal & vegetable fats	1 610	5	ND–0.006	—
Coffee, tea & cocoa	3 505	5	0.0001–1.8	—
Sugar, honey & sweets	3 908	5	ND–0.03	—
Spices	2 237	5	0.006–0.2	—

Table 7 (contd)

Food category	Total no. of samples	No. of countries/region contributing data	Range of national or regional mean cadmium concentrations (mg/kg)	Codex MLs (mg/kg)
Alcoholic beverages	3 443	4	ND–0.004	—
Drinking-water (bottled & tap)	21 472	3	ND–0.0004	0.003 (natural mineral water)
Total no. of samples	155 496			

ND, not detected

[a] MLs for vegetables: 0.05 mg/kg for brassica, bulb and fruiting vegetables, excluding tomatoes and fungi; 0.1 mg/kg for stalk, stem and legume vegetables; and 0.2 mg/kg for leafy vegetables.

[b] ML for cephalopods and bivalves, excluding scallops and oysters.

7. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

7.1 National and regional estimates of dietary exposure

New information on national estimates of dietary exposure to cadmium was submitted by Australia, China, Japan and the USA. EFSA submitted dietary exposure estimates for Europe. Additional information on national dietary exposure for Chile, Lebanon and the Republic of Korea was obtained from the scientific literature. National and regional exposure estimates were expressed on either a daily or weekly basis, as these estimates are based on 1- to 7-day food consumption surveys. During the meeting, the Committee concluded that a provisional tolerable monthly intake (PTMI) was appropriate for cadmium (see section 10). For contaminants such as cadmium that are widely distributed in foods at approximately constant levels, day-to-day variability in dietary exposure over the long term would be low, so extrapolating dietary exposure from a daily or weekly basis to a monthly basis would not have a substantial impact on exposure estimates. Therefore, the national and regional exposure estimates as reported below were extrapolated to a monthly basis by multiplying daily exposures by 30 or weekly exposures by 4.

7.1.1 Australia

Food Standards Australia New Zealand has conducted two recent TDSs that included assessments of cadmium in foods: the 20th Australian TDS in 2000–2001 and the 23rd Australian TDS in 2008–2009. Analytical results from the 23rd Australian TDS were described and summarized in the previous section of this report. As dietary exposure estimates from the most recent TDS have not yet been completed, those from the 20th TDS (2000–2001) are presented in Table 8.

Table 8. Dietary exposure to cadmium in Australia (2000–2001)

Population subgroup	Average body weight (kg)	Exposure (µg/kg bw per month)	
		Lower bound (ND = 0)	Upper bound (ND = LOR)
Infants 9 months	9.2	3.9	20.4
Toddlers 2 years	14	5.4	17.1
Boys 12 years	49	3.3	8.7
Girls 12 years	52	2.7	6.6
Adult males 25–34 years	82	2.4	7.2
Adult females 25–34 years	66	2.1	6.6

Lower- and upper-bound estimates of exposure were calculated for six population subgroups. Both estimates were based on mean food consumption data from the 1995 Australian National Nutrition Survey. The lower-bound estimates were based on median cadmium concentrations in foods, assuming that results below the LOR were equal to zero, whereas upper-bound estimates assumed that results below the LOR were equal to the LOR. Table 8 summarizes the range of exposure estimates per kilogram of body weight per month. Estimates of exposures for infants and toddlers range (from lower to upper bound) from 3.9 to 20.4 µg/kg bw per month. Exposures for boys and girls 12 years of age range from 2.7 to 8.7 µg/kg bw per month, whereas those for adults range from 2.1 to 7.2 µg/kg bw per month. No information was provided regarding contributions of specific food categories to overall exposure.

7.1.2 China

China completed its most recent TDS in 2007, from which the mean cadmium concentrations in 12 food group composites from 12 geographic regions (provinces) were reported above (Table 6). Dietary exposures were estimated from these cadmium occurrence data, but assuming a value of one half the LOD for non-detects rather than zero, as reported in Table 6.

Estimated regional cadmium exposures ranged from 0.5 µg/kg bw per month in Ningxia province to 36.5 µg/kg bw per month in Sichuan province (Table 9). The national average cadmium exposure was estimated to be 9.9 µg/kg bw per month.

Based on the national average estimate of exposure, the food categories that contributed most to cadmium exposure were cereals/grains (32%) and vegetables (25%). For Sichuan province, which had the highest cadmium exposure, cereals accounted for 85% of the total exposure; the cadmium level in the cereals composite sample from this province was 0.067 mg/kg, which is about 6 times the national mean concentration in cereals.

Table 9. Dietary exposure to cadmium in China (2007)

Province	Mean exposure (μg/kg bw per month)[a]	Major sources of exposure (% of total exposure)
Heilongjiang	6.8	Meat (93%)
Liaoning	5.2	Seafood (88%)
Hebei	2.6	Legumes (36%) + seafood (34%)
Shanxi	1.2	Vegetables (62%)
Henan	2.7	Cereals (50%) + vegetables (45%)
Ningxia	0.5	Cereals (39%) + potatoes (23%)
Shanghai	4.7	Vegetables (69%)
Fujian	14.7	Cereals (40%) + vegetables (37%)
Jiangxi	22.4	Cereals (92%)
Hubei	9.7	Meat (53%) + vegetables (41%)
Sichuan	36.5	Cereals (85%)
Guangxi	10.3	Cereals (37%) + legumes (24%)
National average	9.9	Cereals (32%) + vegetables (25%)

[a] Based on mean cadmium concentration (ND = LOD/2) and body weight of 63 kg.

Although cereals were the main source of dietary exposure to cadmium in most provinces, vegetables, meat and seafood were significant sources in several provinces. Vegetables accounted for 62% and 69% of total cadmium exposure in Shanxi and Shanghai provinces, respectively. Meat was the major source of cadmium in the diet in Heilongjiang and Hubei provinces. Seafood contributed 88% of the total dietary cadmium in Liaoning province.

7.1.3 Europe

Dietary exposure estimates for Europe were calculated for the recent EFSA assessment on cadmium in foods (EFSA, 2009a). The cadmium occurrence data summarized in Table 6 were used in the exposure estimates, after applying the sampling adjustment factor to the mean cadmium concentrations to correct for the unbalanced proportion of samples analysed in food categories in relation to their relative consumption amounts.

Several food consumption databases were used in order to estimate exposure for different population subgroups: the EFSA Concise European Food Consumption Database (EFSA, 2008), French data on food consumption by ovolactovegetarians and Italian data on food consumption by children.

The EFSA Concise European Food Consumption Database was compiled from data provided by 16 European countries on food consumption by adults. (Note that the countries that provided the cadmium occurrence data summarized in Table 6 are not necessarily the same countries represented in the consumption database.) The EFSA database includes mean consumption estimates for each of 15 broad food categories for each of the 16 countries. The cadmium occurrence data submitted for the EFSA assessment were aggregated to these same food categories so that the consumption and occurrence data could be linked. Dietary exposure was estimated by multiplying mean consumption per food category for each country by the adjusted (weighted) mean cadmium concentration for each food category. A body weight of 60 kg was used as the default for all countries when converting exposure estimated from a per person basis to exposure per kilogram of body weight.

Mean exposure for adults ranged from 7.6 µg/kg bw per month (Bulgaria) to 11.8 µg/kg bw per month (Germany), with an estimated median European exposure of 9.1 µg/kg bw per month (Table 10). As the same mean cadmium concentrations per food category were used in all calculations, differences in exposure estimates reflect variability in national consumption patterns only.

National estimates of high exposures were also estimated for the adult population based on the EFSA Concise European Food Consumption Database by summing the 95th percentile exposures (consumers only) from the two food categories contributing most to exposure and the mean exposure (whole population) for other food categories. High exposure estimates ranged from 10.2 µg/kg bw per month (Finland) to 15.6 µg/kg bw per month (Slovakia), with an overall European estimate of 12.1 µg/kg bw per month.

Regarding cadmium exposures for certain subpopulations, food consumption data from Italy were used to estimate exposure for children. Exposure for children 0.5–12 years of age was estimated to be 11.9 and 22.0 µg/kg bw per month at the mean and 95th percentile, respectively. For estimating dietary exposure for vegetarians, detailed French consumption data for ovolactovegetarians were used to represent vegetarians as a whole. From the French consumption data, it was shown that vegetarians consume greater amounts of nuts, oilseeds, pulses and cereals. Average exposure of vegetarians in Europe to cadmium was modelled by using results of exposure estimates for adults but replacing consumption of meat and fish groups with added consumption of nuts and oilseeds. The resulting estimate of average exposure was 21.60 µg/kg bw per month; high exposure was not estimated for this subgroup.

EFSA reported cadmium exposures from specific food categories (Table 11) for adults only. These were estimated by multiplying the median consumption values (consumers only) across the 16 European countries that submitted consumption data by the adjusted mean cadmium occurrence values for each food category. Food categories contributing most to adult exposure to cadmium included cereals and grains; vegetables, nuts and pulses; and edible offal and offal products. As these estimates were based on consumers-only consumption data, they could not be summed to calculate the total exposure or the relative contributions of each food category to total exposure.

Table 10. Dietary exposure to cadmium in Europe (2003–2007)

Country or subpopulation	Exposure (µg/kg bw per month)[a]	
	Mean	High[b]
Adults		
Belgium	9.3	13.1
Bulgaria	7.6	12.5
Czech Republic	9.5	12.3
Denmark	9.0	11.2
Finland	7.8	10.2
France	9.1	12.5
Germany	11.8	14.3
Hungary	8.6	10.9
Iceland	8.3	12.4
Ireland	10.2	13.8
Italy	8.2	10.7
Netherlands	9.0	11.8
Norway	9.2	11.3
Slovakia	9.2	15.6
Sweden	9.3	11.6
United Kingdom	8.6	11.5
Adults, all countries (median)	9.1	12.1
Vegetarians	21.6	NA
Children 0.5–12 years	11.9	22.0[c]

NA, not available

[a] Based on mean European cadmium concentration (ND = LOD/2) (weighted) and mean national food consumption (whole population).

[b] Sum of 95th percentile exposure (consumers only) for the two food categories with highest exposure plus mean exposure (whole population) for the other food categories.

[c] 95th percentile.

Table 11. Main sources of dietary exposure for adults in Europe

Food category	Mean cadmium occurrence[a] (mg/kg)	Median consumption[b] (consumers only) (g/day)	Cadmium exposure (consumers only) (µg/day)
Cereals/grains	0.016	257	4.2
Vegetables, nuts, pulses	0.019	194	3.7
Meat + offal combined	0.017	151	2.5
Offal only	0.126	24	3.0
Meat	0.008	132	1.0
Starchy vegetables/roots	0.021	129	2.7
Alcoholic beverages	0.004	413	1.7
Fish and seafood	0.027	62	1.7
Sugars	0.026	43	1.1
Milk and dairy products	0.004	287	1.1
Coffee, tea, cocoa	0.002	601	1.1
Juices, soft drinks, bottled water	0.001	439	0.4
Miscellaneous foods	0.024	14	0.3
Fats (vegetable + animal)	0.006	38	0.2
Tap water	0.000	349	0.1
Eggs	0.003	25	0.08

[a] Mean (ND = LOD/2) (adjusted).
[b] Median of national estimates, consumers only.

7.1.4 USA

The USFDA conducts its TDS continuously, and foods are routinely analysed for cadmium. Cadmium levels in samples collected between 2004 and 2008 (Table 6) and consumption data from the 2003–2006 NHANES were used to estimate dietary exposure. Monte Carlo simulations were run for 14 population subgroups using the full distribution of both consumption and cadmium occurrence data; for the latter, all samples with results below the LOD were assigned a value of zero.

Estimates of mean and 90th percentile exposures are reported in Table 12. Infants and children 2 years and 6 years of age had the highest cadmium exposures, with means of 9.4, 12.9 and 10.9 µg/kg bw per month, respectively. Exposures at the 90th percentile were 17.6, 21.5 and 17.1 µg/kg bw per month, respectively.

Mean exposures for teenagers and adults were similar, ranging from 4.1 to 5.5 µg/kg bw per month. Boys 14–16 years of age had the highest 90th percentile exposures, at 10.1 µg/kg bw per month.

Table 12. Dietary exposure to cadmium in the USA (2004–2008)

Population subgroup	Exposure (µg/kg bw per month)[a]	
	Mean	90th percentile
M + F 6–11 months	9.4	17.6
M + F 2 years	12.9	21.5
M + F 6 years	10.9	17.1
M + F 10 years	7.2	12.7
M 14–16 years	5.5	10.1
F 14–16 years	5.3	8.9
M 25–30 years	5.3	9.5
F 25–30 years	4.6	8.6
M 40–45 years	4.7	7.2
F 40–45 years	4.5	8.4
M 60–65 years	4.7	8.9
F 60–65 years	4.3	7.2
M 70+ years	4.1	6.9
F 70+ years	4.5	8.3

F, female; M, male

[a] Based on distributions of cadmium occurrence data (ND = 0) and individual consumption data and self-reported body weights of consumption survey participants.

7.1.5 Estimates of dietary exposure from the published literature

In addition to the dietary exposure estimates that were submitted for the present assessment, information on dietary exposure to cadmium in four countries (Chile, Japan, Lebanon and the Republic of Korea) was obtained from the literature.

Results of a TDS conducted in Chile in 2001–2002 showed an estimated cadmium exposure of 21 µg/day or 0.3 µg/kg bw per day, based on a body weight of 68 kg (Munoz et al., 2005). This was extrapolated for the present assessment to an estimated exposure of 9 µg/kg bw per month. The major sources of cadmium in the diet were fish and shellfish, spices and cereals.

In a study by Matsudo (2007), cadmium exposure in Japan was estimated to be 21.1 µg/person per day, calculated as 0.4 µg/kg bw per day using a body weight of 53 kg. This was extrapolated to 12 µg/kg bw per month.

A TDS was conducted in Lebanon in 2004 in order to estimate dietary exposure of urban adults to heavy metals and radionuclides (Nasreddine et al., 2006). Five market baskets consisting of 77 foods were collected in Beirut, and the foods were prepared “as consumed” before analysis. Cadmium levels in those foods

Table 13. National estimates of dietary exposure to cadmium for adults

Country or region	Treatment of ND occurrence data in exposure estimates	Mean exposure (μg/kg bw per month)	High exposure (μg/kg bw per month)
Australia	ND = 0 and ND = LOD	2.2–6.9	—
Chile	Not specified	9	—
China	ND = LOD/2	9.9	—
Europe	ND = LOD/2	9.1[a]	12.1[b]
Japan	Not specified	12	—
Lebanon	ND = LOQ/2	5.2	6.9[c]
Republic of Korea	ND = LOD	7.7	—
USA[d]	ND = 0	4.6	8.1

[a] Median of mean exposure estimates for 16 European countries.
[b] Sum of 95th percentile exposure (consumers only) for the two food categories with highest exposure plus mean exposure (whole population) for the other food categories.
[c] Calculated from mean food consumption and highest cadmium concentrations in each food category.
[d] Calculated from distributions of both food consumption and cadmium occurrence data; high exposure equals 90th percentile of exposure.

were multiplied by national food consumption data, resulting in estimated mean and maximum exposures of 1.2 and 1.6 μg/kg bw per week (or 0.17 and 0.23 μg/kg bw per day), respectively. These were extrapolated to 4.8 and 6.4 μg/kg bw per month. Grains, vegetables and drinking-water were the major sources of cadmium in the diet.

A study in the Republic of Korea reported an estimated cadmium exposure of 14.3 μg/person per day or 0.26 μg/kg bw per day, based on a body weight of 55 kg (Lee et al., 2006). On a monthly basis, this would result in an exposure of 7.7 μg/kg bw. The two major sources of dietary exposure to cadmium were vegetables (especially seaweed) and fish.

7.1.6 Summary of national estimates of dietary exposure to cadmium

In summary, the national estimates of mean cadmium exposure for adults ranged from 2.2 to 12 μg/kg bw per month (Table 13). Estimates of high exposure for adults reported for Europe, Lebanon and the USA were 12.1, 6.9, and 8.1 μg/kg bw per month, respectively.

For Australia, Europe and the USA, mean dietary exposure for children 0.5–12 years of age ranged from 2.7 to 12.9 μg/kg bw per month; the highest exposure for this age group was reported for Europe (22.0 μg/kg bw per month). Dietary exposure for vegetarians, as reported by EFSA, was estimated to be 21.6 μg/kg bw per month. Overall, the food categories that contributed most to cadmium exposure

were cereals/grains, vegetables, meat and poultry offal, and seafood (especially molluscs).

7.2 Regional estimates of dietary exposure

The Codex Alimentarius Commission guidelines for conducting exposure assessments for contaminants in foods (FAO/WHO, 2010) recommend that regional dietary exposure estimates should be calculated using regional average contaminant values and the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets. Such estimates were not calculated for the present meeting because occurrence data were submitted by countries that represented only 2 of the 13 GEMS/Food clusters. Furthermore, national exposure estimates based on national food consumption data were submitted by the countries that also submitted the majority of new occurrence data. As the national estimates provided more refined estimates than could be calculated with the consumption cluster diets, only the national estimates were considered in this assessment.

8. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

8.1 Biomarker studies

The predominant sites of cadmium accumulation that contribute to body burden are the liver, kidney and several other tissues, but particularly muscle, skin and bone. The highest concentrations are found in the liver and renal cortex. The primary toxic effect resulting from chronic cadmium exposure is impaired tubular reabsorption of filtered solutes. However, the critical concentration of cadmium in the renal cortex that is likely to produce renal dysfunction remains a source of investigation and discussion. Nevertheless, excess urinary excretion of low molecular weight proteins and solutes appears to be associated with reduced tubular reabsorption. It should be noted that low molecular weight proteins (e.g. β2MG, α1MG, RBP and *N*-acetyl-β-D-glucosaminidase) are nonspecific for a given cadmium exposure and may not necessarily be adverse, but may indicate potential renal impairment. Clinically, impaired kidney function is usually apparent through increased levels of protein, amino acids, uric acid, calcium, copper and phosphorus in urine and/or serum. After prolonged cadmium exposure, increased levels of high molecular weight proteins in urine or decreased serum clearance of creatinine are indicative of glomerular dysfunction, which is generally associated with progressive renal damage.

A number of investigators have examined different data sets and cut-off criteria to estimate a safe cadmium body burden as a function of cadmium concentration in urine. BMD analyses of data from populations living in areas in Sweden (Suwazono et al., 2006) or Japan (Uno et al., 2005; Kobayashi et al., 2006) that are not polluted with cadmium or in cadmium-polluted areas in Japan (Shimizu et al., 2006) have been completed. These analyses used urinary cadmium level as a biomarker of cadmium exposure and the prevalence of abnormal levels of β2MG,

α1MG (also known as pHC or protein heterogeneous in charge), total protein, *N*-acetyl-β-D-glucosaminidase, RBP, albumin or GFR as biomarkers of renal tubular effects. As summarized in Table 14, the BMDs for urinary cadmium levels vary widely between the studies, depending on the renal biomarker and the cut-off level used.

8.2 Pivotal data from human clinical/epidemiological studies

To overcome the limitations inherent in a "key study" analysis, EFSA (2009b) undertook a comprehensive systematic literature review (published between January 1966 and October 2008), using Cochrane methodology (see Higgins & Green, 2008), to compile a database for the purpose of deriving a BMD and its 95% confidence lower bound (BMDL) using cut-off points relevant to clinical changes in target organs. Most published studies reported a relationship between urinary cadmium levels and renal biomarkers of cadmium toxicity. The most frequently studied low molecular weight protein biomarker of renal dysfunction was β2MG. The database compiled by EFSA covered approximately 30 000 predominately non-occupationally exposed individuals (99%) reported in 35 studies, but β2MG and cadmium concentrations in urine were expressed only as group means with standard deviations. The majority of the individuals were of Asian descent (93.5%) and female (75%). The age distribution was approximately equally divided around 50 years (i.e. ≥50 years: 51.5%; <50 years: 48.5%). The data for the population aged 50 years and over in the 35 studies were assigned to concentration classes, resulting in 98 groups containing matched pairs of urinary cadmium and β2MG levels. The 98 groups ranged in size from 3 to 908 individuals, with a median of 56.

EFSA (2009b) noted that there were few studies on effects of cadmium on bone; of those, most were considered to be heterogeneous and unsuitable. They also considered associations between cadmium exposure and other non-renal health effects, including diabetes, hypertension, carcinogenicity, reproductive outcomes and neurotoxicity. EFSA (2009b) found the results of these studies to be too preliminary to serve as the basis for its evaluation. Further work is needed to clarify the contribution of exposure to cadmium to these diseases.

8.3 General modelling considerations

8.3.1 Data check

The database containing the 35 suitable studies and described in detail by EFSA (2009b) was checked for consistency and accuracy. Only one alteration was made to the database. In the study reported by Hotz et al. (1999), it was considered unnecessary to transform the reported means and standard deviations to geometric values because it appeared as though they were already geometric values.

Table 14. Overview of recent studies reporting benchmark dose estimations for cadmium levels in urine and effects on renal tubular function

Study population	Cut-off level	Sample size (age in years)	Effect biomarker	BMD model	Urinary cadmium critical dose level (β2MG, μg/g creatinine; NAG, U/g creatinine; PROT, α1MG, mg/g creatinine)		Reference
					BMD_5 / $BMDL_5$	BMD_{10} / $BMDL_{10}$	
General population (Japan)	84% upper limit value of the target population	410 M; 418 F (40–59 years) Urine sampling occurred 1997–1998	β2MG NAG PROT	Quantal linear model	β2MG M: 0.5 / 0.4 F: 0.9 / 0.8 NAG M: 0.3 / 0.3 F: 0.8 / 0.6 PROT M: 0.9 / 0.6 F: 3.2 / 1.8	β2MG M: 1.0 / 0.7 F: 1.8 / 1.3 NAG M: 0.7 / 0.6 F: 1.6 / 1.2 PROT M: 1.9 / 1.2 F: 6.6 / 3.6	Uno et al. (2005)
General population (Japan)	84% upper limit value of the target population of those who had never smoked	1114 M; 1664 F (≥50 years), non-smokers	β2MG NAG PROT	Log-logistic model	β2MG M: 2.9 / 2.4 F: 3.8 / 3.3 NAG M: 4.8 / 3.3 F: 4.7 / 3.7 PROT M: 3.6 / 3.1 F: 4.8 / 4.2	β2MG M: 5.0 / 4.0 F: 6.6 / 5.5 NAG M: 8.3 / 5.7 F: 8.3 / 6.4 PROT M: 5.6 / 4.9 F: 7.5 / 6.6	Kobayashi et al. (2006)

Table 14 (contd)

Study population	Cut-off level	Sample size (age in years)	Effect biomarker	BMD model	Urinary cadmium critical dose level (β2MG, µg/g creatinine; NAG, U/g creatinine; PROT, α1MG, mg/g creatinine)		Reference
					BMD_5 / $BMDL_5$	BMD_{10} / $BMDL_{10}$	
General population (Sweden)	95th percentile of effect biomarkers on the "hypothetical" control distribution at a urinary cadmium level of zero	790 F (53–64 years)	NAG α1MG		NAG 0.64 / 0.5 α1MG 0.63 / 0.49	NAG 1.08 / 0.83 α1MG 1.05 / 0.81	Suwazono et al. (2006)
Residents in cadmium-polluted (Kakehashi River basin) and non-polluted areas (Japan)	84% upper limit values from a group of 424 males and 1611 females who did not smoke and lived in three different areas not polluted with cadmium	3178 M + F and 294 M + F (≥50 years) from polluted and non-polluted areas	β2MG	Quantal linear model	β2MG M: 1.5 / 1.2 F: 1.4 / 1.1	β2MG M: 3.1 / 2.5 F: 2.9 / 2.3	Shimizu et al. (2006)
				Log-logistic model	β2MG M: 3.7 / 2.9 F: 2.6 / 1.5	β2MG M: 5.1 / 4.2 F: 4.2 / 2.7	

[a] BMD_x, benchmark dose for an x% response; $BMDL_x$, lower limit on the benchmark dose for an x% response; F, female; M, male; NAG, *N*-acetyl-β-D-glucosaminidase; PROT = protein; U = unit. One unit will hydrolyse 1.0 µmol of *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide per minute at pH 5.0 and 25 °C.

8.3.2 Selection of mathematical model

Three different models were considered:

1) the Hill model, which is a four-parameter sigmoidal model (minimum, maximum, median effective dose [ED_{50}] and a shape parameter);
2) an exponential model with a threshold, which has three parameters (minimum, threshold and slope);
3) a biexponential model with four parameters (minimum, slope 1, slope 2 and a breakpoint).

All three models provided a similar visual goodness of fit, but the biexponential model was selected because it best showed the obvious transition or breakpoint between the slope observed at low and high concentrations of cadmium in urine (Figure 1). As the breakpoint is indicative of the onset of pathological changes in renal tubular dysfunction, the Committee considered this model to be suitable to characterize the urinary cadmium/β2MG dose–response relationship. The biexponential model is essentially the same as the piecewise-linear model described by EFSA (2009b). As individual subject data were not available and because much of the variation in outcome is attributable to within-group variation of urinary cadmium, it is not possible to model potential variation in the cause–effect relationship between the two biomarkers. This precluded the calculation of a BMD defined by a population percentile (e.g. a BMD_{10}), although a population average BMD associated with a specific urinary concentration (e.g. 300–1000 µg/g creatinine) could be calculated. In addition, an additional parameter was used to account for differences between Asians and Caucasians.

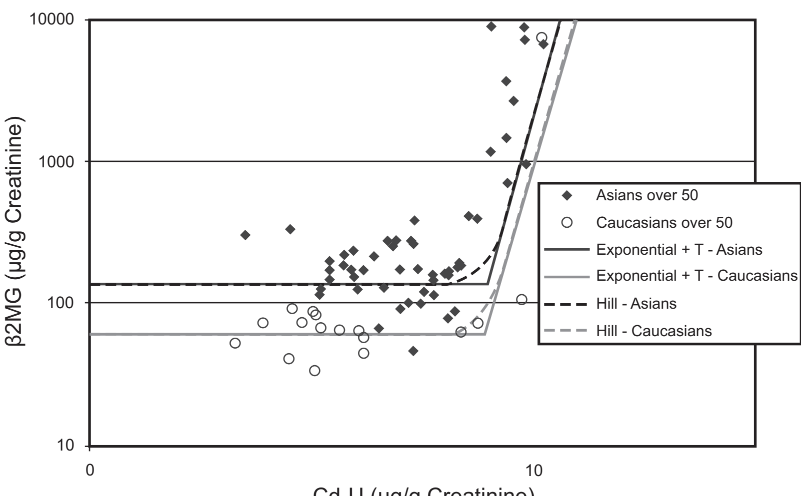
Parameter estimates were generated with linear regression, minimizing the sum of squares of the logs of the residuals. Log residuals were used in order to prevent domination of the parameter estimates by very high β2MG levels.

From Figures 1 and 2, it is apparent that Caucasians excrete less β2MG than Asians for an equivalent concentration of cadmium. A possible explanation for this difference may be the way in which urinary β2MG and urinary cadmium excretion are expressed as a function of excreted creatinine. Creatinine excretion is known to be affected by BMI and protein intake. This source of variation needs to be considered when comparing urinary cadmium data expressed as a function of creatinine between sexes and populations (Suwazono et al., 2005; Gerchman et al., 2009). In general, the mean or median BMI for Asian populations is lower than that observed for non-Asian populations (WHO Expert Consultation, 2004).

8.3.3 Use of the biexponential model in the evaluation

As described in section 8.3.2, the Committee chose the breakpoint for the second slope, which characterizes where the urinary β2MG concentration begins to rapidly increase with increasing urinary cadmium concentration, as the basis of the evaluation. The breakpoint derived for the population aged 50 years and over corresponds to 5.24 (5th–95th percentiles 4.94–5.57) µg/g creatinine. Hence, the breakpoint value of 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine and associated uncertainty are similar to those reported by EFSA (2009b)

Figure 1. Data and models with log–log plot

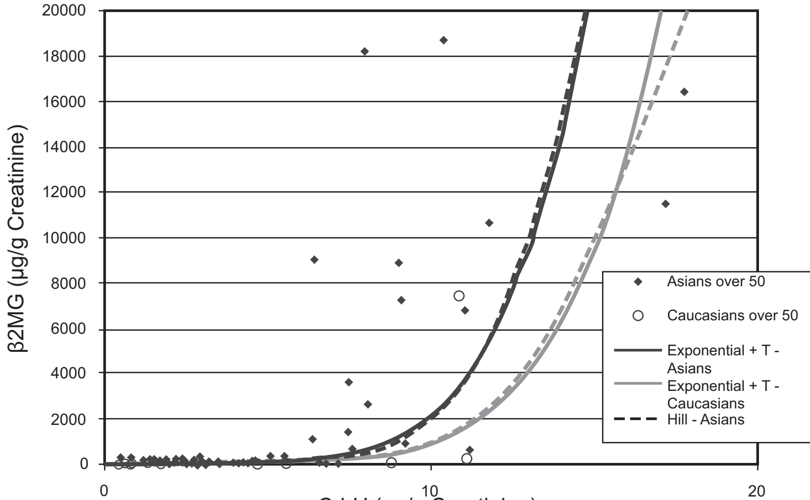


for the entire data set without covariates (i.e. 5.54; 95% CI 5.24–5.82). An inspection of the plotted data using the biexponential model (Figure 3) illustrates that this urinary cadmium concentration of 5.24 μg/g creatinine corresponds to the point at which the urinary β2MG concentration rises dramatically.

8.3.4 Toxicodynamic variability

Toxicodynamic variability in the dose–response relationship (i.e. variability in toxic response of the kidney to cadmium that has reached the target organ) is not taken into account by the model, because the data represent only a population average rather than individual data points. The lack of empirical evidence of a response below a urinary cadmium concentration of 5.24 (5th–95th percentiles 4.94–5.57) μg/g creatinine indicates that the variance is small. Toxicodynamic variability in the model was accounted for by introducing a log triangular distribution to represent individuals with increased or decreased susceptibility. The extreme values of the distribution were defined with a maximum variability in either direction (increased or decreased susceptibility) that ranged from 1 to 3, with a median of 2. The value of 3 approximately corresponds to the toxicodynamic component of a conventional 10-fold uncertainty factor for interindividual variability (IPCS, 2005). Individual subjects were presumed to have a critical concentration (breakpoint) somewhere between the range defined by the mean multiplied or divided by the maximum value. As the same maximum value was used for both increased and reduced individual susceptibility, the adjustment resulted in broadened distributions of both population variability and uncertainty without affecting the geometric central estimates.

Figure 2. Data and models with linear plot



8.3.5 Toxicokinetic modelling

The relationship between urinary cadmium concentration and dietary cadmium exposure was characterized by a one-compartment model reported by Amzal et al. (2009), which was based on a long-term study of Swedish women. Amzal et al. (2009) compared a complex eight-compartment model with a simplified one-compartment model and found that the simplified model provided an adequate description of the toxicokinetic relationship, while also allowing for an accounting of population variability. This one-compartment model consisted of two toxicokinetic parameters (cadmium half-life and a constant that subsumed several physiological parameters) and a statistical parameter for variation in apparent half-life that was used to account for individual variability.

This one-compartment model was used by the Committee for the evaluation. The confidence intervals associated with each of the parameters provided by Amzal et al. (2009) were used to generate the confidence intervals associated with the model estimates. The calculated relationship between dietary cadmium exposure and urinary cadmium concentration is linear; therefore, the outcome may be expressed as a population distribution of the ratio with confidence intervals (Figure 4).

Figure 3. The EFSA biexponential (piecewise-linear) dose–response model for urinary cadmium and β2MG concentrations

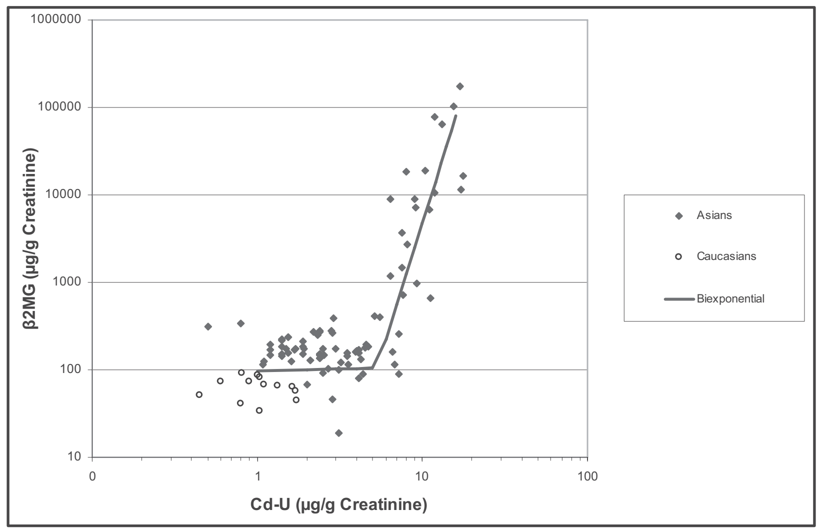


Figure 4. Population distribution of urinary to dietary cadmium ratios

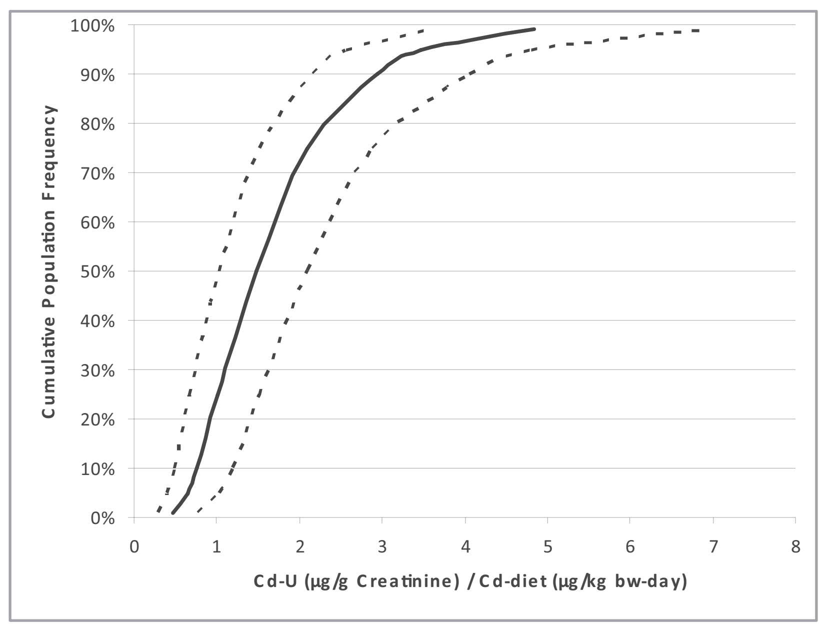
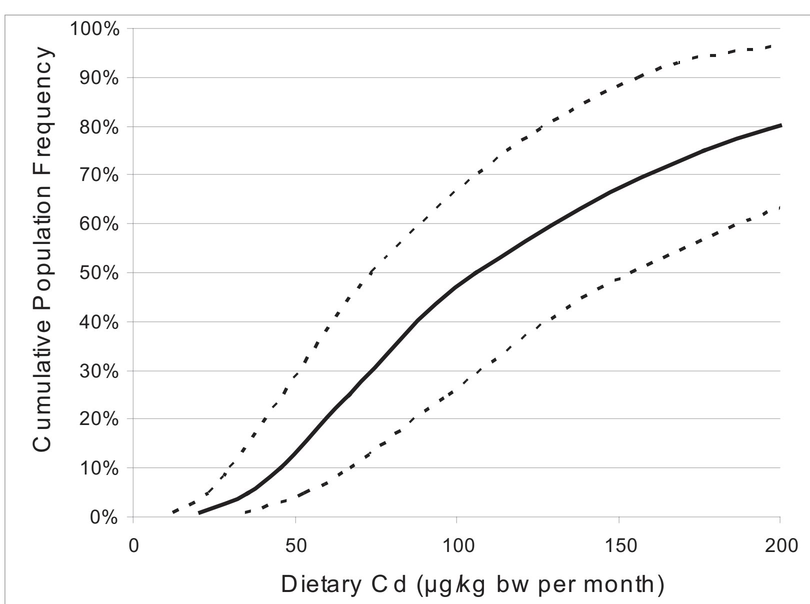


Figure 5. Population distribution of dietary cadmium exposure with 5th–95th percentile confidence intervals



8.4 Estimating the relationship between urinary cadmium excretion and dietary cadmium exposure

A two-dimensional Monte Carlo simulation was used to estimate the population percentiles with associated 5th to 95th percentile confidence intervals from the variability and uncertainty in the breakpoint, the adjustment for toxicodynamic variability and the toxicokinetic model (Figure 5). The simulation employed 1000 iterations for the variability dimension and 300 for the uncertainty dimension. The dietary cadmium exposure (µg/kg bw per day) that equates to a urinary cadmium concentration of 5.24 (5th–95th percentiles 4.94–5.57) µg/g creatinine was estimated to be 1.2 (5th–95th percentiles 0.8–1.8) µg/kg bw per day at the 5th population percentile. This is equivalent to 36 (5th–95th percentiles 24–54) µg/kg bw per month. The Committee decided to use the lower bound of the CI to account for particularly susceptible individuals so that they would remain below the point at which renal pathology is indicated by increased urinary β2MG levels.

9. COMMENTS

9.1 Absorption, distribution, metabolism and excretion

In previously reviewed studies, the Committee noted that most ingested cadmium passes through the gastrointestinal tract largely without being absorbed. In mice, rats and monkeys, the absorption of cadmium from the gastrointestinal tract

depends on the type of cadmium compound, dose and frequency, age and interaction with various dietary components. A recent study has shown that expression of *DMT1* and *MTP1* genes is upregulated in response to iron-deficient diets. This upregulation may explain the observation that both the urinary cadmium excretion and kidney cadmium concentration were significantly higher in women with low iron stores (serum ferritin concentration below 30 µg/l).

The oral bioavailability of cadmium in laboratory animals ranges from 0.5% to 3.0%, on average. Following absorption, cadmium binds to metallothionein, but this binding can be overloaded at relatively moderate doses. Cadmium is distributed mainly to the liver, kidneys and placenta. The cadmium concentrations in liver and kidneys are comparable after short-term exposure, but the kidney concentration generally exceeds the liver concentration following prolonged exposure, except at very high exposures. Cadmium present in liver and kidney accounts for more than half of the body burden. The retention of cadmium in various tissues is variable, and its release appears to be multiphasic. The apparent half-life estimates range between 200 and 700 days for mice and rats and up to 2 years in the squirrel monkey.

In humans, about 50% of the cadmium body burden is found in kidneys. Other major bioaccumulating organs or tissues contributing to the body burden are liver (15%) and muscle (20%). The quantity of cadmium in bone is small. The slow excretion of cadmium results in a long biological half-life, which has been estimated to be between 10 and 33 years. A recent estimate, based on long-term dietary exposure data covering a period of 20 years from a Swedish cohort of 680 women aged between 56 and 70 years, indicated an apparent half-life of kidney cadmium of 11.6 years, with a standard deviation of 3.0 years (Amzal et al., 2009). A one-compartment toxicokinetic model was applied to these dietary exposure data. The average daily dietary exposure was reported to be 14 µg (0.2 µg/kg bw), and the mean urinary cadmium level was 0.34 µg/g creatinine. Based on the model, the population distribution of the daily dietary cadmium exposure corresponding to a given level of urinary cadmium could be obtained (see section 9.9.3).

9.2 Toxicological data

In previously reviewed studies, the Committee noted that long-term oral exposure to cadmium resulted in a variety of progressive histopathological changes in the kidney, including epithelial cell damage of proximal tubules, interstitial fibrosis and glomerular basal cell damage with limited tubular cell regeneration. Biochemical indications of renal damage were seen in the form of low molecular weight proteinuria, glucosuria and aminoaciduria. Tubular dysfunction also caused an increase in the urinary excretion of cadmium.

9.3 Observations in humans

A number of new epidemiological studies have assessed factors influencing cadmium concentrations in kidney and urine following environmental exposure, as well as the relationship between cadmium exposure and several health effects.

The kidney is the critical target organ for the long-term effects of cadmium, showing a variety of progressive histopathological changes, including epithelial cell damage in the proximal tubule, interstitial fibrosis and glomerular basal cell damage. The earliest manifestation of cadmium-induced nephrotoxicity is renal tubular dysfunction, which most often manifests as the urinary excretion of low molecular weight proteins and enzymes, such as β2MG, RBP, α1MG and *N*-acetyl-β-D-glucosaminidase. Urinary β2MG level has been the most widely used marker of renal tubular dysfunction.

Several studies monitoring populations following a reduction in cadmium exposure have attempted to address the question of the reversibility of early renal changes. A modest increase in urinary excretion of β2MG or RBP, in the range of 300–1000 μg/g creatinine, is unlikely to indicate compromised renal function and is usually reversible after cadmium exposure is reduced. With β2MG or RBP excretion above 1000 μg/g creatinine, proteinuria due to renal tubular dysfunction becomes irreversible, although GFR is normal or only slightly impaired; when the urinary excretion of these proteins is increased up to 10 000 μg/g creatinine, renal tubular dysfunction progresses to overt nephropathy usually associated with a lower GFR. These values have been used as cut-off criteria to estimate cadmium nephrotoxicity (measured by urinary β2MG excretion) as a function of cadmium concentration in urine. Although there is good evidence demonstrating relationships between urinary excretion of cadmium and various renal biomarkers (e.g. urinary β2MG or RBP concentration), the health significance of these nonspecific biomarkers in relation to cadmium-induced renal damage remains somewhat uncertain. These biomarker changes in the lower range (i.e. 300–1000 μg/g creatinine) might reflect an early renal response to cadmium, which may be purely adaptive or reversible.

Previously reviewed studies have shown that effects on bone generally arise only after kidney damage has occurred and are likely to be secondary to resulting changes in calcium, phosphorus and vitamin D metabolism. Recent studies have evaluated the association between cadmium and bone mineral density or osteoporosis in populations with low-level cadmium exposure. Although these studies found a significant inverse association between the score of bone mineral density and urinary excretion of cadmium at low levels of exposure, they did not assess renal damage. In one of these studies, in Sweden, the incidence of forearm fractures was significantly increased (by 18%) per unit of urinary cadmium (1 μg/g creatinine). In a Belgian study, a significant relative risk of fractures of 1.73 was associated with a doubling of mean cadmium excretion in the urine (1.66 versus 0.83 μg/g creatinine) among women. There was no association between fractures and cadmium levels among men. Another study in Belgium that investigated the association between urinary cadmium and bone mineral density also measured markers of bone resorption, renal tubular dysfunction and calcium metabolism. In this study, even in the absence of renal tubular dysfunction, urinary cadmium level was associated with reduced bone mineral density, increased calciuria and reduced levels of serum parathyroid hormone. However, four additional studies failed to show any association between urinary cadmium and bone mineral density or calcium metabolism, or the association was no longer significant after controlling for age, body weight and smoking, in the absence of renal tubular damage. The

assessment of the association between urinary cadmium and bone mineral density is based upon different types of epidemiological designs, including prospective and cross-sectional studies, with variable power and different degrees of control of the relevant confounders. Although the overall evidence at present points to an association between urinary cadmium and a decrease in bone mineral density, it is unclear whether the effect is secondary to renal tubular dysfunction. Therefore, the data do not provide a basis for a dose–response analysis of the direct effects of cadmium on bone mineral density.

Cadmium has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (group 1), with sufficient evidence for lung cancer and limited evidence for kidney, liver and prostate cancer. Most of the evidence is derived from high cadmium exposure of workers exposed through inhalation. Some case–control studies have reported associations of bladder cancer with increased levels of blood cadmium, breast cancer with increased urinary excretion of cadmium and prostate cancer with increased levels of cadmium in toenails; the relationship between cadmium concentration in toenails and dietary exposure is unknown. A prospective study in Sweden reported a significantly increased risk of endometrial cancer in relation to dietary intake of cadmium in postmenopausal women.

In several cross-sectional studies, increased levels of cadmium measured in blood or urine have been found to be associated with various cardiovascular end-points, including myocardial infarction, stroke, heart failure, hypertension and changes in measures of arterial function (aortic pulse wave velocity and carotid, brachial and femoral pulse pressures). The epidemiological evidence for an association between cardiovascular diseases and cadmium is weak.

Prospective studies of the relationship between mortality and environmental exposure to cadmium were also available. In one study, based on a representative sample of the population of the USA with 9 years of follow-up, a doubling of the urinary cadmium level (0.64 versus 0.32 µg/g creatinine) was observed. This was associated with a 28% increased mortality by all causes, 55% increased mortality by cancer, 21% increased mortality by cardiovascular diseases and 36% increased mortality by coronary heart disease, which were statistically significant among men. No significant effects were observed among women. In a study from Belgium of subjects from a cadmium-polluted area and a control area with a follow-up of 20 years, a doubling of the mean urinary cadmium concentration (1.36 versus 0.68 µg/g creatinine) was significantly associated with 20% increased risk of mortality by all causes, 43% increased mortality for cancer and 44% increased mortality for non-cardiovascular diseases. Two prospective studies assessed mortality, renal tubular dysfunction and environmental exposure to cadmium in cohorts of residents in highly polluted areas in Japan. One of them reported a significant increase of 41% in mortality for subjects with β2MG excretion greater than or equal to 1000 µg/g creatinine, compared with the regional reference death rate, after 20 years of follow-up. The other study, with a follow-up of 15 years, found a significant increase in overall mortality of 27% in men and 46% in women with β2MG urinary levels above 1000 µg/g creatinine; moreover, among subjects with β2MG urinary levels between

300 and 1000 μg/g creatinine, there was a significantly increased risk of death by cerebral infarction, digestive diseases (men) and heart failure (women).

9.4 Analytical methods

Analytical methods for the determination of cadmium in foods, water and biological materials are well established; the detection techniques include FAAS, ETAAS, BIFF-AAS, HG-AFS, ICP-OES and ICP-MS. HR-CS-ETAAS allows direct analysis of solids with improved LODs. In recent years, the use of DRC-ICP-MS has allowed the removal of the interferences with a minimum loss of sensitivity. Although ETAAS has been extensively used, ICP-MS could be considered as the method of choice, as it offers lower LODs and wide dynamic range and allows simultaneous determination of several elements. Additionally, ICP-MS offers high specificity through spectral interpretation and isotopic information. Microwave-assisted acid digestion has been the preferred sample preparation technique, although other techniques, such as ashing and slurry preparation, have been used.

Most data submitted were obtained using the above methods, which were validated. Laboratories followed good quality assurance programmes; some had also participated in proficiency testing schemes and achieved good *z*-scores.

9.5 Sampling protocols

General guidance for sampling is described in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (FAO/WHO, 2004).

9.6 Prevention and control

There have been worldwide efforts to reduce cadmium exposure, including implementation of MLs for cadmium in foods, food additives and water. Other prevention and control measures include controlling cadmium levels in fertilizers and feeds and following good agricultural and manufacturing practices.

9.7 Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed new cadmium occurrence data submitted by EFSA, covering 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Estonia, France, Germany, Greece, Iceland, Ireland, Italy, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom), as well as data submitted by 11 other countries (Australia, Brazil, Canada, Chile, China, France, Ghana, Japan, Singapore, the USA and Viet Nam). The food industry also submitted data on cadmium levels in products that are distributed and used worldwide. The total number of analytical results (single or composite samples) was 155 496, with 84.4% coming from Europe, 5.2% from North America, 1.5% from Asia, 1.4% from Latin America, 0.3% from the Pacific region and 0.1% from Africa. The data submitted by industry accounted for 7.0% of the data.

A summary of the new occurrence data by food category is provided in Table 7 (see section 6.2). For all food categories, calculations of mean concentrations

included results below the LOD or LOQ (i.e. non-detects or ND), although the values assigned to those results varied by country. National average concentrations of cadmium range between not detected and 0.04 mg/kg in most food categories. Higher national mean concentrations, ranging from 0.1 to 4.8 mg/kg, were reported for vegetables (including dried); meat and poultry offal; shellfish/molluscs; nuts and oilseeds; coffee, tea and cocoa; and spices.

9.8 Food consumption and dietary exposure assessment

New information on national estimates of dietary exposure to cadmium was submitted by Australia, China, Japan and the USA. EFSA submitted dietary exposure estimates for Europe. Additional information on national dietary exposure for Chile, Lebanon and the Republic of Korea was obtained from the scientific literature. National and regional exposure estimates were expressed on either a daily or weekly basis, as these estimates are based on 1- to 7-day food consumption surveys. During the meeting, the Committee concluded that a PTMI was appropriate for cadmium (see section 10). For contaminants such as cadmium that are widely distributed in foods at approximately constant levels, day-to-day variability in dietary exposure over the long term would be low, so extrapolating dietary exposure from a daily or weekly basis to a monthly basis would not have a substantial impact on exposure estimates. Therefore, the national and regional exposure estimates were extrapolated to a monthly basis by multiplying daily exposures by 30 or weekly exposures by 4.

Mean cadmium exposure for adults ranged from 2.2 to 12 µg/kg bw per month (see Table 13 in section 7.1.6). Estimates of high exposures reported for Europe, Lebanon and the USA ranged from 6.9 to 12.1 µg/kg bw per month. For Australia and the USA, dietary exposure for children 0.5–12 years of age ranged from 3.9 to 20.6 µg/kg bw per month. Dietary exposure for vegetarians, as reported by EFSA, was estimated to be 23.2 µg/kg bw per month.

The food categories that contributed most to cadmium exposure were reported by Chile, China, Europe, Lebanon and the Republic of Korea. For Chile, the major sources of cadmium in the diet were fish and shellfish, spices and cereals/grains. For China, the main contributors to dietary exposure to cadmium on a national basis were cereals/grains and vegetables; meat and seafood were found to be the main dietary sources of cadmium in several regions within China. Cereals/grains, vegetables/nuts/pulses and animal offal were the main dietary sources of cadmium in Europe. In the Republic of Korea, the main sources of cadmium in the diet were rice, vegetables/seaweed and seafood. The major sources of cadmium in the Lebanese diet were reported to be cereals/grains and vegetables.

The Codex Alimentarius Commission guidelines for conducting exposure assessments for contaminants in foods (FAO/WHO, 2010) recommend that regional dietary exposure estimates should be calculated using regional average contaminant values and the GEMS/Food consumption cluster diets. Such estimates were not calculated for the present meeting because occurrence data were submitted by countries that represented only 2 of the 13 GEMS/Food clusters. Furthermore, national exposure estimates based on national food consumption data

were submitted by the countries that also submitted the majority of new occurrence data. As the national estimates provided more refined estimates than could be calculated with the GEMS/Food consumption cluster diets, only the national estimates were considered in this assessment.

9.9 Dose–response analysis

The basis of the current PTWI is an estimate of a critical cadmium concentration in the kidney cortex at or below which there is no observed increase in β2MG concentrations in urine. A toxicokinetic model was used to estimate the dietary exposure required to reach this critical cadmium concentration in the kidney cortex. An alternative approach is to identify a threshold level of a urinary biomarker of renal tubular damage, such as β2MG, and then use a toxicokinetic model to calculate the dietary exposure corresponding to that threshold level.

9.9.1 Biomarker meta-analysis

In order to determine a dose–response relationship between a suitable biomarker and urinary cadmium levels for the general population, the data available in published studies were compiled and used for a meta-analysis to characterize the relationship between urinary β2MG and urinary cadmium levels (EFSA, 2009b). Urinary β2MG level was chosen as the most suitable biomarker for the meta-analysis because it is widely recognized as a marker for renal pathology and consequently had the largest number of available data. The database covers approximately 30 000 predominantly non-occupationally exposed individuals (99%) reported in 35 studies, but the data are expressed only as group means with standard deviations. The majority of these non-occupationally exposed individuals were of Asian descent (93.5%) and female (75%). The age distribution was approximately equally divided above and below 50 years (i.e. ≥50 years: 51.5%; <50 years: 48.5%). As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved after 45–60 years of exposure. Therefore, data relating β2MG excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older should provide the most reliable basis to determine a critical concentration of cadmium in the urine. The data for the population aged 50 years and over in the 35 studies were categorized according to urinary cadmium concentration, resulting in 98 groups containing matched pairs of urinary cadmium and β2MG levels. The 98 groups ranged in size from 3 to 908 individuals, with a median of 56.

The Committee identified the biexponential model as being suitable to characterize the cadmium–β2MG dose–response relationship. In the model, the first (low urinary cadmium concentration) slope is virtually flat, and only the second (high urinary cadmium concentration) slope was considered by the Committee to be indicative of renal pathology (see Figure 3 in section 8.3.3 above). Therefore, the Committee chose the breakpoint for the second slope, which is the point at which the urinary β2MG concentration begins to rapidly increase with increasing urinary cadmium level, as the basis of the evaluation. This breakpoint derived for the population aged 50 years and over corresponds to 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram of creatinine (Figure 3).

9.9.2 Toxicodynamic variability

Toxicodynamic variability in the dose–response relationship is not taken into account by the model, because the data represent only a population average rather than individual data points. The lack of empirical evidence of elevated β2MG levels below a urinary cadmium concentration of 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine indicates that the variance is small.

Toxicodynamic variability in the model was accounted for by incorporating a maximum variability that ranges from 1 to 3. The value of 3 approximately corresponds to the toxicodynamic component of the conventional 10-fold uncertainty factor for interindividual variability (IPCS, 2005). Individual subjects were presumed to have a critical concentration (breakpoint) somewhere within the range defined by the mean multiplied or divided by the maximum value. As the same maximum value was used for both increased and reduced individual susceptibility, the adjustment resulted in broadened distributions of both population variability and uncertainty without affecting the geometric central estimates.

9.9.3 Toxicokinetic modelling

A one-compartment model was used to characterize the relationship between urinary cadmium concentration and dietary cadmium exposure (see section 9.1 above). This model included a statistical parameter for variation in apparent half-life. The calculated relationship between dietary cadmium exposure and urinary cadmium concentration is linear; therefore, the outcome may be expressed as population distribution of the ratio with confidence intervals (see Figure 4 in section 8.3.5 above).

9.9.4 Estimating the relationship between urinary cadmium excretion and dietary cadmium exposure

A two-dimensional Monte Carlo simulation was used to estimate the population percentiles with associated 5th–95th percentile confidence intervals from the variability and uncertainty in the breakpoint, the adjustment for toxicodynamic variability and the toxicokinetic model (see Figure 5 in section 8.4 above). The dietary cadmium exposure (µg/kg bw per day) that equates to 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine in urine was estimated to be 1.2 (5th–95th percentiles 0.8–1.8) µg/kg bw per day at the 5th population percentile. This is equivalent to 36 (5th–95th percentiles 24–54) µg/kg bw per month. The Committee decided to use the lower bound of the confidence interval to account for particularly susceptible individuals so that they would remain below the dietary exposure at which renal pathology is indicated.

10. EVALUATION

Since cadmium was last considered by the Committee, there have been a number of new epidemiological studies that have reported cadmium-related biomarkers in urine following environmental exposure. The Committee noted that a large meta-analysis of studies that measured the dose–response relationship between β2MG and cadmium excretion in urine was available. As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved

after 45–60 years of exposure. Therefore, data relating β2MG excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older provided the most reliable basis on which to determine a critical concentration of cadmium in the urine. An analysis of the group mean data from individuals who were 50 years of age and older showed that the urinary excretion of less than 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine was not associated with an increased excretion of β2MG. Higher urinary cadmium levels were associated with a steep increase in β2MG excretion.

To determine a corresponding dietary exposure that would result in a urinary cadmium concentration at the breakpoint of 5.24 (5th–95th percentiles 4.94–5.57) µg of cadmium per gram creatinine, a one-compartment toxicokinetic model was used. The lower bound of the 5th population percentile dietary cadmium exposure that equates to the breakpoint was estimated to be 0.8 µg/kg bw per day or about 25 µg/kg bw per month.

The Committee noted that the existing health-based guidance value for cadmium was expressed on a weekly basis (PTWI), but, owing to cadmium's exceptionally long half-life, considered that a monthly value was more appropriate. The PTWI of 7 µg/kg bw was therefore withdrawn.

In view of the long half-life of cadmium, daily ingestion in food has a small or even a negligible effect on overall exposure. In order to assess long- or short-term risks to health due to cadmium exposure, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a PTMI. The PTMI established was 25 µg/kg bw.

The estimates of exposure to cadmium through the diet for all age groups, including consumers with high exposure and subgroups with special dietary habits (e.g. vegetarians), examined by the Committee at this meeting are below the PTMI.

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LEAD (addendum)

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1. EXPLANATION

Lead (Pb) occurs in Earth's crust primarily as the mineral galena (lead(II) sulfide) and, to a lesser extent, as anglesite (lead(II) sulfate) and cerussite (lead carbonate). It occurs in the environment both naturally and, to a greater extent, from anthropogenic activities such as mining and smelting, battery manufacturing and the use of leaded petrol (gasoline). Lead contamination of food arises mainly from the environment or from food processing, food handling and food packaging. Atmospheric lead can contaminate food through deposition on agricultural crops. Water is another source of lead contamination of food. Although lead exists in both organic and inorganic forms, only inorganic lead has been detected in food.

Lead was previously evaluated by the Committee at its sixteenth, twenty-second, thirtieth, forty-first and fifty-third meetings (Annex 1, references *30*, *47*, *73*, *107* and *143*). At the sixteenth meeting, the Committee established a provisional tolerable weekly intake (PTWI) of 3 mg of lead per person, equivalent to 50 µg/kg body weight (bw), stating that this did not apply to infants and children. At its twenty-second meeting, the Committee retained the PTWI for adults, noting that establishing a PTWI for children was not yet possible owing to the lack of relevant scientific data. The health risks associated with exposure of infants and children to lead were evaluated at the thirtieth meeting, and a PTWI of 25 µg/kg bw was established for this population group, based on the information that a mean daily exposure to lead of 3–4 µg/kg bw for infants and children was not associated with an increase in blood lead levels. At the forty-first meeting, the Committee withdrew the previous PTWI of 50 µg/kg bw for adults and extended the PTWI of 25 µg/kg bw to all age groups. In these previous evaluations, it was emphasized that the PTWI applied to lead from all sources. At its fifty-third meeting, the Committee was asked to assess the risk of dietary exposure of infants and children to lead. It concluded that current concentrations of lead in food would have very little impact on the neurobehavioural development of infants and children but stressed that a full risk assessment of lead should take other sources of exposure into account.

At its present meeting, the Committee considered information on lead related to the toxicology, epidemiology, exposure assessment and analytical methodology, in particular for a dose–response analysis below blood lead levels of 10 µg/dl, at the request of the Codex Committee on Contaminants in Food.

The literature relating to lead is extensive, and the present Committee used the recent review of the European Food Safety Authority (EFSA, 2010) as the starting point for its evaluation, together with newer studies that were considered to

be informative. Only brief summaries of toxicological effects are given, but studies of the effects critical for the risk assessment are evaluated in more detail. The main emphasis is on studies in humans.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and elimination

Exposure to lead and lead chemicals can occur through ingestion, inhalation and dermal contact. Gastrointestinal absorption of ingested lead varies depending on physiological factors such as age, fasting, nutritional calcium and iron status, pregnancy and physicochemical characteristics of particles (size, solubility and lead species) (EFSA, 2010).

Several studies have reported that lead absorption from the gastrointestinal tract appears to be higher in children than in adults (Alexander, Clayton & Delves, 1974; Ziegler et al., 1978; Rabinowitz, Kopple & Wetherill, 1980; Heard & Chamberlain, 1982; James, Hilburn & Blair, 1985), although another study suggested that children aged 6–11 years and their mothers absorb a similar percentage of ingested lead (Gulson et al., 1997). Experimental studies with Rhesus monkeys and rats have provided additional evidence for age-dependent differences in gastrointestinal absorption (Forbes & Reina, 1972; Kostial et al., 1978; Pounds, Marlar & Allen, 1978; Aungst, Dolce & Fung, 1981).

Fasting humans absorb much larger fractions of lead than do their fed counterparts. The presence of food in the gastrointestinal tract decreases the absorption of water-soluble lead (Rabinowitz, Kopple & Wetherill, 1980; Heard & Chamberlain, 1982; Blake & Mann, 1983; Blake, Barbezat & Mann, 1983; James, Hilburn & Blair, 1985; Maddaloni et al., 1998). Fasting subjects absorbed approximately 63% of a tracer dose of lead acetate in water, whereas fed subjects absorbed 3% (Heard & Chamberlain, 1982; James, Hilburn & Blair, 1985).

Iron is believed to impair lead uptake in the gut, whereas iron deficiency is associated with increased blood lead concentrations in children (Cheng et al., 1998; Bárány et al., 2005). Studies in rats also showed that iron deficiency increased lead absorption, possibly by enhancing its binding to iron-binding carriers (Barton et al., 1978; Morrison & Quaterman, 1987; Bannon et al., 2003). Diets with low levels of calcium have been shown to increase lead absorption in children (Ziegler et al., 1978; Mahaffey et al., 1982) and in laboratory animals (Mykkänen & Wasserman, 1981, 1982).

Absorption through the respiratory tract is influenced by the particle size distribution and the ventilation rate. In adults, the rate of deposition of airborne lead is approximately 30–50%. This rate is dependent on the size of the particles and the ventilation rate of the individual. Smaller particles (<1 μm) have been shown to have greater deposition and absorption rates than larger particles (USEPA, 1986). Once deposited in the lower respiratory tract, lead appears to be almost completely absorbed (Morrow et al., 1980).

Dermal absorption of lead compounds is substantially lower than absorption by inhalation or oral routes of exposure. Dermal absorption of lead in humans has been estimated to be 0.06% during normal use of lead-containing preparations (Moore et al., 1980).

Inorganic lead is minimally absorbed through the skin, but organic lead, such as tetraethyl lead (as in leaded gasoline), which is still legally allowed to be used in aircraft, watercraft and farm machinery, is well absorbed through the skin (Patrick, 2006).

Once absorbed, 96–99% of circulating lead in blood is bound to erythrocytes. At low blood lead concentrations, whole blood lead levels increase linearly with serum levels. However, at higher blood lead concentrations, owing to saturation of lead-binding sites in the erythrocytes, the relationship between serum lead and blood lead is non-linear. The distribution of lead in the body is route independent; in adults, approximately 90% of the total body burden of lead is in the bones, compared with approximately 70% in children, but its concentration increases with age (EFSA, 2010).

Bone lead can contribute to elevated blood levels long after the exposure ceases (Fleming et al., 1997). Studies have reported that conditions such as ageing (Drasch, Bohm & Baur, 1987), osteoporosis (Gulson, Palmer & Bryce, 2002), pregnancy (Lagerkvist et al., 1996; Maldonado-Vega et al., 1996; Schuhmacher et al., 1996; Franklin et al., 1997; Gulson et al., 1997), lactation (Gulson et al., 2003, 2004), menopause and postmenopause (Hernandez-Avila et al., 2000; Gulson, Palmer & Bryce, 2002; Berkowitz et al., 2004; Nash et al., 2004; Popovic et al., 2005) increase bone resorption and consequently also increase lead levels in blood. Lead can be transferred from the mother to the fetus (Goyer, 1990; Graziano et al., 1990; Carbone et al., 1998) and also from the mother to infants via maternal milk (Gulson et al., 1998b; Ettinger et al., 2006).

The unexcreted fraction of absorbed lead is distributed among blood, soft tissues and bones (Barry, 1975). Half-lives for inorganic lead in blood and bone are approximately 30 days and between 10 and 30 years, respectively (Rabinowitz, 1991).

It has been reported that liver is the largest repository of soft tissue lead, followed by kidney cortex and medulla, pancreas, ovary, spleen, prostate, adrenal gland, brain, fat, testis, heart and skeletal muscle. Despite lead's faster turnover in these tissues, its concentrations are relatively constant (Barry, 1975; Treble & Thompson, 1997). Regardless of the route of exposure, lead is excreted primarily in urine and faeces (Rabinowitz, 1991). Minor routes of excretion include sweat, saliva, hair, nails and breast milk (Hursh & Suomela, 1968; Hursh et al., 1969; Rabinowitz, Wetherill & Kopple, 1976; Kehoe, 1987; Stauber et al., 1994).

2.1.2 Biotransformation

Metabolism of inorganic lead consists primarily of reversible ligand reactions, including the formation of complexes with amino acids and non-protein thiols and binding to various proteins (Goering & Fowler, 1985; Goering, 1993).

Organic lead compounds are metabolized to inorganic lead both in humans and in experimental animals. Organic or alkyl lead, such as tetraethyl and tetramethyl lead, undergo oxidative dealkylation to the highly neurotoxic metabolites triethyl and trimethyl lead, respectively. In the liver, the reaction is catalysed by a cytochrome P450–dependent mono-oxygenase system (IARC, 2006).

2.1.3 Effects on enzymes and other biochemical parameters

The toxicity of lead may be attributed to the affinity of lead for thiol groups (–SH) (Vallee & Ulmer, 1972) and other organic ligands in proteins.

Lead has been known to alter the haematological system by inhibiting the activities of enzymes essential for haem biosynthesis, such as δ-aminolaevulinic acid synthase (ALAS), δ-aminolaevulinic acid dehydratase (ALAD) and ferrochelatase. The enzyme most sensitive to the effect of lead is ALAD (EFSA, 2010).

Inhibition of ALAD by lead results in increased circulating aminolaevulinic acid (ALA), a weak γ-aminobutyric acid (GABA) agonist that decreases GABA release by presynaptic inhibition, which may account for some of the behavioural disorders seen in patients with porphyria and perhaps in lead toxicity. Inhibition of ALAD activity occurs over a wide range of blood lead concentrations beginning at less than 10 µg/dl (ATSDR, 2007).

Simmonds, Luckhurst & Woods (1995) observed a decrease in ALAD activity in erythrocytes in rats given lead acetate at 1000 mg/l in the drinking-water for 6 days. Blood lead concentrations increased to 44 µg/dl after the first day and remained within 10 µg/dl of that value until the end of the exposure period.

It has been reported that lead indirectly stimulates the mitochondrial enzyme ALAS, which catalyses the condensation of glycine and succinyl coenzyme A to form ALA. The activity of ALAS is the rate-limiting step in haem biosynthesis; ALAS is induced by negative feedback from the depression of haem synthesis (EFSA, 2010).

Lead is also known to inhibit the activity of the zinc-containing mitochondrial enzyme ferrochelatase, which catalyses the insertion of iron(II) into the protoporphyrin ring to form haem (USEPA, 1986; Goering, 1993). Lead-related anaemia is known to be a late complication when blood lead levels exceed 50 µg/dl (USEPA, 1986; Goering, 1993).

Other enzymes have also been reported to be inhibited by lead. Lead was shown to inhibit Na^+,K^+-adenosine triphosphatase (ATPase) activity and to increase intracellular Ca^{2+} levels, possibly with activation of protein kinase C (Kramer, Gonick & Lu, 1986; Watts, Chai & Webb, 1995; Hwang et al., 2001), resulting in hypertension (Carmignani et al., 2000; Ni et al., 2004; Vaziri & Sica, 2004) and subsequent depletion of nitric oxide, which is involved in the regulation of blood pressure (Gonick et al., 1997; Vaziri et al., 1997; Vaziri, 2008).

The activity of dihydrobiopterin reductase, an enzyme involved in the synthesis of catecholamines, is reduced by lead in rat brain. The activity of nicotinamide adenine dinucleotide synthetase in erythrocytes may also be inhibited by lead (Annex 1, reference *144*).

Lead also competitively interferes with divalent cations, such as calcium, magnesium and zinc. Subsequent impairment of mitochondrial oxidative phosphorylation and the intracellular messenger system affects endocrine and neuronal function and smooth muscle contraction (ATSDR, 2007).

Lead is reported to compete with calcium for binding sites on cerebellar phosphokinase C, thereby affecting neuronal signalling (Markovac & Goldstein, 1988). Furthermore, high lead exposure in children has been shown to decrease circulating levels of the active form of vitamin D, 1,25-dihydroxyvitamin D, resulting in perturbation of calcium homeostasis (Mahaffey et al., 1982; Rosen & Chesney, 1983).

2.1.4 Physiologically based pharmacokinetic (PBPK) modelling

The relationship between dietary exposure to lead and blood lead levels has been previously evaluated by the Committee (Annex 1, reference *144*). For infants, the evaluation is based on data from a study of a group of Scottish infants exposed to lead from drinking-water (Lacey, Moore & Richards, 1985). In this study, the kinetic relationship between exposure to lead and blood lead levels was analysed. An analysis conducted by the United States Environmental Protection Agency (USEPA) concluded that there is a linear relationship between exposure and blood lead levels, with a linear slope of 0.16 µg/dl per 1 µg/day of dietary exposure (USEPA, 1989). This analysis is the basis for the USEPA's toxicokinetic model used in the EFSA (2010) evaluation. The previous evaluation by the Committee (Annex 1, reference *144*) presented a reanalysis of the same data that included an intercept parameter to account for unquantified sources of exposure other than drinking-water and concluded that a range between 0.05 and 0.10 µg/dl per 1 µg/day of dietary exposure was appropriate for low levels of lead exposure. In the present analysis, the Committee employed a range of 0.05–0.16 µg/dl per 1 µg/day for children.

The Committee (Annex 1, reference *144*) also previously evaluated the relationship between dietary exposure to lead and blood lead levels in adults. Based on a similar study of drinking-water in Scotland (Sherlock et al., 1982), the Committee identified a range of 0.023–0.07 µg/dl per 1 µg/day for adults.

2.2 Toxicological studies

2.2.1 Acute toxicity

Lead has been described as a classic chronic poison. Health effects are generally not observed after a single exposure, and oral median lethal doses (LD_{50} values) for lead salts have been reported to be greater than 2000 mg/kg bw. The lowest observed lethal doses in experimental animals after multiple short-term oral exposures to lead acetate, lead chlorate, lead nitrate, lead oleate, lead oxide or lead sulfate range from 300 to 4000 mg/kg bw (Annex 1, reference *143*).

Acute kidney damage was reported in male rats following intraperitoneal administration of lead acetate (0, 0.05, 0.15 and 0.30 mmol/kg bw as Pb^{2+}) to groups of five male and five female rats. Minimal kidney damage, as shown by increased urinary γ-glutamyl transferase activity, was observed only in males given the highest

dose. It was also observed that in all animals and at all doses, natriuria was significantly decreased on the first day (from 4 h after administration). Such changes evoke mild tubular abnormalities, but glomerular disturbances may also be involved (IARC, 2006).

2.2.2 Short-term studies of toxicity

There are very few short-term studies of the toxicity of lead reported in the literature. In a study of exposure by the inhalation route, Bizarro et al. (2003) observed that CD-1 male mice exposed to a mist containing lead acetate at a concentration of 0.01 mol/l in deionized water intermittently for 4 weeks showed a time-related increase in the fraction of damaged mitochondria in Sertoli cells, which, according to the investigators, could lead to a transformation process that may interfere with spermatogenesis.

Sundström & Karlsson (1987) reported that lead administered to newborn rats postnatally on days 1–15 by daily intraperitoneal injections of lead nitrate at a dose of 10 mg/kg bw was found to cause haemorrhagic encephalopathy in the cerebellum at 15 days.

Sokol (1987) reported that Wistar rats given 0.3% (3000 mg/l) lead acetate in their drinking-water for 30 days manifested a hyper-responsiveness to stimulation with both gonadotropin-releasing hormone and luteinizing hormone, whereas they manifested a blunted response to naloxone, indicating that lead exerts its toxic effects at hypothalamic or supra-hypothalamic sites.

2.2.3 Long-term studies of toxicity and carcinogenicity

The studies on the long-term toxicity of lead in experimental animals are extensive, and most findings are in agreement with the observations in humans (ATSDR, 2007). Chronic oral exposure to inorganic lead has effects on multiple organs, such as kidney and liver, and systems, including cardiovascular, haematological, immune, reproductive and nervous systems. Increased mortality, weight loss and depression of weight gain have also been reported in rats (IARC, 2006).

Studies of the carcinogenicity of lead in experimental animals have been reviewed by the International Agency for Research on Cancer (IARC), and no new studies have been published since the last monograph (IARC, 2006). Inorganic lead salts are the main lead compounds tested via oral exposure, mostly in rats.

A substantial body of literature has demonstrated the association between lead exposure and renal tumours in rats. With an extremely low spontaneous incidence in rodents, renal tumours have been consistently observed in rats exposed to lead acetate or lead subacetate for 1 year or longer. The exposure levels in these studies, however, are considered very high compared with human dietary exposure (EFSA, 2010). A 2-year study in rats found 500 mg/kg in diet to be the highest lead acetate concentration at which no renal tumours occurred and 100 mg/kg in diet to be the concentration at which no renal pathological lesions were observed. Renal tumours have also been found in lead-treated mice, but the results

are not as consistent as those in rats. In one study using hamsters, no tumours developed following lead exposure (IARC, 2006).

Brain gliomas, also rarely spontaneous in rodents, seem to be associated with chronic inorganic lead exposure as well. Lead-induced brain gliomas were reported in three independent studies, each using a different strain of rat (IARC, 2006).

Results of genotoxicity (see section 2.2.4) and mechanistic studies suggest indirect mechanisms, including the inhibition of deoxyribonucleic acid (DNA) repair, interference with cellular redox regulation and induction of oxidative stress, and deregulation of cell proliferation for lead carcinogenicity (Beyersmann & Hartwig, 2008). It is therefore possible that lead can augment the effects of other carcinogens, and lead has been examined together with various organic carcinogens in a number of studies. Oral exposure to lead salts enhanced the incidence of renal tumours induced by *N*-ethyl-*N*-hydroxyethylnitrosamine and *N*-nitrosodimethylamine. However, there is not enough evidence showing that lead increases the incidence of chemical-induced skin tumours and lung tumours (IARC, 2006).

In summary, there is sufficient evidence for the carcinogenicity of inorganic lead compounds in experimental animals (IARC, 2006). Lead induces renal and brain tumours in experimental animals and may also act as a tumour promoter together with other renal carcinogens.

2.2.4 Genotoxicity

(a) In vitro studies

When tested in bacterial and yeast systems, lead compounds usually give negative results. The few positive results might have resulted because of the mutagenic effects of the anions, such as chromium in lead chromate. In cell-free systems, lead acetate causes DNA strand breaks, increases 8-hydroxy-deoxyguanosine and inhibits tubulin assembly (IARC, 2006; EFSA, 2010).

Genotoxic assays using different animal cells and measuring different genotoxic end-points are summarized in Table 93 in the IARC (2006) monograph. Mutagenesis assays have given equivocal results. Among those showing positive results, the type of mutation, the extent of mutagenicity and the effective doses varied with experimental conditions. Several studies revealed an increase in mutation frequency stimulated by lead in combination with other mutagens, such as ultraviolet C (UVC) irradiation. Lead-induced inhibition of DNA repair was also observed in X-ray or UVC-treated cells. As for chromosomal aberrations, results are mostly negative, except for lead chromate, the positive results for which may be due to the action of chromate, but not lead. Equivocal results have been published for DNA strand breaks and sister chromatid exchange. A dose-dependent increase of micronuclei was induced by lead chloride and lead acetate, but not by lead nitrate.

The above evidence indicates that it is not likely that lead interacts directly with DNA. The mechanisms of lead genotoxicity probably involve multiple indirect

effects, such as the production of reactive oxygen species, the generation of protein crosslinks and the inhibition of DNA repair.

(b) In vivo studies in experimental animals

Results from in vivo animal studies have been summarized in Table 92 in the IARC (2006) monograph. Most rat studies have demonstrated DNA damage and micronucleus formation in kidney cells and aneuploidy in bone marrow cells. However, studies with mice and monkeys have not yielded consistent results.

(c) In vivo studies in humans

Genetic effects such as DNA damage, chromosomal aberrations and micronucleus formation have been observed in individuals occupationally exposed to lead, whereas there is no evidence showing similar effects related to non-occupational exposure. A complete list of the human studies can be found in Table 91 in the IARC (2006) monograph. Depending on the genotoxic end-point, the effect is noted when blood lead concentration falls in the range of 15–65 µg/dl (IARC, 2006). A major limitation in these studies is the occupational co-exposure to other genotoxic metals, such as cadmium, and therefore the contribution of lead alone is difficult to evaluate. In addition, many studies did not consider smoking as a potential confounder, making it difficult to derive a dose–response relationship.

2.2.5 Reproductive and developmental toxicity

A number of studies in male rats and other rodent species indicate that blood lead concentrations above 30–40 µg/dl for at least 30 days are associated with reductions in spermatogenesis and serum testosterone levels. Equivocal results have been reported for end-points such as reproductive organ histopathology, spermatozoal end-points and levels of pituitary hormones. It appears that certain animal species and strains are quite resistant to the reproductive toxicity of lead, probably as a result of the physiology-based differences in lead accumulation and distribution in target organs (Apostoli et al., 1998). Increased DNA damage, cytotoxicity and reactive oxygen species have been found in male germ cells, yet it is still not clear whether the reproductive toxicity of lead is caused by its direct interaction with reproductive organs or by the impairment of the pituitary testicular axis, or both.

In female rats chronically exposed to lead, irregular estrous cycles and morphological changes in ovaries have been observed. Ronis et al. (1996) reported that exposure of the dams to lead acetate caused a significant dose-responsive decrease in birth weight of rat litters. In addition, reduced serum testosterone level and dose-dependent pubertal delay were observed in male offspring. Delayed vaginal opening in female offspring and suppression of circulating estradiol were also observed.

Exposure to lead in early development results in several endocrine disruptions, accompanied by delayed puberty, suppression of prepubertal growth and suppression of the male pubertal growth spurt. Studies on hormonal changes

suggest that functions of the hypothalamic–pituitary–gonadal axis can be affected when related structures are undergoing rapid proliferation (IARC, 2006).

2.2.6 Special studies

(a) Neurological and behavioural effects

In rodent and non-human primate models, it has been demonstrated that chronic exposure to low-level lead affects learning abilities and behaviour, particularly in the developing animals. The magnitude of these effects appears to be strongly dependent on the developmental period in which exposure takes place (IARC, 2006). Deficits in reversal or repeated learning have consistently been observed in lead-exposed animals, the magnitude of which varied by species and stimulus dimensions (EFSA, 2010). It has also been suggested that lead is associated with some symptoms of attention deficit hyperactivity disorder (ADHD), particularly impulsivity and inattention (EFSA, 2010).

Effects of lead on visual and hearing functions have also been observed in experimental animals. Outcomes of chronic exposure to lead include the impairment of scotopic visual function (night blindness), decreased amplitudes and prolonged latencies of flash-evoked visual potentials, and reduction of ocular motor function. However, there is not enough evidence for loss of the spatial and temporal contrast sensitivity functions of the visual system (IARC, 2006; EFSA, 2010). The effects of lead on auditory functions have been studied in monkeys, but the association remains unclear. Changes in the auditory brainstem-evoked potentials and pure tone detection were observed in some, but not all, studies. It seems that the results were influenced by the age of the test animals and the level and length of the exposures (IARC, 2006).

Although the exact mechanism or site of action is not clear, effects of lead on motor function and aggressive behaviour have been reported and reviewed by IARC (2006). Results of behavioural tests performed primarily in rats and monkeys have suggested that the impaired performance is the result, at least in part, of a combination of distractibility, inability to inhibit inappropriate responding and perseverance of behaviours that are no longer appropriate (ATSDR, 2007).

(b) Nephrotoxicity

The renal effects of lead in animal models occur as a result of both acute and chronic exposures. Studies on the mechanisms of lead nephrotoxicity suggest the involvement of oxidative stress. Chronic exposure to lead induces the formation of characteristic intranuclear inclusion bodies in the proximal tubular epithelial cells in various animal species. These inclusion bodies may function as an intracellular depot of non-diffusible lead.

In rats, the lowest chronic lead dose related to detectable renal effects is 5 mg/l in drinking-water. At this exposure level, pathological changes in renal proximal tubular cells, formation of intranuclear inclusion bodies, inhibition of renal mitochondrial respiration and swollen mitochondria were seen after 9 months of exposure. At higher doses, various nephropathy symptoms were observed,

including increased glomerular filtration rate (GFR), focal tubular atrophy, interstitial fibrosis, elevated urinary *N*-acetyl-β-D-glucosaminidase and glutathione *S*-transferase. Many symptoms appear only at certain stages of exposure. For example, the hyperfiltration effect is observed only during the first 3 months of lead exposure (EFSA, 2010). A comparative study of prenatal and postnatal exposure suggested that lead exposure during kidney development resulted in more severe damage compared with later lead exposure and that exposure to lead starting at weaning was more nephrotoxic than exposure starting 2 months later (Vyskocil, Cizkova & Tejnorova, 1995).

(c) Cardiovascular effects

The cardiovascular effects of lead in experimental animals have been reviewed by IARC (2006) and EFSA (2010). Low-level (<100 mg/l in drinking-water) chronic lead exposure has consistently been associated with a hypertensive effect in rats. However, the effects of high-level lead on blood pressure are equivocal, making it difficult to derive a dose–response relationship. In some studies, high-level exposure failed to demonstrate increased blood pressure, suggesting a biphasic response (Victery, 1988). In one study, exposure of spontaneously hypertensive rats to low-level lead resulted in an enhanced susceptibility to ischaemia-induced arrhythmias, although this effect was less marked when the exposure level was higher (IARC, 2006). In rats given lead acetate at 100 mg/l in their drinking-water, increased heart rate and heart contraction were seen (Reza et al., 2008). Observations in a large number of experimental animal studies suggest that the cardiovascular effects are secondary to lead-induced nephropathy. However, there is also evidence suggesting that direct actions, such as the alteration in intracellular calcium concentration and the inactivation of nitric oxide synthase, may also play a role (Vaziri, 2008).

(d) Immunotoxicity

Lead reduces resistance to bacterial and viral infections and decreases antibody production in different experimental animals (IPCS, 1995). The immunological effects of lead in experimental animals have been previously reviewed (IARC, 2006). Decreased B-lymphocyte responsiveness, suppressed humoral antibody titres and inhibited mitogenic responses of lymphocytes have been observed in mice. Lead also attenuates the delayed-type hypersensitivity (DTH) response, causes the shift of immune responses away from thymus-dependent T helper 1 (Th1)–associated responses towards Th2-dependent responses and increases serum immunoglobulin E levels (Farrer et al., 2008). Lead nitrate has effects on the proliferative responses of B and T lymphocytes of many animal species (IARC, 2006). The underlying mechanisms for lead-induced immunomodulation have remained elusive. Overall, available data suggest that nitric oxide, produced via the modulation of inducible nitric oxide synthase activity, is a key mediator in lead-induced immunomodulation (Farrer et al., 2008).

In rats exposed to lead in utero, depressed cell-mediated immune function was observed, as shown by a decrease in DTH reactions. Exposure of pregnant females to moderate levels of lead produces chronic immunomodulation in their

offspring. Rat embryos may be more sensitive to lead-induced immunotoxic effects when exposed during late gestation, with the effects on DTH function being more pronounced in females. Dietert et al. (2004) reviewed the recent findings pertaining to the effects of lead on the developing immune system compared with effects on the adult immune system. Several rodent studies have shown that the adult blood lead level required for immunotoxicity appears to be significantly greater than the minimum blood lead level needed for embryonic- or neonatal-associated immune impairment, suggesting that the sensitivity to lead immunotoxicity during embryonic and neonatal periods is much greater than that in the adult.

(e) Effects on haematopoietic system

The effects of lead on the haematopoietic system in experimental animals have been studied and reviewed extensively (IPCS, 1995; IARC, 2006; ATSDR, 2007). Lead is known to be a potent inhibitor of haem synthesis. A reduction in haem-containing enzymes could compromise energy metabolism.

A number of studies have shown that the haem biosynthesis enzyme ALAS is induced by lead as a feedback regulation. Haem oxygenase in liver and kidney is also induced, leading to increased haem degradation. The mechanism of alteration in the haem synthesis pathway is suggested to be lead-induced oxidative stress (Gautam & Flora, 2010).

2.3 Observations in humans

2.3.1 Biomarkers of exposure

Blood is the tissue used most frequently to estimate exposure to lead and its association with health outcomes. This is largely because blood is easily sampled and the methods for measuring blood lead concentration are well developed. The elimination half-life of lead in blood is approximately 30–40 days in adults, however, so the blood lead level provides information primarily about an individual's exposure in recent months. The exposure averaging time will vary among individuals, depending on the extent to which endogenous pools of lead, representing past exposure, are contributing to blood lead. Under conditions of steady-state exposure, only a small percentage of total body burden of lead is in blood (~5%), and nearly all of this is bound to erythrocytes (96–99%), with the balance in plasma. The ratio of erythrocyte to plasma lead decreases as lead levels increase owing to saturation of binding sites on erythrocytes. Typically, whole blood lead concentration is measured. Although the fraction in plasma is thought to be more relevant than whole blood lead to lead's toxicity, it has rarely been used as the exposure biomarker owing to the analytical challenges and the cost of measuring such low concentrations accurately.

Because 90% of the body lead burden in adults, and 70% in children, is in bone, methods based on X-ray fluorescence (primarily K-line) have been developed for measuring the concentration of lead in bone, expressed as grams of lead per gram of bone mineral (Hu et al., 2007). The half-life of lead in bone varies depending on bone type, being longer in cortical bone (approximately 30 years) than in

trabecular bone (5–10 years). The bones in which measurements are most frequently made are tibia, patella and calcaneus. The technology required to make these measurements is limited to only a small number of laboratories, however. Moreover, the correlation between bone lead level and a concurrent blood lead level is often poor because of the very different exposure averaging times that these two biomarkers capture. For instance, in one study of 50- to 70-year-olds with primarily only environmental exposures to lead, the correlation between bone and blood lead levels was 0.12 (Martin et al., 2006). The correlation can be much higher in individuals who, in the past, had substantial occupational exposures to lead, for whom bone stores contributed to blood lead in later life (i.e. after occupational exposure ended) (Morrow et al., 2007). These considerations make it difficult to base risk assessments, particularly for food, on the dose–response relationships derived from studies that relied on bone lead level as the exposure biomarker in cohorts with only environmental exposures to lead.

The X-ray fluorescence methods currently available have been used almost exclusively with adults, as they are not sufficiently sensitive for measuring bone lead in children, presumably because of the rapid remodelling of bones that occurs during a child's growth. Some epidemiological studies have measured the lead concentration in shed deciduous teeth as an alternative assay of mineralized tissue. Efforts have been made to reconstruct temporal features of a child's exposure history from the spatial distribution of stable lead isotopes in a tooth (Gulson & Wilson, 1994).

Lead is excreted in urine, but urinary lead levels have not been widely used in health outcome studies. Lead diuresis in response to a chelating agent challenge is used clinically, however, to determine the need for therapeutic chelation and to monitor treatment efficacy. The relationship between blood lead and urinary lead levels under other conditions is weak (Gulson et al., 1998a).

Because of the possibility of external contamination by ambient lead, the concentration of lead in hair is not viewed as an acceptable biomarker.

(a) Blood lead level during pregnancy

Lead crosses the placenta by passive diffusion, and blood lead levels in newborns are generally 80–90% of the maternal venous level at delivery. The kinetics of lead during pregnancy are complex. The blood lead level during pregnancy changes as a result of a variety of factors, even if external exposures remain stable. These factors include changes in plasma volume, red cell mass and the redistribution of lead among the different body pools caused by pregnancy-related physiological changes. During the first half of pregnancy, blood lead level declines by approximately 15%, most likely due to haemodilution, organ growth and increased GFR or increased lead excretion (Rothenberg et al., 1994). After the 20th week of pregnancy, however, an increase of 14–40% in blood lead level, in both whole blood and plasma, has been observed in numerous cohorts (Hertz-Picciotto et al., 2000; Tellez-Rojo et al., 2004). Considerable evidence now supports the hypothesis that this is the result, at least in part, of increased influx of lead into the blood by means of the mobilization of bone lead stores. The timing of this increase coincides with the increased requirement for calcium to support fetal ossification.

Other studies suggest that bone lead is mobilized at rates that are consistent with the pattern of bone loss in menopausal women (Symanski & Hertz-Picciotto, 1995; Nash et al., 2004). In pregnant women, plasma lead level was highest in women who had both a high bone lead level and greater bone resorption activity (Tellez-Rojo et al., 2004). Studies evaluating changes in the ratios of stable lead isotopes over the course of pregnancy provide compelling evidence that lead stored in deep pools, such as bone, is, indeed, redistributed to the blood compartment during pregnancy (Gulson et al., 2003; Manton et al., 2003). There are several case reports of women who were lead poisoned in early life and, when they became pregnant, again developed symptoms of lead toxicity (e.g. Riess & Halm, 2007). Some epidemiological evidence (Hertz-Picciotto et al., 2000; Gulson et al., 2004) and one randomized trial conducted during lactation, when calcium needs exceed those during pregnancy (Hernandez-Avila et al., 2003), suggest that increased calcium intake can reduce the amount of mobilization of bone lead.

(b) Estimates of blood lead levels by region

For the World Health Organization's (WHO) Global Burden of Disease 2005 project, an updating of the estimates of the global burden of disease for the period 1990–2005, A. Pruss-Ustun (unpublished data, 2010) derived estimates of the distribution of blood lead levels for the 21 regions being considered in the project (Table 1). Where possible, separate estimates are provided by age (child versus adult), sex and area of residence (urban versus rural). Clearly, the average level of exposure varies considerably across regions, with the means for some subgroups exceeding 10 μg/dl. In an analysis to estimate the global burden of lead-related disease, Fewtrell, Kaufmann & Pruss-Ustun (2003) noted that, worldwide, 40% of children had a blood lead level greater than 5 μg/dl, 20% had a level greater than 10 μg/dl and less than 10% of children had a blood lead level greater than 20 μg/dl. However, 99% of the children with a level above 20 μg/dl lived in developing countries, demonstrating the regional disparities in exposure that exist.

(c) Sources of exposure

Lead is a "multimedia" contaminant, with sources or pathways that include air, water, soil, dust, food, paint and consumer products. This complexity can make source attribution very challenging. Airborne lead is largely attributable to industrial emissions. The reduction in the use of lead as a petrol additive, which began around 1980 worldwide, had a substantial impact on the blood lead distribution. WHO estimated that, in the years following phase-down of lead in petrol, the mean blood lead level in a country declined by an average of 7.8% per year (Fewtrell, Kaufmann & Pruss-Ustun, 2003). This value varies depending on the relative importance of the different sources or pathways in a particular population, however. Studies from several regions suggest that exposures can continue to be elevated for a large percentage of children even after the removal of lead from gasoline. For example, after the phase-out of leaded petrol in India, the percentage of children less than 12 years of age in Mumbai who had a blood lead level above 10 μg/dl fell to half the percentage measured in 1997, but one third (33.2%) of children still had a level above this value, and the geometric mean blood lead level was 8.4 μg/dl

Table 1. Estimated blood lead levels worldwide,[a] 1990 and 2005

	Blood lead level (μg/dl)																		
	AsiaC	AsiaE	AsiaPhi	AsiaS	AsiaSE	Aus	Car	EurC	EurE	EurW	LAA	LAC	LAS	LAT	NAHI	NAME	OC	SSAfrica
1990																		
Children, urban mean	10.7	8.5	3.8	12.0	6.8	6.3	8.4	6.2	7.0	6.8	12.7	13.9	10.9	11.1	2.7	11.4	4.7	9.7
Children, rural mean	2.9	7.5	1.9	8.2	5.2	2.4	2.2	4.2	4.7	3.3	8.5	8.5	4.6	4.6	2.7	7.3	4.7	1.9
Adults, urban mean	10.7	9.1	3.6	13.9	5.5	6.3	8.8	6.2	7.0	6.9	12.7	12.7	10.9	11.1	2.2	11.4	2.2	9.4
Adults, rural mean	2.9	8.1	1.9	8.2	5.2	2.4	2.2	4.2	4.7	3.3	10.3	10.3	4.6	4.6	2.2	7.3	2.2	1.9
Urban GSD	1.6	1.7	1.8	1.6	1.5	1.6	2.1	2.0	1.5	1.4	1.4	1.4	1.5	1.3	NA	1.7	1.9	1.6
Rural GSD	2.0	1.7	1.8	1.7	1.7	2.0	2.2	2.7	1.6	1.8	1.9	1.9	1.8	1.8	NA	2.0	1.9	2.8
Children GSD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.2	NA	NA	NA
Adults GSD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.1	NA	NA	NA
2005																		
Children, urban mean	3.7	4.6	2.1	9.0	5.4	2.1	9.7	3.4	3.0	1.6	3.2	5.4	3.8	4.5	1.9	6.5	2.6	6.8
Children, rural mean	2.4	4.1	1.9	8.2	5.3	2.4	2.2	2.9	3.0	1.6	3.6	3.6	2.2	2.2	1.9	6.5	2.6	1.9
Adults, urban mean	3.7	4.2	3.2	10.2	4.6	2.1	9.7	3.8	3.0	2.9	3.2	6.0	3.8	4.5	1.5	6.5	0.6	6.6
Adults, rural mean	2.4	3.8	1.9	8.2	5.3	2.4	2.2	2.9	3.0	2.9	3.6	3.6	2.2	2.2	1.5	6.5	0.6	1.9
Urban GSD	2.4	1.9	1.8	1.6	1.7	2.4	1.8	1.5	1.5	NA	2.1	1.6	1.9	1.6	NA	1.8	2.4	1.9
Rural GSD	1.6	1.9	1.8	1.7	1.7	2.0	2.2	1.5	1.5	NA	1.8	1.8	2.2	2.2	NA	1.8	2.4	2.6
Children GSD	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.9	NA	NA	NA	NA	2.17	NA	NA	NA
Adults GSD	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.6	NA	NA	NA	NA	2.18	NA	NA	NA

Table 1 (contd)

GSD, geometric standard deviation; mean, geometric mean; NA, not available or applicable

[a] The country groupings used in the table are as follows:

AsiaC: Asia, Central

Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Mongolia, Tajikistan, Turkmenistan, Uzbekistan

AsiaE: Asia, East

China, Democratic People's Republic of Korea

AsiaPHI: Asia Pacific, High Income

Brunei, Japan, Republic of Korea, Singapore

AsiaS: Asia, South

Afghanistan, Bangladesh, Bhutan, India, Nepal, Pakistan

AsiaSE: Asia, Southeast

Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Maldives, Mauritius, Mayotte, Myanmar, Philippines, Seychelles, Sri Lanka, Thailand, Timore Leste, Viet Nam

Aus: Australasia

Australia, New Zealand

Car: Caribbean

Anguilla, Antigua and Barbuda, Aruba, Bahamas, Barbados, Belize, Bermuda, British Virgin Islands, Cayman Islands, Cuba, Dominica, Dominican Republic, French Guiana, Grenada, Guadaloupe, Guyana, Haiti, Jamaica, Martinique, Montserrat, Netherlands Antilles, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Suriname, Trinidad and Tobago, Turks and Caicos Islands

EurC: Europe, Central

Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Montenegro, Poland, Romania, Serbia, Slovakia, Slovenia, The former Yugoslav Republic of Macedonia

EurE: Europe, Eastern

Belarus, Estonia, Latvia, Lithuania, Republic of Moldova, Russian Federation, Ukraine

EurW: Europe, Western

Andorra, Austria, Belgium, Channel Islands, Cyprus, Denmark, Faeroe Islands, Finland, France, Germany, Gibraltar, Greece, Greenland, Holy See, Iceland, Ireland, Isle of Man, Israel, Italy, Liechtenstein, Luxembourg, Malta, Monaco, Netherlands, Norway, Portugal, Saint Pierre et Miquelon, San Marino, Spain, Sweden, Switzerland, United Kingdom

Table 1 (contd)

LAA: Latin America, Andean
Ecuador, Peru, Plurinational State of Bolivia
LAC: Latin America, Central
Colombia, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Venezuela (Bolivarian Republic of)
LAS: Latin America, Southern
Argentina, Chile, Falkland Islands (Malvinas), Uruguay
LAT: Latin America, Tropical
Brazil, Paraguay
NAHI: North America, High Income
Canada, United States of America
NAME: North Africa / Middle East
Algeria, Bahrain, Egypt, Iran (Islamic Republic of), Iraq, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Syrian Arab Republic, Tunisia, Turkey, United Arab Emirates, West Bank and Gaza Strip, Western Sahara, Yemen
OC: Oceania
American Samoa, Cook Islands, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia (Federated States of), Nauru, New Caledonia, Niue, Northern Mariana Islands, Palau, Papua New Guinea, Pitcairn, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, Wallis and Futuna Islands
SSAC: Sub-Saharan Africa, Central
Angola, Central African Republic, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon
SSAE: Sub-Saharan Africa, East
Burundi, Comoros, Djibouti, Eritrea, Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Rwanda, Somalia, Sudan, Uganda, United Republic of Tanzania, Zambia
SSAS: Sub-Saharan Africa, Southern
Botswana, Lesotho, Namibia, South Africa, Swaziland, Zimbabwe
SSAW: Sub-Saharan Africa, West
Benin, Burkina Faso, Cameroon, Cape Verde, Chad, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Saint Helena, Sao Tome and Principe, Senegal, Sierra Leone, Togo
NB: For the purpose of lead exposure estimates, all sub-Saharan regions have been pooled into one (SSAfrica) because of a lack of data.

(Nichani et al., 2006). After the phase-out in Uganda, 20% of 4- to 8-year-old children still had a blood lead level greater than 10 µg/dl (Graber et al., 2010). In a pooled analysis of studies of Chinese children, the mean blood lead level after the phase-out was 8.1 µg/dl, with 24% of children having a level greater than 10 µg/dl (He, Wang & Zhang, 2009).

Some sources of lead exposure are specific to particular regions or cultures. Some traditional medicines (e.g. Ayervedic preparations) and spices from Asia have been reported to have high lead content and to have caused lead toxicity (Kales & Saper, 2009; Shamshirsaz et al., 2009; Lin et al., 2010), as have some cosmetics used primarily in the Middle East and South Asia (e.g. surma) (Rahbar et al., 2002). In Mexico, the preparation of food in lead-glazed ceramics that were not fired at sufficiently high temperatures has been identified as a major risk factor for elevated lead exposure (Villalobos et al., 2009). With the large-scale migration across national boundaries and international transport of foods, this problem has contributed to increased exposure in Hispanic subpopulations elsewhere (Handley et al., 2007; Lynch, Elledge & Peters, 2008). Electronic wastes sent to developing countries for recycling of materials have been reported to be associated with greater lead exposure to children in proximity to the recovery facilities (Albalak et al., 2003; Huo et al., 2007).

2.3.2 Biomarkers of effect

A variety of biomarkers of effect have been used in studies on the health effects of exposure to lead. Because lead inhibits the activity of certain enzymes in the haem pathway, such as ALAD and ferrochelatase, the accumulation products ALA and free erythrocyte protoporphyrin (or zinc protoporphyrin) have been used. For studies in which renal function was the end-point of interest, biomarkers of function such as GFR, serum creatinine level and serum uric acid concentration have been used. Studies of cardiovascular health have relied on biomarkers such as blood pressure, heart rate variability and pulse pressure. Studies of nervous system toxicity have employed biomarkers such as nerve conduction velocity, postural balance, tremor and intelligence quotient (IQ). Typically, these biomarkers have been considered both as continuously distributed variables and as categorical variables, with category boundaries specified according to judgements about clinical significance.

2.3.3 Clinical observations

High-dose exposure is associated with toxic effects on several organ systems, affecting haematopoiesis, renal function and, most prominently, particularly in children, the central nervous system. The haematological effects include anaemia, which is attributable to the inhibition of the enzymes ALAD and ferrochelatase. Effects on the kidney include an acute renal nephropathy involving proximal tubule dysfunction due to impairment of mitochondrial respiration and a more chronic nephropathy that is associated with reductions in GFR and atrophy of proximal and distal tubules. The neurological effects include encephalopathy characterized by brain oedema and haemorrhage due to microvascular damage.

Clinical signs and symptoms at presentation are variable and occur at blood lead levels that differ widely across individuals. In some people, blood lead levels of several hundred micrograms per decilitre have been reported to be asymptomatic, whereas others with a blood lead level of 100 μg/dl might present with an encephalopathy. Many of the symptoms and signs are nonspecific and might include abdominal colic, nausea, vomiting, constipation, anorexia, changes in consciousness, lethargy, irritability, paraesthesias and other signs of peripheral neuropathy, and pallor.

2.3.4 Epidemiological studies

(a) Mortality

Recent prospective cohort studies provide reasonably consistent evidence, in both men and women, that higher lead levels are associated with higher all-cause mortality and that deaths from cardiovascular diseases are largely responsible for the associations.

In the first of several studies in the USA that relied on data from the National Health and Nutrition Examination Survey (NHANES) to evaluate this association, Lustberg & Silbergeld (2002) followed up, in 1992, individuals who participated in NHANES II (1976–1980) (n = 4292, 30–74 years of age). A baseline blood lead level of 20–29 μg/dl was associated with an adjusted hazard ratio (HR) for all-cause mortality of 1.46 (95% confidence interval [CI] 1.14–1.86), using individuals with a baseline blood lead level below 10 μg/dl as the reference group. The HRs were also significant for circulatory mortality (1.39, 95% CI 1.01–1.91) and cancer (1.68, 95% CI 1.02–2.78). The HRs for individuals with baseline blood lead levels of 10–19 μg/dl were increased, but not significantly.

Menke et al. (2006) used NHANES III data (1988–1994) to consider the association between blood lead level and all-cause and cause-specific mortality in the adult population in the USA. The follow-up interval was 12 years in the 13 946 individuals with a blood lead level less than 10 μg/dl (mean 2.6 μg/dl). The causes of death considered in the analyses were cardiovascular disease, myocardial infarction, stroke, cancer and lung cancer. Cox proportional hazard regression was used to estimate HRs for individuals in baseline blood lead tertiles, adjusting for age, race, sex, diabetes mellitus, body mass index, smoking, alcohol consumption, physical activity, income, C-reactive protein, total cholesterol, education, urban residence, postmenopausal status, hypertension and kidney function (GFR <60 ml/min per 1.73 m^3). Comparing individuals in the highest versus the lowest tertile, the adjusted HR for all-cause mortality was 1.25 (95% CI 1.0–1.5, P for trend = 0.002). The associations with baseline blood lead level were also significant for cardiovascular deaths (HR 1.6, 95% CI 1.1–2.2), myocardial infarction (HR 1.9, 95% CI 1.0–3.4) and stroke (HR 2.5, 95% CI 1.2–5.3). Spline regressions, used to describe the shapes of the relationships, suggested that the increase in mortality was evident at blood lead levels greater than 2 μg/dl.

Schober et al. (2006) also evaluated mortality among participants in the NHANES III survey (n = 9757). Compared with the group with a baseline blood lead

level below 5 μg/dl, individuals with a baseline blood lead level of 5–9 μg/dl had a significantly increased adjusted risk of all-cause mortality (HR 1.24, 95% CI 1.05–1.48), as did individuals with a baseline blood lead level of 10 μg/dl or higher (HR 1.59, 95% CI 1.28–1.98). The HRs were similar in these two exposure strata for deaths from cardiovascular disease or cancer.

The associations between lead biomarkers and total and cause-specific mortality were evaluated in approximately 868 men enrolled in the United States Veterans Administration Normative Aging Study (Weisskopf et al., 2009). The mean age at baseline was 67.3 years (standard deviation [SD] 7.3 years), and a mean length of follow-up was 8.9 (SD 3.9) years. Blood lead level at baseline, which averaged 5.6 (SD 3.4) μg/dl, was not associated with mortality. Adjusting for age, smoking and education, lead concentration in patella was significantly associated with all-cause, cardiovascular (HR 5.6, 95% CI 1.7–18.3) and ischaemic heart disease deaths. For example, the HR for men in the highest tertile of patella lead, compared with men in the lowest tertile, was 2.5 (95% CI 1.2–5.4). Adjustment for hypertension, race, alcohol use, physical activity, body mass index, high-density lipoprotein, cholesterol and diabetes mellitus did not affect the results appreciably. Exploration of the functional forms of the associations suggested linear dose–response relationships. The HRs associated with tibia lead concentration were greater than 1 for these three end-points, but the 95% CIs included 1.

Khalil et al. (2009b) followed up 533 women, aged 65–87 at baseline, for a mean of 12 (SD 3) years. The mean blood lead level was 5.3 (SD 2.3) μg/dl (range 1–21 μg/dl). For the purposes of analyses, the mortality among women with a baseline blood lead level less than 8 μg/dl was compared with the mortality of women with a level of 8 μg/dl or greater. For all-cause mortality, the HR for women with a blood lead level of 8 μg/dl or greater was 1.6 (95% CI 1.0–2.5, $P = 0.04$). For deaths from cardiovascular diseases, the HR was 3.1 (95% CI 1.2–7.7, $P = 0.02$). Blood lead level was not significantly associated with stroke, cancer or non-cardiovascular deaths.

(b) Cancer

IARC (2006) recently reviewed studies of the carcinogenicity of inorganic lead. These included studies of both occupational and environmental (i.e. general population) exposures. The following section summarizes IARC's conclusions about the evidence.

(i) Occupational exposures

In one study conducted on primary smelter workers in Sweden, a 2-fold excess in the number of lung cancer cases was observed. However, these workers were also exposed to arsenic, a known risk factor for lung cancer. In a Finnish study of workers from a variety of industries, blood lead level, measured as part of surveillance programmes, showed a modest, but non-significant, dose–response relationship between blood lead level and incidence of lung cancer. In five other studies, involving battery and smelter workers in the USA, the United Kingdom and Italy, no consistent increase in risk was found as exposures increased. Data on smoking were not available for some of these cohorts.

In five of the cohorts considered with regard to lung cancer, the association between blood lead level and stomach cancer was also evaluated. Compared with the reference populations, the numbers of stomach cancer cases were 30–50% higher in the workers than in the reference populations, but data necessary to conduct dose–response analyses were not available. In addition, it was not possible to consider the possible contributions of other risk factors for stomach cancer, such as ethnicity, diet, socioeconomic status or *Helicobacter pylori* infection.

In one of five cohort studies, a 2-fold excess of kidney cancers was reported, but in the other four studies, the rates were close to the expected values. It was noted that the numbers of cases in these cohorts were small.

In four cohort studies that evaluated tumours of the brain and nervous system, no excess risk was observed among the exposed workers, compared with the reference population. A nested case–control study of Finnish workers reported a significant association between blood lead level and risk for glioma. As with kidney cancers, however, the numbers of cases on which these conclusions are based were small.

(ii) Environmental exposures

One study analysed data for a subgroup of participants in NHANES II, which was conducted between 1976 and 1980 (Jemal et al., 2002). The mean blood lead level was 7.3 µg/dl in the lowest quartile and 19.7 µg/dl in the highest quartile. Adjusting for a variety of baseline variables (age, poverty, alcohol and tobacco use, region and year of examination), risk of cancer mortality across blood lead quartiles was not significant for men or women, nor were site-specific cancer risks. The numbers of cases observed for some specific cancers were small, however.

Lustberg & Silbergeld (2002) analysed a somewhat different subgroup of NHANES II participants, including non-Caucasians but excluding participants whose blood lead level was 30 µg/dl or higher. In analyses that adjusted for baseline values of age, sex, race, education, income, smoking, body mass index, exercise and region, a positive association was found between blood lead level and risk of cancer. Using individuals with a blood lead level below 10 µg/dl as the referent group, the relative risk observed among individuals with a blood lead level of 10–19 µg/dl was 1.46 (95% CI 0.87–2.48). The relative risk among individuals with a blood lead level of 20–29 µg/dl was 1.68 (95% CI 1.02–2.78). The relative risks for lung cancer in these two blood lead groups were 1.70 (95% CI 0.60–4.81) and 2.20 (95% CI 0.80–6.06), respectively. The IARC working group expressed concern about possible residual confounding resulting from the investigators' failure to adjust for duration of smoking.

Based on its evaluation, IARC concluded that although the evidence for carcinogenicity is sufficient in animals, there is *limited evidence* in humans for the carcinogenicity of inorganic lead and that inorganic lead compounds are *probably carcinogenic* to humans (group 2A).

Since the completion of the IARC evaluation, additional studies have been published in which the association between lead exposure and mortality from cancer has been evaluated, but the results do not indicate that any revision to the IARC

conclusions is required. In a study using the data on 13 946 participants in NHANES III, conducted between 1988 and 1994, Menke et al. (2006) evaluated the association between blood lead level (mean of 2.6 µg/dl) and overall cancer mortality and mortality from lung cancer. In analyses adjusting for age, race/ethnicity, sex, diabetes mellitus, body mass index, smoking, alcohol consumption, physical activity, income, C-reactive protein, total cholesterol, education, residence, postmenopausal status, hypertension and kidney function, the HR for individuals in tertile 2 of blood lead level (≥1.94–3.62 µg/dl) was 0.72 (95% CI 0.46–1.12). The HR for individuals in tertile 3 of blood lead level (3.63 µg/dl) was 1.10 (95% CI 0.82–1.47). The *P* for trend was 0.10. The HRs for lung cancer in tertiles 2 and 3 were 0.70 (95% CI 0.34–1.42) and 0.79 (95% CI 0.40–1.58), respectively.

Among 868 men participating in the United States Veterans Administration Normative Aging Study, Weisskopf et al. (2009) found that neither baseline blood lead level nor patella lead level, measured using K-line X-ray fluorescence, was significantly associated with cancer mortality. For baseline blood lead level, adjusting for age, smoking and education, the HR for individuals in tertile 2 (4–6 µg/dl) was 1.03 (95% CI 0.42–2.55), and the HR for individuals in tertile 3 (>6 µg/dl) was 0.53 (95% CI 0.20–1.39) (*P* for trend = 0.15). For patella lead level, the HR for individuals in tertile 2 (22–35 µg/g bone) was 0.82 (95% CI 0.26–2.59), and the HR for individuals in tertile 3 (>35 µg/g bone) was 0.32 (95% CI 0.08–1.35) (*P* for trend = 0.14).

In a case–control study of primary smelter workers, arsenic exposure, but not lead exposure, was a risk factor for lung cancer in 141 cases and age-matched controls (Lundstrom et al., 2006).

Alatise & Schrauzer (2010) reported that women newly diagnosed with infiltrating ductal carcinoma of the breast had higher levels of blood and hair lead and that hair lead level correlated significantly with tumour volume.

In a study of 362 patients with brain tumours (glioma or meningioma) and 494 controls, gene–environment interactions were found. Specifically, cumulative lead exposure as a main effect, estimated on the basis of job history, was not associated with glioblastoma multiforme and meningioma, but polymorphisms in the *RAC2* and *GPX1* genes (for glioblastoma multiforme) and the *GPX1* and *XDH* genes (for meningioma) were observed to modify the association (Bhatti et al., 2009).

(c) Renal function

In a sample of 769 adolescents (12–20 years old) in NHANES III, Fadrowski et al. (2010) evaluated the association between blood lead level and GFR, estimated on the basis of both serum cystatin C level and serum creatinine level. The former is considered preferable to the latter as a marker of kidney function, as it appears to be less dependent than creatinine-based estimates on age, sex, height and muscle mass. The median blood lead level was 1.5 µg/dl (interquartile range 0.7–2.9 µg/dl). Models were adjusted for age, sex, race/ethnicity, urban/rural, tobacco smoke exposure, annual household income and educational level of family reference person. Participants with a blood lead level in the highest quartile (≥3 µg/dl) had a 6.6 ml/min per 1.73 m^3 lower cystatin C–estimated GFR (95% CI of −0.7 to −12.6 ml/min per 1.73 m^3) compared with those in the first quartile

(<1 µg/dl), with a significant trend ($P = 0.009$). Restricted quadratic spline analyses showed no departures from linearity and no threshold. The associations were qualitatively similar but weaker using creatinine to estimate GFR, suggesting that previous studies that depended on creatinine-based estimates of kidney function might have underestimated the association between GFR and blood lead level. Many of the previous studies had been conducted in adults with chronic kidney disease or hypertension. This study extends the association to the general population of adolescents in the USA in whom significant co-morbidities were absent. A limitation of this study is its cross-sectional design, leaving open the possibility of reverse causation (i.e. kidney disease causes decreased excretion of lead). This appears unlikely, however, as at least some prospective studies have shown that baseline blood lead level is associated with subsequent decline in kidney function (Kim et al., 1996), particularly among participants with diabetes or hypertension (Tsaih et al., 2004).

Using adult (≥20 years old) participants in NHANES III ($n = 15\,211$), Muntner et al. (2003) evaluated the associations between blood lead level and two indices of renal dysfunction: serum creatinine (cut-points representing the 99th percentiles for race/sex) and chronic kidney disease (GFR <60 ml/min per 1.73 m^3). Models were adjusted for age, sex, systolic blood pressure, diabetes mellitus, current smoking, history of cardiovascular disease, body mass index, alcohol consumption, household income, marital status and health insurance. Significant associations between blood lead level and kidney dysfunction were found among individuals with hypertension ($n = 4813$), but not among those without hypertension ($n = 10\,398$). Among those with hypertension, the adjusted odds ratio (OR) for elevated serum creatinine level for individuals in the highest quartile of blood lead level (6.0–56.0 µg/dl) was 2.4 (95% CI 1.5–4.0), and the adjusted OR for chronic kidney disease for the same individuals was 2.6 (95% CI 1.5–4.5). For both outcomes, the adjusted ORs were also significant for individuals in quartiles 2 (2.5–3.8 µg/dl) and 3 (3.9–5.9 µg/dl), with the trend across quartiles significant ($P < 0.001$). Muntner et al. (2005) subsequently reported similar associations using data from NHANES 1999–2002 ($n = 9961$). Adjusting for the same set of covariates plus race/ethnicity, they found that individuals in the highest quartile of blood lead level (2.47 µg/dl) were 2.7 (95% CI 1.5–5.0) times more likely than individuals in quartile 1 of blood lead level (<1.1 µg/dl) to have chronic kidney disease (defined as a GFR <60 ml/min per 1.73 m^3).

In a sample of adults from Taiwan, China ($n = 1565$), Lai et al. (2008) evaluated the associations between blood lead level and two indices of renal dysfunction: serum creatinine level (levels above 1.2 mg/dl considered abnormal) and serum uric acid level (levels above 7 mg/dl in males and 6 mg/dl in females considered abnormal). Adjusting for age, sex, occupation, education, marital status, smoking, alcohol, betel nut chewing, hypertension and lipid levels, they found that the ORs for individuals with blood lead levels in the highest tertile (>7.5 µg/dl; 0.8% of individuals had blood lead levels above 10 µg/dl) were 1.9 (95% CI 1.2–3.1) for elevated serum creatinine level and 2.7 (95% CI 1.6–4.5) for hyperuricaemia (both $P < 0.01$).

Lin and colleagues reported a series of studies on patients with chronic kidney disease, evaluating whether the rate of decline in kidney function over time differs depending on lead burden. Some studies have been observational. For example, Yu, Lin & Lin-Tan (2004) followed 121 patients for 4 years, classifying their baseline ethylenediaminetetraacetic acid (EDTA)-chelatable lead as “low” (urinary lead level below 80 μg/72 h urine collection following a provocative chelation dose) or “high” (urinary lead level between 80 and 600 μg/72 h). Significantly more patients with high compared with low baseline lead burdens experienced a doubling of serum creatinine level or required haemodialysis ($P = 0.001$). Each microgram per decilitre increase in baseline blood lead level, which was 4.9 μg/dl in the high-chelatable lead group and 3.4 μg/dl in the low-chelatable lead group, was associated with a reduction of 4.0 ml/min per 1.73 m^3 in GFR over the period of observation. Other studies by this group involved random assignment of patients with chronic kidney disease to receive therapeutic chelation, with decline in kidney function as the primary end-point. For instance, in one study involving 64 patients, whose baseline chelatable lead levels ranged from 80 to 600 μg/72 h, the patients randomized to active treatment received EDTA for up to 3 months, with additional rounds of treatment if indicated. The mean baseline blood lead levels of the chelation and placebo groups were 6.1 and 5.9 μg/dl, respectively. At the end of 2 years, the mean estimated GFR had increased by 2.1 ml/min per 1.73 m^3 in the chelated group and declined by 6.0 ml/min per 1.73 m^3 in the placebo group ($P < 0.01$) (Lin et al., 2003). In a subsequent study involving 108 patients with chronic kidney disease with chelatable lead levels between 20 and 80 μg/72 h and baseline blood lead levels of 1.2–4.6 μg/dl, the mean change in GFR was 6.6 ml/min per 1.73 m^3 in the chelated group and −4.6 ml/min per 1.73 m^3 in the placebo group ($P < 0.001$) (Lin et al., 2006a). This group conducted a study similar in design on 87 patients with type II diabetes and diabetic nephropathy, baseline chelatable lead levels between 30 and 373 μg/72 h and a mean blood lead level of 6.5 μg/dl (range 1.6–19.1 μg/dl) (Lin et al., 2006b). In the 12-month observation period following random assignment of patients to chelation or placebo, the rate of decline in GFR was 5.0 (SD 5.7) ml/min per 1.73 m^3 in the chelation group and 11.8 (SD 7.0) ml/min per 1.73 m^3 in the placebo group. Baseline blood and chelatable lead levels were both significant predictors of progressive nephropathy in patients with diabetes.

A case–control study compared the blood and tibia lead levels of 55 African Americans with end-stage renal disease (patients receiving chronic haemodialysis treatment) with those of 53 age- and sex-matched controls (Muntner et al., 2007). The cause of end-stage renal disease was hypertension for 40% of the cases, diabetes for 36%, glomerulosclerosis for 6% and unknown for 18%. The mean blood lead level was significantly higher among cases (6 versus 3 μg/dl, $P < 0.001$), with 67% of cases (compared with 6% of controls) having a level of 5–9 μg/dl and 15% (compared with no controls) having a level of 10 μg/dl or greater. The tibia lead levels of cases were somewhat higher than those of controls, but the difference was not significant. The authors suggested that this finding, along with the fact that blood and tibia lead levels were more highly correlated for cases than for controls, might indicate greater bone turnover in the cases, resulting in higher blood lead levels.

The association between lead exposure and renal function in children has not been studied extensively. The few data available suggest that higher blood lead levels are associated with increased GFR (as estimated by serum creatinine or cystatin C levels), suggesting a paradoxical effect, perhaps a hyperfiltration phenomenon (Staessen et al., 2001; de Burbure et al., 2006).

Several factors have been found to modify the association between blood lead level and kidney function, although the evidence is inconsistent. Among these are certain genetic polymorphisms, including ALAD, the vitamin D receptor and nitric oxide synthase (Weaver et al., 2003, 2006; Wu et al., 2003). Among adults who participated in NHANES 1999–2006 (n = 14 778), Navas-Acien et al. (2009) found that higher cadmium exposure resulted in more striking positive associations between blood lead level and renal dysfunction. Adjusting for survey year, age, sex, race/ethnicity, body mass index, education, smoking, cotinine, alcohol consumption, hypertension, diabetes mellitus and menopausal status, individuals with blood lead and blood cadmium levels that placed them in the highest quartile for both metals had ORs of 2.3 (95% CI 1.7–3.2) for albuminuria and 2.0 (95% CI 1.3–3.1) for reduced GFR (estimated based on serum creatinine). The same ORs calculated without taking blood cadmium level into account were much smaller: 1.2 (95% CI 1.0–1.5) and 1.6 (95% CI 1.2–2.1), respectively. For individuals in the highest quartiles of both metals, the OR associated with having both indicators of kidney dysfunction was 4.1 (95% CI 1.6–10.7).

(d) Cardiovascular system

Increased blood pressure/hypertension have long been recognized as a consequence of occupational exposure to lead, raising the question of whether a similar, but more modest, association between lead burden and other cardiovascular outcomes is evident as well at the lower lead exposures experienced by the general population. A variety of reviews (e.g. Navas-Acien et al., 2007) have evaluated the evidence with regard to blood pressure, generally finding that it supports the presence of a positive relationship, with the magnitude of the increase in blood pressure per microgram per decilitre being modest, approximately 1 mmHg (0.13 kPa). The CIs of the estimates derived from consideration of the integrated evidence are mostly positive values but usually include 0 as well (e.g. in the USEPA [2006] integrative analysis, the 95% CI was −3.9 to 11 for systolic pressure and −1.3 to 7.3 for diastolic pressure).

Some analyses suggest that the association between lead exposure and blood pressure varies across sociodemographic strata. Limiting analyses of NHANES III data to women aged 40–59 years (n = 2165), Nash et al. (2003) reported that, compared with women with blood lead levels in the lowest quartile (0.5–1.6 µg/dl), women in the highest quartile (4–31 µg/dl) had an adjusted OR of 3.4 (95% CI 1.3–8.7) for diastolic hypertension (>90 mmHg [12 kPa]) and an adjusted OR of 1.5 (95% CI 0.7–3.2) for systolic hypertension (>140 mmHg [19 kPa]). The associations were strongest for postmenopausal women. In analyses of NHANES III data stratifying by race, Vupputuri et al. (2003) found significant adjusted associations between blood lead level and blood pressure in black males and females: each 3.3 µg/dl increase was associated with a 0.82 mmHg (0.11 kPa)

increase in systolic blood pressure in black males (95% CI 0.19–1.44 mmHg [0.025–0.19 kPa]) and a 1.55 mmHg (0.21 kPa) increase in systolic pressure in black females (95% CI 0.47–2.64 mmHg [0.063–0.35 kPa]). No associations were found in white males or females.

Glenn et al. (2003, 2006) conducted two longitudinal studies, in occupationally exposed cohorts, of the association between changes in blood lead level and changes in systolic blood pressure. In a cohort of 496 current and former workers followed for 4 years, the baseline blood lead level was 4.6 ± 2.6 µg/dl. Adjusting for covariates, systolic blood pressure increased 0.64 mmHg (0.085 kPa) (standard error [SE]: 0.25 mmHg [0.033 kPa]), 0.73 mmHg (0.097 kPa) (SE: 0.26 mmHg [0.035 kPa]) and 0.61 mmHg (0.081 kPa) (SE: 0.27 mmHg [0.036 kPa]) for each standard deviation increase in blood lead level at baseline, tibia lead at year 3 or peak past tibia lead levels, respectively (Glenn et al., 2003). In a cohort of 575 Korean workers also followed for 4 years, the baseline blood lead level was 31.4 ± 14.2 µg/dl. Adjusting for covariates, systolic blood pressure increased 0.9 mmHg (0.12 kPa) (95% CI 0.1–0.6 mmHg [0.013–0.08 kPa]) for each 10 µg/dl increase in blood lead per year (Glenn et al., 2006).

Park et al. (2009) used a prediction model, developed using data from the United States Veterans Administration Normative Aging Study, for predicting bone lead level from blood lead level to reanalyse the association between lead and hypertension in NHANES III. The association was stronger using estimated bone lead level compared with using blood lead level, suggesting that use of a biomarker of shorter-term exposure to lead might result in an underestimate of the association. Among the potential mechanisms proposed to underlie this association are lead-related impairments in renal function, oxidative stress, effects on the renin–angiotensin system and suppression of nitric oxide. Another potential mechanism is suggested by the finding, in a random sample of 1140 50- to 70-year-olds in the Baltimore Memory Study, that blood lead level is significantly correlated with homocysteine levels after adjustment for age, sex, race/ethnicity, education, tobacco use, alcohol consumption and body mass index (Schafer et al., 2005). The same finding was reported in a smaller cross-sectional study in occupationally exposed workers (Chia et al., 2007a).

Published studies used by WHO in estimating the global burden of disease attributable to lead indicate that relative risks of ischaemic heart disease and cerebrovascular stroke associated with small increases in blood pressure (0.4–3.7 mmHg [0.053–0.49 kPa] systolic blood pressure) have been estimated to be in the range of 1.01–1.4, with higher relative risks at younger ages. A large meta-analysis that included 61 prospective studies showed that increases in blood pressure, even among individuals who are clinically normotensive, are associated with increased vascular mortality (Prospective Studies Collaboration, 2002). This analysis included 12.7 million person-years of follow-up for 958 000 adults who were free of known vascular disease at baseline. Blood pressure was significantly associated with age-specific mortality rates for stroke, ischaemic heart disease and other vascular causes, with the relationships apparent throughout the blood pressure range, extending down to 115 mmHg (15 kPa) systolic blood pressure and 75 mmHg (10 kPa) diastolic blood pressure. The association was log-linear, such

that the proportional difference in risk associated with a given increase in blood pressure was similar across the entire range. For example, among those of middle age, reducing systolic blood pressure by 2 mmHg (0.3 kPa) would be estimated to produce a 10% reduction in stroke mortality and a 7% decrease in mortality from ischaemic heart disease.

As noted previously, studies relating lead exposure to overall mortality have tended to find that deaths from cardiovascular disease are largely responsible for the association (Lustberg & Silbergeld, 2002; Schober et al., 2006). The evidence regarding lead and clinical cardiovascular end-points is mixed, however, with most of the studies focusing on individuals with occupational exposure. In cross-sectional analyses of NHANES 1999–2002, an association was reported between concurrent blood lead level and the risk of peripheral artery disease (Navas-Acien et al., 2004; Muntner et al., 2005). Other studies have shown a non-significant elevation in risk of stroke. In a study of non-occupational exposure and heart rate variability in a sample of people from the Republic of Korea (n = 331, comparing blood lead levels below 1.39 µg/dl with those above 3.45 µg/dl), Jhun, Kim & Paek (2005) reported inverse associations between low-frequency, high-frequency and total power spectrum, but these associations were not significant in adjusted analyses. In community-exposed adult men (n = 413) in the United States Veterans Administration Normative Aging Study, Park et al. (2006) also did not find significant adjusted associations between higher tibia or patella lead levels and indices of heart rate variability, although they did find significant associations between patella lead level and heart rate variability (higher low-frequency power and the ratio of low- to high-frequency power) among men with metabolic syndrome. No such associations were found for tibia lead level, however. The authors interpreted these findings as evidence that oxidative stress induced by lead exposure in these men was responsible for autonomic dysfunction in the cardiovascular system. In analyses of the same cohort (n = 593), Perlstein et al. (2007) found a significant association between tibia lead level and pulse pressure (the difference between systolic and diastolic pressures), an index of arterial stiffening, but not between blood lead level and pulse pressure. One mechanism of arterial stiffening is thought to be vascular oxidative stress. Men with tibia lead levels greater than the median value (19.0 µg/g) had pulse pressures that were 4.2 mmHg (0.56 kPa) higher (95% CI 1.9–6.5 mmHg [0.25–0.87 kPa]), compared with men with tibia lead levels below the median, adjusting for age, race, diabetes, family history of hypertension, education, waist circumference, alcohol intake, smoking, height, heart rate, fasting glucose and ratio of total cholesterol to high-density lipoprotein cholesterol. Patella lead levels were also measured, but results were not reported.

Limited data are available on the association between lead exposure and blood pressure in children. Among the children participating in the Kosovo prospective lead study (Factor-Litvak et al., 1996), in whom blood lead levels were substantially elevated (mean of 37 µg/dl in children in the exposed town, 8.7 µg/dl in children in the unexposed town), an increase in blood lead level of 10 µg/dl was associated with a very small increase in systolic blood pressure (0.5 mmHg [0.07 kPa], 95% CI −0.2 to 1.3 mmHg [−0.027 to 0.17 kPa]) and diastolic blood pressure (0.4 mmHg [0.05 kPa], 95% CI −0.1 to 0.9 mmHg [−0.013 to 0.12 kPa]). In contrast,

Gerr et al. (2002) reported significant adjusted associations between higher tibia lead levels and higher systolic and diastolic blood pressures in young adults, half of whom had grown up in the vicinity of a lead smelter. Subjects in the highest quartile of tibia lead level (>10 µg/g) had systolic pressures that were 4.3 mmHg (0.57 kPa) higher than those in the lowest quartile (<1 µg/g) and diastolic pressures that were 2.8 mmHg (0.37 kPa) higher. Although the current blood lead levels were low for subjects in all tibia lead quartiles and were unrelated to either systolic or diastolic blood pressure, the subjects with tibia lead levels in the highest quartile were estimated to have had a mean childhood blood lead level of 65 µg/dl. No association between blood lead level and blood pressure was found in children 12–33 months of age (*n* = 780) who participated in the Treatment of Lead-Exposed Children (Chen et al., 2006), a randomized clinical trial in which oral succimer was administered to children with baseline blood lead levels of 20–44 µg/dl. Children were followed up for 5 years post-chelation.

A series of studies in 9-year-old children (*n* = 108) suggest that early lead exposure (blood lead level measured at a mean of 2.6 years: mean 4.0 µg/dl, range 1.5–13.0 µg/dl) mediates the association between lower family socioeconomic status and greater salivary cortisol response to acute stress (Gump et al., 2005, 2007, 2008, 2009).

(e) Reproduction

A recent review (Bellinger, 2005) on lead and pregnancy concluded that fertility is reduced in couples during periods in which the male has a blood lead level greater than 40 µg/dl or a blood lead level greater than 25 µg/dl for several years. The reduced fertility is manifested as fewer live births, reduced likelihood of conception or increased time to pregnancy. Although the evidence regarding lead and spontaneous abortion is limited, in one well-designed study of 668 women in Mexico (Borja-Aburto et al., 1999), the risk was doubled (OR 2.3) at maternal blood lead levels of 5–9 µg/dl and was 5-fold higher (OR 5.4) at maternal blood lead levels of 10–14 µg/dl.

High-dose lead exposure has long been recognized as a risk factor for eclampsia (Troesken, 2006), and a case–control study conducted in the Islamic Republic of Iran of women and newborns suggested that risk of pre-eclampsia is increased among women with blood lead levels largely below 20 µg/dl (Vigeh et al., 2006). Hypertension is a clinical feature of pre-eclampsia, so several studies have investigated the link between blood pressure during pregnancy and lead. Two case–control studies suggest that the risk of pregnancy hypertension is increased at blood lead levels below 10 µg/dl (Sowers et al., 2002; Magri, Sammut & Savona-Ventura, 2003). A prospective cohort study (Rothenberg et al., 2002) found that lead concentration in the calcaneus, but not lead concentration in the tibia or blood lead level, was significantly associated with third-trimester hypertension. In a study conducted in Kosovo (Factor-Litvak et al., 1993), the OR for proteinuria was 4.5 (95% CI 1.5–13.6) for women in the highest decile of pregnancy blood lead level (>40 µg/dl), although the OR rose above 1 for women with a blood lead level greater than 5.8 µg/dl.

Increased paternal or maternal lead exposure has been linked to the risk of a congenital malformation in offspring, although the evidence is somewhat inconsistent across studies. In some studies demonstrating such an association, exposure status was based solely on job title rather than on a lead biomarker. An increased risk of neural tube defects has been reported in offspring of women residing in an area with high lead levels in water (Bound et al., 1997). A study conducted using data from a regional birth defect surveillance programme in the USA found that men who were presumed (on the basis of self-report, an industrial hygiene assessment or job exposure matrix) to have been exposed to lead in the 3-month period prior to conception through the first trimester had an OR of 1.83 (95% CI 1.00–3.42) of delivering a child with total anomalous pulmonary venous return (Jackson et al., 2004). For maternal exposure during this interval, the OR was 1.57 (95% CI 0.64–3.47).

Evidence continues to mount that higher prenatal exposure to lead impairs fetal growth. In a cohort of Mexican women in whom bone lead level was measured 1 month postpartum, higher tibia lead levels were significantly associated with shorter birth length (infants in the highest quintile of maternal tibia lead level had an OR of 1.79 [95% CI 1.10–3.22]) (Hernandez-Avila et al., 2002). In the same cohort, infants of mothers with higher patella lead levels, measured 1 month postpartum, had a significantly smaller head circumference (Hernandez-Avila et al., 2002) as well as lower weight at 1 month of age and less weight gain between birth and 1 month (Sanin et al., 2001). Similar relationships between cord blood lead level (mean 3.9 [SD 3.6] µg/dl) and birth weight and length have been reported in a study conducted in Brazil (Zentner et al., 2006). In a study of 262 pregnancies in California (Jellife-Pawlowski et al., 2006), women with a blood lead level greater than 10 µg/dl during pregnancy were at increased risk of delivering an infant that was preterm (OR 3.2, 95% CI 1.2–7.4) or small for gestational age (OR 4.2, 95% CI 1.3–13.9). Second-trimester blood lead level was a particularly strong predictor of length of gestation (−1.0 days for each microgram per decilitre above 10 µg/dl).

(f) Nervous system

(i) Nerve conduction velocity

A recent meta-analysis was conducted investigating the association between blood lead level and peripheral nerve conduction velocities, latencies and amplitudes in adults (Krieg, Chrislip & Brightwell, 2008). Forty-nine studies, which included 2825 individuals exposed to lead, primarily as a result of occupation, and 1629 controls, were included in the analysis. The nerves measured in these studies included the median, ulnar and radial nerves in the arm and the deep and superficial peroneal, posterior tibial, aural and fibular nerves in the leg. Mixed models were used to estimate the slopes of the dose–effect relationships. The slopes of the relationships were generally negative for velocities, positive for latencies and flat for amplitudes. The lowest blood lead levels at which relationships were found ranged from 33.0 µg/dl (conduction velocity of the median sensory nerve) to 64.0 µg/dl (distal motor latency of the median nerve). The authors noted that these should not be interpreted as estimates of a biological threshold but merely as estimates of the

blood lead levels at which the exposure-related changes in nerve function reached statistical significance. Increasing the number or the precision of the nerve conduction measurements or increasing the number of subjects with lower blood lead levels might have produced lower estimates.

(ii) Postural balance

The association between blood lead level and postural sway, measured using the Neuromotor Test System (CATSYS), was evaluated in a cohort of 181 Japanese workers (121 lead-exposed, 60 controls) (Iawata et al., 2005). Analyses were adjusted for age, height, smoking and alcohol use. Most postural sway measures were significantly greater in the lead-exposed workers and significantly related, in multiple regression analyses, to blood lead level. Benchmark dose (BMD) modelling produced lower bounds on the BMD (BMDLs) in the range of 12–17 µg/dl for the different indices of sway.

(iii) Essential tremor

In a case–control study, the mean blood lead level of 100 patients with essential tremor (3.3 [SD 2.4] µg/dl) was significantly higher than the mean blood lead level of 143 controls (2.6 [SD 1.6] µg/dl) (Louis et al., 2003). In a logistic regression analysis adjusting for age and current cigarette smoking, the OR was 1.19 (95% CI 1.03–1.57, $P = 0.02$). The adjusted OR was somewhat greater when patients with a family history of essential tremor were excluded (OR 1.38, 95% CI 1.15–1.64, $P = 0.001$). This association was also found in a subsequent study of 105 essential tremor cases and 105 controls (Dogu et al., 2007). In this study, the mean blood lead level of cases was 3.2 (SD 1.9) µg/dl, compared with a mean blood lead level of 1.6 (SD 0.8) µg/dl in the controls. Adjusting for age, sex, education, cigarette smoking, cigarette pack-years and alcohol use, the OR was 4.19 (95% CI 2.59–6.78, $P < 0.001$). In addition, the correlation between tremor severity and blood lead level was 0.48 ($P < 0.001$), although this relationship was not found when the analysis was restricted to essential tremor cases.

Neither of these studies involved incident cases of essential tremor, and the cross-sectional design used in each study makes it uncertain whether the higher blood lead levels measured in the cases preceded or followed the diagnosis of essential tremor. More importantly, as the authors noted, even if elevated lead exposure preceded the diagnosis and the role of lead can be considered causal, these studies cannot be used to identify the critical blood lead level. If a blood lead level as low as 3 µg/dl were sufficient to cause essential tremor, the observed prevalence in the population would be much higher than it is (1–6%). Given that the mean age of the participants was greater than 50 years in the Louis et al. (2003) study and greater than 66 years in the Dogu et al. (2007) study, it is possible that their blood lead levels had been considerably higher in the past.

(iv) Amyotrophic lateral sclerosis

Past lead exposure has been associated with the risk of amyotrophic lateral sclerosis (ALS) in case–control studies (Kamel et al., 2002, 2008; Fang et al., 2010)

and one case report (Oh et al., 2007). Information on lead exposure was obtained for 109 cases recruited from two hospitals in Boston, Massachusetts, USA, and from 256 community controls frequency matched on age, sex and residence (Kamel et al., 2002). Bone and blood lead levels were measured in most cases (*n* = 107) and a subset (*n* = 41) of controls. Self-reported occupational exposure to lead was associated with an OR of 1.9 (95% CI 1.1–3.3). The risk of ALS increased, as well, with increasing patella lead level (OR 3.6, 95% CI 0.6–20.6, for each 1 µg/g increase), increasing tibia lead level (OR 2.3, 95% CI 0.4–14.5, for each 1 µg/g increase) and increasing blood lead level (OR 1.9, 95% CI 1.4–2.6, for each 1 µg/dl increase). A follow-up study using Cox proportional hazard analysis found a weak association between longer survival and high baseline blood lead level (HR 0.9, 95% CI 0.8–1.0), baseline patella lead level (HR 0.5, 95% CI 0.2–1.0) and baseline tibia lead level (HR 0.3, 95% CI 0.1–0.7) (Kamel et al., 2008). In an additional study involving 184 cases and 194 controls, a doubling of blood lead level was associated with a 1.9-fold (95% CI 1.3–2.7) increase in risk of ALS, adjusting for age and an index of bone resorption (C-terminal telopeptides of type 1 collagen) (Fang et al., 2010). Additional adjustment for an index of bone formation (procollagen type 1 amino-terminal peptide) did not affect the results.

(v) Adult cognitive function

Seeber, Meyer-Baron & Schaper (2002) reviewed two meta-analyses involving data from 24 studies of neurobehavioural performance of workers occupationally exposed to lead. They concluded that although the evidence is not entirely consistent across studies, deficits in different domains are present at blood lead levels between 37 and 52 µg/dl. Several recent studies have reported on the associations between measurements of bone lead levels and cognitive function, both in occupationally exposed adults and in the general adult population. Khalil et al. (2009a) administered a battery of neuropsychological tests to 83 lead battery plant workers and 51 controls who had previously been part of a cohort of 469 individuals administered the same battery 22 years earlier. Current mean blood lead levels were 12 µg/dl for the workers and 3 µg/dl for the controls. Tibia lead level was associated with lower scores, both cross-sectionally and longitudinally, in that it predicted declines in performance over the follow-up interval in the workers, but not in the controls, adjusting for baseline scores, age, education, years of employment and lifestyle factors. The domains most strongly related to cumulative lead exposure were spatial ability, executive functions and learning/memory. Other studies of occupationally exposed workers have also reported that bone lead levels predict decline in test scores over time (Schwartz et al., 2005).

Similar findings have also been reported in cohorts drawn from the general population. Using the Baltimore Memory Study, a longitudinal cohort study of urban adults of diverse ethnicity, Bandeen-Roche et al. (2009) evaluated the association between tibia lead levels and performance on a battery of neuropsychological tests (*n* = 943–1140 for the baseline and two follow-up assessments). Previous cross-sectional analyses of these data had revealed relationships between tibia lead levels and test scores (Shih et al., 2006). In adjusted longitudinal analyses, higher tibia lead levels were significantly associated with greater decline in eye–hand

coordination over time. Tibia lead level–associated deficits were also found in multiple other skills, including executive functioning, verbal memory and learning. Weisskopf et al. (2004, 2007) reported similar findings in the United States Veterans Administration Normative Aging Study, with the domains of response speed, visuospatial and visuomotor being most strongly associated with bone lead level measured 3.5 years earlier, but not with concurrent blood or bone lead levels.

Most studies of lead and adult cognitive function have been conducted in males. Weuve et al. (2009) assessed biomarkers of lead exposure (tibia, patella, blood) in 587 women 47–74 years of age drawn from the Nurses' Health Study cohort in the USA. Five years later, their cognitive function was assessed. Mean blood lead level at baseline was 2.9 (SD 1.9) µg/dl. All three biomarkers were inversely associated with women's test scores.

Several types of variables have been investigated as potential effect modifiers of the association between increased lead exposure and adult cognition. In a study of workers, Bleecker et al. (2007b) found that among pairs of workers matched in terms of lifetime weighted blood lead level, the inverse association between lead and test scores was more pronounced, at least in certain domains (not motor function), among the members of the pairs that had low "cognitive reserve", operationalized as poorer reading achievement. They suggested that greater cognitive reserve is protective against lead's adverse effects. Analyses of both the Baltimore Memory Study (Glass et al., 2009) and the Veterans Administration Normative Aging Study suggest that greater levels of stress, either self-reported (Peters et al., 2010) or operationalized as the level of psychosocial hazards in the neighbourhood of residence, render an individual more vulnerable to the adverse effects of lead. Finally, several genetic polymorphisms have been investigated as potential effect modifiers. Stewart et al. (2002) reported that the slope of the inverse association between tibia lead level and cognitive test score was steeper among workers carrying at least one *ε4* allele of the apolipoprotein E gene than among workers not carrying one. Analysing NHANES III data, Krieg et al. (2009, 2010) reported that effect modifications of the association between lead and cognition by both vitamin D receptor genotypes and ALAD genotypes were complex and differed as a function of age (e.g. 12–16 years, 20–59 years, >60 years). Other studies have investigated the role of ALAD polymorphisms. Chia et al. (2004, 2007b) suggested that workers carrying the *ALAD2* allele are, to some extent, protected against lead neurotoxicity. In contrast, among men in the Veterans Administration Normative Aging Study, those with the *ALAD2* allele showed a stronger inverse association between blood lead level and performance on the Mini-Mental Status Examination (Weuve et al., 2006) and poorer scores on a spatial copying test (Rajan et al., 2008). In the same cohort, however, carriers of the *ALAD1* allele were at greater risk of lead-associated changes in mood (Rajan et al., 2007).

(vi) Brain imaging

Several studies suggest that white matter is particularly vulnerable to injury as a result of lead exposure. Stewart et al. (2006) found that increasing tibia lead levels were significantly associated with grade of white matter lesion in 536 former

organolead workers (for a 1 µg/g increase in lead concentration, adjusted OR associated with having a lesion of grade 5+ was 1.04, 95% CI 1.02–1.06, $P = 0.004$). Because the workers were all at least 15 years removed from occupational exposure, these changes likely represent progressive or persistent structural lesions.

T.J. Hsieh et al. (2009) used diffusion-tensor imaging to compare the integrity of white matter in workers occupationally exposed to lead ($n = 19$) in Taiwan, China, with that of age- and sex-matched community controls ($n = 18$). The mean blood lead level of the workers was 11.5 (SD 1.5) µg/dl, compared with 3.2 (SD 1.2) µg/dl in the controls ($P < 0.001$). Tibia and patella lead levels were also measured. The fractional anisotropy values of the workers and controls differed significantly bilaterally in parietal, occipital and temporal white matter (all $P < 0.05$). Moreover, significant correlations were found between fractional anisotropy values in these regions and the three lead exposure indices. Fractional anisotropy values for the genu and splenium of the corpus callosum did not differ, nor did mean diffusion values in any of the regions measured. These findings suggest that white matter is injured, as reduced fractional anisotropy is widely interpreted as an indication of axonal damage (fibre orientation and organization) and demyelination. This hypothesis is supported by the results of a study of workers at a primary lead smelter, with blood lead levels that averaged 29 µg/dl (range 16–42 µg/dl), conducted by Bleecker et al. (2007a). Damage to white matter, presenting as hyperintensities on T2-weighted magnetic resonance imaging, mediated, to some extent, the inverse association between lead exposure and motor performance.

There is also evidence that grey matter volume in the adult brain is associated with past lead exposure. In the study of occupational lead exposures conducted by Stewart et al. (2006), significant associations were found between higher tibia lead levels and reduced total brain volume, total grey matter and volumes in several specific regions, including frontal, the cingulate gyrus and the insula. These analyses were adjusted for age, education, height and apolipoprotein ε4 status. Cecil et al. (2008) and Brubaker et al. (2010) reported on structural and volumetric imaging studies in young adulthood (mean age 21 [SD 1.5] years) of individuals enrolled prior to birth in the Cincinnati Prospective Lead Study. Significant inverse linear associations were found between annual mean blood lead level measured in the interval from 3 to 6 years of age and grey matter volume, with the magnitude of volume loss increasing with age. The associations were most striking in the frontal regions, particularly the anterior cingulate cortex and ventrolateral prefrontal cortex. Associations were more striking for males than for females. In diffusion-tensor imaging studies of 91 subjects in this cohort, Brubaker et al. (2009) found diverse changes in white matter that were significantly associated with childhood blood lead levels. Specifically, reduced fractional anisotropy and axial diffusivity were found throughout the white matter, as well as changes in the genu, body and splenium of the corpus callosum. Together, these changes suggest lead-related alterations in myelination and in axonal integrity. Finally, using functional magnetic resonance imaging in members of this study cohort ($n = 42$), Yuan et al. (2006) reported that significant lead-associated changes in activation patterns in the left frontal cortex and left middle temporal gyrus were found as subjects completed a verb generation task.

As found with respect to white matter changes, there is some evidence that the lead-associated changes in brain volume might mediate the lead-associated changes observed in adults' cognitive function. In their cohort of former organolead workers, Schwartz et al. (2007) found that larger volumes of different brain regions were associated with better scores on tests of visuoconstruction, processing speed, visual memory, executive functioning and eye–hand coordination. Furthermore, for the three domains for which test scores were significantly associated with peak tibia lead level (visuoconstruction, eye–hand coordination, executive functioning), volumetric mediation was found. Specifically, the magnitudes of the associations were reduced when volumes of the regions of interest were included as covariates in regression models relating tibia lead level to test scores (Caffo et al., 2007).

(vii) Child IQ and neuropsychological function

To enable a more powerful exploration of the quantitative characteristics of the dose–response relationship between children's blood lead levels and their IQ scores, particularly at blood lead levels below 10 µg/dl, the data from seven prospective cohort studies were pooled (Lanphear et al., 2005). The studies were conducted in the USA (Boston, Rochester, Cincinnati, Cleveland), Mexico City, Kosovo and Port Pirie, South Australia. The analyses included a total of 1333 children, followed from infancy to 5–10 years of age, for whom serial assessments of blood lead level were available. Four indices of lead exposure history were compared in terms of their relationship to IQ: concurrent (the blood lead level measured closest in time to the IQ test), maximum blood lead level measured prior to the IQ test, average lifetime blood lead level (mean blood lead level between 6 months of age and the measurement of IQ) and early childhood blood lead level (mean blood lead level from 6 to 24 months of age). Adjustments were made for 10 covariates measured in each study: HOME Inventory (a measure of the home environment and parental practices and attitudes), sex, birth weight, birth order, maternal education, maternal IQ, maternal age, marital status, prenatal smoking and prenatal alcohol use. A variety of functional forms relating blood lead indices to IQ were evaluated in terms of their relative fit to the data. Of the four blood lead variables, concurrent blood lead level provided the best fit to the data and was selected as the primary exposure metric. A restricted cubic spline model indicated that a log-linear model provided the best fit and suggested that a decline of 6.9 (95% CI 4.2–9.4) IQ points occurred over the blood lead level range of 2.4–30 µg/dl (the upper and lower 5th percentiles of the blood lead distribution). Moreover, a restricted spline model, which does not impose any assumptions about the shape of the dose–response relationship, suggested that the steepest declines in IQ were apparent at blood lead levels below 10 µg/dl. An IQ decrement of 3.9 (95% CI 2.4–5.3) points was associated with an increase in blood lead level from 2.4 to 10 µg/dl; a decrement of 1.9 (95% CI 1.2–2.6) points was associated with an increase in blood lead level from 10 to 20 µg/dl; and a decrement of 1.1 (95% CI 0.7–1.5) points was associated with an increase in blood lead level from 20 to 30 µg/dl. Piecewise linear models were also fit to specific ranges of blood lead levels, defined a priori. Among children for whom the maximum blood lead level measured was below 7.5 µg/dl (n = 103), the regression coefficient for concurrent blood lead level was −2.94 (95% CI −5.16 to −0.71), compared with a regression

coefficient of −0.16 (95% CI −2.4 to −0.08) for children with a maximum blood lead level greater than or equal to 7.5 μg/dl (*P* = 0.015). Sensitivity analyses indicated that the results did not depend unduly on the data from any one study, as the coefficient for concurrent blood lead level changed only by −2.6% to +8.6% when the data from any one of the seven studies were excluded.

The importance of a lead-associated shift of a relatively small number of points in mean IQ can best be appreciated by examining the impact on the population IQ distribution (Bellinger, 2004). Consider the example of a downward shift of 3 points from the expected value of 100 to 97. A reduction of this size would be of uncertain importance to an individual child. However, if all other characteristics of the IQ distribution remain the same (i.e. its dispersion), the shift in the mean would result in an increase of 8% in the number of children with an IQ below 100 and a 57% increase in the number of children with an IQ below 70, the criterion often used to identify those with an intellectual disability. It would also result in a 40% reduction in the number of children with a score of 130 or higher. A downward shift of 5 points in the mean IQ would result in a doubling of the number of children with a score below 70. Using data from a study of low-level lead exposure, Needleman et al. (1982) demonstrated these phenomena empirically. Rose & Day (1990) provided similar demonstrations for changes in mean body mass and the prevalence of obesity, as well as changes in systolic blood pressure and the prevalence of hypertension. A mean reduction of 5 mmHg (0.7 kPa) in systolic blood pressure would result in a reduction of 50% in the prevalence of hypertension, for example.

Although no explanation has been found for a supralinear relationship between blood lead level and child IQ, the same relationship has been reported in several independent studies since the publication of the international pooled analyses (e.g. Kordas et al., 2006; Tellez-Rojo et al., 2006). Tellez-Rojo et al. (2006) evaluated blood lead levels and neurodevelopmental outcome data at 12 and 24 months from 294 children participating in a prospective study conducted in Mexico City, Mexico (note: this is not the Mexico City study that was included in the international pooled analysis). Analyses were restricted to children whose blood lead levels were less than 10 μg/dl at both 12 and 24 months. Adjusting for covariates, blood lead level at 24 months was significantly associated, inversely, with both mental and motor development scores at 24 months, whereas blood lead level at 12 months was inversely associated with the motor development score at 24 months of age, but not with concurrent mental or motor development. The results were stable when adjustment was made for prenatal (cord) blood lead level. For both mental and motor development scores at 24 months, the coefficients that were associated with concurrent blood lead level were significantly larger among children with blood lead levels less than 10 μg/dl than among children with blood level levels greater than or equal to 10 μg/dl.

Numerous other studies have reported adverse neurodevelopmental outcomes in children at blood lead levels below 10 μg/dl. In cross-sectional analyses of 534 6- to 10-year-old children, Surkan et al. (2007) found, using children with blood lead levels of 1–2 μg/dl as the reference group and adjusting for age, race, socioeconomic status and caregiver IQ, that children with blood lead levels of 5–10 μg/dl had a 5-point deficit in IQ (*P* = 0.03), a 7.8-point deficit in reading, a 6.9-point deficit in mathematics as well as deficits in specific neuropsychological domains, such as spatial attention and executive functions.

In a cohort of 246 7.5-year-old African American children (mean blood lead level of 5.4 μg/dl, range 1–25 μg/dl), Chiodo, Jacobson & Jacobson (2004) found significant covariate-adjusted inverse associations between blood lead level and performance on a variety of neuropsychological tests. The domains for which significant associations were observed included intelligence, reaction time, visuomotor integration, fine motor skills and executive functions. Non-parametric regression analyses failed to identify non-linearities for 12 of 15 end-points. For the remaining three end-points, the deviation from linearity was a steeper slope at the lowest blood lead levels. On the basis of analyses exploring the significance of different cut-point values, the authors concluded that a threshold is not readily apparent, with most associations apparent at blood lead levels as low as 3–5 μg/dl.

Chandramouli et al. (2009) investigated the associations between blood lead level at 30 months of age and academic performance and behaviour of 488 7- to 8-year-old children participating in the Avon Longitudinal Study of Parents and Children. The mean blood lead level was 4.2 μg/dl, with 21% of children having a level of 5–10 μg/dl and 6% having a level exceeding 10 μg/dl. Adjusting for covariates, which included sex, child IQ, maternal education, home ownership, maternal smoking, home facilities, paternal socioeconomic status, Family Adversity Index and parenting attitudes, blood lead level was inversely related to reading, writing, spelling and mathematics scores (*P*-values of 0.004, 0.001, 004 and 0.053, respectively). The results of analyses in which blood lead level was categorized (2–5, 5–10, >10 μg/dl) indicated that the associations became significant when the blood lead level exceeded 5 μg/dl.

Solon et al. (2008) reported a cross-sectional study of 877 children, ages 6 months to 5 years, from the Philippines. This was a population-based stratified random sample of children, in whom the mean blood lead level was 7.1 μg/dl. In age-stratified analyses (0.5–3 years, 3–5 years) and adjusting for covariates, each 1 μg/dl increase in blood lead level was associated with a 3.3-point decline in neurodevelopmental score in the younger age group and a 2.5-point decline in the older age group. Nutritional factors, notably folate status and haemoglobin levels, appear to be effect modifiers, with deficiency states exacerbating the inverse associations between lead and neurodevelopment.

In a cohort of 261 8- to 11-year-old children from the Republic of Korea, with a mean blood lead level of 1.7 μg/dl (range 0.42–4.91 μg/dl), blood lead level was inversely associated with IQ score (coefficient −0.18, $P = 0.003$), adjusting for age, sex, maternal education, paternal education, income, maternal smoking during pregnancy, exposure to second-hand smoke after birth, birth weight, maternal age at birth and blood manganese level (Kim et al., 2009). In addition, an additive interaction was observed between blood lead and blood manganese levels, such that the inverse associations between blood lead level and IQ scores were more strongly inverse among children with blood manganese levels greater than the median value (14 μg/l) compared with children with blood manganese levels below the median value.

Miranda and colleagues have linked existing state-wide databases in North Carolina, USA, including blood lead surveillance data and children's scores on an

end-of-grade (Grade 4) reading test. In a study involving 8603 children, they reported that children with a higher blood lead level were at significantly increased risk of failing the reading test, with the association evident at blood lead levels as low as 2 μg/dl (Miranda et al., 2007). In a subsequent study of blood lead screening results at ages 9–36 months and end-of-grade (again Grade 4) reading score, data were available on 57 678 children from all 100 counties in North Carolina. Using children with a blood lead level of 1 μg/dl as the referent group and creating dummy variables for groups of children with each integer unit increase in blood lead level, Miranda et al. (2009) found significant inverse coefficients for all blood lead categories. For instance, for children with a blood lead level of 2 μg/dl, the linear regression coefficient was −0.30 (95% CI −0.58 to −0.01); for children with a blood lead level of 5 μg/dl, the coefficient was −0.80 (95% CI −1.08 to −0.51); and for children with a blood lead level greater than 10 μg/dl, the coefficient was −1.75 (95% CI −2.09 to −1.41). Quantile regression analyses revealed a significant difference in the association between blood lead level and reading score depending on location in the reading score distribution. Specifically, the difference between the reading scores of children with high versus low blood lead levels was greater among children who were performing in the lower tail of the reading distribution. In other words, higher blood lead levels had a disproportionately greater impact on children who, for reasons other than lead, were at risk of reading difficulties. This implies an effect modification, such that the impact of a given level of lead exposure can vary depending on a child's circumstances, specifically the presence or absence of other risk factors for the outcome of interest.

The initial reports of an apparently steeper slope of the relationship between blood lead level and IQ at blood lead levels below 10 μg/dl compared with above 10 μg/dl generated concern that this reflects only a statistical artefact. Bowers & Beck (2006) argued, for instance, that "the dose–response curve between an environmental measure that is lognormally distributed and any cognitive score that is normally distributed will by necessity have a non-linear slope" (p. 523). This contention was dismissed by several respondents to this paper (e.g. Hornung, Lanphear & Dietrich, 2006; Jusko et al., 2006; Bergdahl, 2006, 2007; Svensgaard et al., 2007), all of whom argued that it was based on inappropriate assumptions. Bowers & Beck (2007) responded to the technical issues raised, but, in an important respect, the issue is moot. In the analyses of the pooled international studies, piecewise linear models were fit to different ranges of the blood lead distribution (e.g. <7.5 μg/dl, <10 μg/dl), with the results showing that the linear slopes were significantly steeper in the lower than in the higher ranges and that the linear fits were adequate within these more restricted ranges. Moreover, it was found that for each of the studies included in the pooled analysis, a linear model provided the best fit across the blood lead range in the individual studies. As had been noted more than a decade before (Schwartz, 1994), the inverse slopes of the studies in which participants tended to have lower blood lead levels appeared to be greater than the slopes in studies involving children with higher blood lead levels. From this observation, it follows, then, that when the individual studies were combined in a pooled analysis, the functional form that would provide the best fit over the more extended blood lead range covered by the studies, in aggregate, is one that is non-linear. This was the result that was obtained when relative fits of different models were compared.

(viii) Attention deficit hyperactivity disorder (ADHD)

Older studies in children consistently identified dose-related increases in behavioural outcomes such as inattention, distractibility and hyperactivity (Needleman et al., 1979; Yule et al., 1984; Thomson et al., 1989; Bellinger et al., 1994). The exposures of the children who participated in these studies were considerably higher than the exposures of contemporary children, and the outcomes were based on teacher or parent reports instead of formal diagnostic evaluations. Recent studies have addressed at least some of these limitations.

In NHANES 1999–2002, parents were asked whether they had ever been told by a health professional that their 6- to 16-year-old child met criteria for ADHD and whether the child was taking a stimulant medication. Braun et al. (2006) found that the OR for children in the fifth quintile in terms of blood lead level (>2 µg/dl), compared with children in the first quintile (<0.8 µg/dl), was 4.1 (95% CI 1.2–14.0). The increase in risk was dose dependent, as the ORs associated with the intermediate three quintiles were 1.1 (95% CI 0.4–3.4), 2.1 (95% CI 0.7–6.8) and 2.7 (95% CI 0.9–8.4), respectively. In these analyses, adjustments were made for age, sex, prenatal and postnatal exposure to environmental tobacco smoke, preschool or child-care attendance, health insurance coverage and serum ferritin level.

The same group analysed data (n = 2588) from NHANES 2001–2004, in which the diagnosis of ADHD in 8- to 15-year-old children was based on the Diagnostic Interview Schedule for Children, a structured interview based on the fourth edition of the American Psychiatric Association’s *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) (Froehlich et al., 2009). Children with a blood lead level that placed them in the upper tertile had an adjusted OR for ADHD of 2.3 (95% CI 1.5–3.8). Children with both prenatal exposure to tobacco and a current blood lead level in the upper tertile were at particularly increased risk (adjusted OR 8.1, 95% CI 3.5–18.7).

In a study of 1778 school-age children in the Republic of Korea, for whom blood lead levels ranged from 0.1 to 10.1 µg/dl (geometric mean 1.8 µg/dl), parents completed the Connors’ scale for ADHD, a screening tool (Ha et al., 2009). Adjusting for age, sex, income, place of residence, parental history of neuropsychiatric disease (but not specifically ADHD) and blood mercury level, the risk of ADHD, defined as a score exceeding a cut-off derived for children from the Republic of Korea, increased linearly with increasing blood lead level. Compared with children with a blood lead level below 1 µg/dl, the OR associated with a blood lead level above 3.5 µg/dl was 1.96 (95% CI 0.76–5.11). The P for trend across blood lead categories was 0.07. A child’s blood lead level was significantly and positively correlated with the number of ADHD symptoms a parent endorsed (P < 0.001), although it appeared from a scatterplot that this association was largely attributable to the small number of children with a blood lead level greater than 5 µg/dl.

In a case–control study conducted among Chinese children aged 4–12 years, 630 children who met diagnostic criteria established by the DSM-IV (revised edition) were matched to 630 controls on age, sex and socioeconomic status (Wang et al., 2008). In a conditional logistic regression analysis in which children with a

blood lead level below 5 μg/dl was the referent group and adjusting for household composition, birth weight, family history of ADHD, pregnancy, labour and delivery complications, medical history, maternal and paternal age, maternal and paternal education, and use of alcohol and cigarettes during pregnancy, risk of ADHD was 5.19 ($P < 0.01$) among children with a blood lead level of 5–10 μg/dl and 7.15 ($P < 0.01$) among children with a blood lead level above 10 μg/dl.

Nigg et al. (2008) implemented a multistage screening and verification process to confirm a diagnosis of ADHD using DSM-IV criteria and to rule out co-morbidities in a sample of 150 8- to 17-year-old children (97 cases and 53 controls). Blood lead levels ranged from 0.40 to 3.47 μg/dl (mean 1.03 μg/dl). Blood lead level was significantly related to the ADHD symptom count for total symptoms and for hyperactivity-impulsivity counts ($P < 0.05$). Adjusting for income and sex, children with ADHD combined subtype had a significantly higher blood lead level than did controls ($P < 0.04$). This group did an additional study of 236 6- to 17-year-olds, 108 of whom met diagnostic criteria for ADHD (Nigg et al., 2010). With adjustment for confounders (e.g. IQ, parental smoking), blood lead level (mean 0.73 μg/dl, maximum 2.2 μg/dl) was associated with risk of ADHD combined subtype, but not the inattentive subtype.

(ix) Adult psychiatric status

Two studies followed up in adulthood children in the Childhood Health and Development Study (Oakland, California, USA) and the New England cohort of the National Collaborative Perinatal Project (Opler et al., 2004, 2008). Cases of schizophrenia spectrum disorder were identified in each cohort, and archived serum samples from pregnancy were analysed for ALA, which accumulates when ALAD is inhibited by lead. Based on the relationship between ALA and blood lead level, cases and controls were stratified into groups with a fetal blood lead level estimated to be greater than or equal to 15 μg/dl or less than 15 μg/dl. In analyses that pooled the data in the two cohorts and adjusted for maternal age at delivery and maternal education, the OR for schizophrenia associated with an estimated blood lead level of 15 μg/dl or higher was 1.92 (95% CI 1.05–3.87).

In NHANES 1999–2004, 1987 20- to 39-year-olds were administered a DSM-IV-based Composite International Diagnostic Interview. Individuals with a current blood lead level in the highest quintile (>2.11 μg/dl; 13 individuals had levels above 10 μg/dl), compared with those in the lowest quintile (<0.7 μg/dl), had 2.3 (95% CI 1.1–4.8) times the odds of meeting criteria for a major depressive disorder and 4.9 (95% CI 1.3–18.5) times the odds of meeting criteria for panic disorder, adjusting for sex, age, race/ethnicity, education and poverty to income ratio (Bouchard et al., 2009). Blood lead level was not significantly associated with generalized anxiety disorder.

(x) Violence and aggression

Lead has long been known to impair behaviour in myriad ways, with an important early case series report (Byers & Lord, 1943) noting that lead-poisoned children exhibited explosive tempers and poor impulse control. Denno (1993)

assembled retrospective evidence that childhood lead poisoning is a risk factor for juvenile crime. Studies exploring the association between lower levels of lead exposure and aggression began with Needleman et al. (1996), who found that children with higher bone lead levels were more likely to receive parent and teacher ratings categorizing their behaviours in the range of clinical concern. Needleman et al. (2002) followed this study up with a case–control study of 216 adjudicated delinquents in Pittsburgh, Pennsylvania, USA. Compared with controls, and adjusting for race, parent education, parent occupation, family size, presence of two biological parents and presence of two parental figures in the home, the OR associated with having an elevated bone lead level was 2.0 (95% CI 1.1–31.0) among the delinquent boys and 7.8 (95% CI 1.7–35.0) among the delinquent girls.

Several studies using ecological designs have reported significant associations between air lead concentrations and homicide rate (Stretesky & Lynch, 2001) and property and violent crime rates (Stretesky & Lynch, 2004) and between lead production and murder rate (Nevin, 2000, 2007). Using aggregated data from Australia, Canada, Finland, France, Germany, Great Britain, Italy, New Zealand and the USA, Nevin (2007) examined the association between preschool blood lead level and different types of crime. Relative fits were compared for models incorporating lags of various durations between measurement of blood lead level and outcome measurement. The lags identified in this way coincided well with the known peak offending ages for various offences (e.g. burglary versus homicide). The ability to adjust for potential confounders in studies of ecological design is limited, however, making inferences about causality tenuous.

Braun et al. (2008) used data for 2619 children 8–15 years of age who participated in NHANES 1999–2002 to evaluate the association between concurrent blood lead level and the diagnosis of conduct disorder. A total of 68 children met DSM-IV criteria, established by parental interview. Adjusting for age, maternal age, sex, race, prenatal tobacco exposure and serum cotinine level and using children in the lowest quartile of blood lead as the referent group (0.8–1.0 μg/dl), significant ORs were found for children in quartile 3 (1.1–1.4 μg/dl) (12.4, 95% CI 2.4–64.6) and quartile 4 (1.5–10 μg/dl) (8.6, 95% CI 1.9–40.0).

In a cross-sectional study of 173 14- to 18-year-olds from Brazil, surface dental enamel lead level was associated, adjusting for familial and sociodemographic confounders, with clinically significant elevation of children's rule-breaking behaviour (by parent report) on the Child Behavior Check List (OR 3.72, 95% CI 0.99–14.04) (Olympio et al., 2010). Enamel lead level was not significantly associated with children's self-report of delinquent behaviours, however.

Three prospective studies of environmental lead exposure in children and later criminal activities have been reported. In the Christchurch Health and Development Study, a birth cohort of 1265 children in New Zealand, dentine lead levels in deciduous teeth, measured at 6–9 years of age, were related, in a dose-dependent manner, to the number of violent/property convictions and self-reported violent/property offences between the ages of 14 and 21 (Fergusson, Boden & Horwood, 2008). The effect sizes for both outcomes were reduced by adjustment for sociodemographic variables and aspects of family functioning, but remained

significant ($P < 0.005$ for convictions and 0.047 for self-reported offences). Additional analyses suggested that educational underachievement (leaving school without qualifications, low grade point average) might mediate the association between increased early lead exposure and criminal behaviour.

Among 488 children in the Avon Longitudinal Study of Parents and Children cohort (Avon, England), a higher blood lead level at 30 months of age was significantly associated with greater antisocial behaviours at age 7–8, as reported by an adult (Chandramouli et al., 2009). Although the association was present when blood lead level was treated as a continuous variable, the increase in these behaviours was most apparent among children whose earlier blood lead levels had exceeded 10 μg/dl.

County records of arrests were collected for 250 19- to 24-year-olds enrolled in a prospective study in Cincinnati, Ohio, USA (Wright et al., 2008). Prenatal blood lead levels and childhood blood lead levels had been measured frequently, providing an unusually detailed blood lead history for the participants up to the age of 6.5 years. The median prenatal blood lead level (first or second trimester) was 7.8 μg/dl (range 2.9–16.0 μg/dl); the median early childhood average blood lead level was 12.3 μg/dl (range 6.0–26.3 μg/dl); and the median blood lead level at 6.5 years was 6.8 μg/dl (range 3.4–18.3 μg/dl). A previous analysis of this cohort had found that adolescents who had had higher blood lead levels in early childhood self-reported more delinquent acts. In analyses of total arrests after age 18, adjusting for maternal IQ, sex, socioeconomic status and maternal education, the rate ratios associated with each 5 μg/dl increase were significant for prenatal blood lead level (1.4, 95% CI 1.1–1.9) and 6-year blood lead level (1.3, 95% CI 1.0–1.6). In analyses of arrests for violent offences, the adjusted rate ratios were significant for average childhood blood lead level (1.3, 95% CI 1.0–1.6) and for 6-year blood lead level (1.5, 95% CI 1.1–1.9). Among the strengths of this study are the prospective collection of data on exposure and potential confounding variables, as well as reliance on administrative records rather than self-report as the basis for assessment of the outcomes (Dietrich et al., 2001).

The biological plausibility of the hypothesis that elevated lead exposure is causally associated with aggression is supported by studies in experimental models, such as cats (Li et al., 2003), primates (Moore et al., 2008) and hamsters (Cervantes et al., 2005).

(f) Sexual maturation

Several cross-sectional studies have reported that higher blood lead levels in children are associated with delayed sexual maturation. Selevan et al. (2003) evaluated the relationship between blood lead level and age at menarche and Tanner stage for pubic hair and breast development in 2186 girls 8–18 years old who participated in NHANES II. Both breast and pubic hair development as well as age at menarche were significantly delayed in African American and Mexican American girls with a blood lead level greater than 3 μg/dl, compared with girls with a blood lead level of 1 μg/dl. Each 1 μg/dl increase in blood lead level was associated with a delay of 2.1–6.0 months in progressing from one Tanner stage to the next in

breast and pubic hair development. The delay in age at menarche among girls with a blood lead level greater than 3 μg/dl was 3.6 months. These indices of maturation were also delayed in white females, but not significantly.

In another set of analyses using NHANES III data, Wu, Buck & Mendola (2003) studied girls 10–16 years of age. Data on age at menarche were available for 1235 girls, and physician-determined data on Tanner stage 2 pubic hair and breast development were available for 1706 girls. Blood lead level was categorized as 0.7–2.0 μg/dl, 2.1–4.9 μg/dl and 5.0–21.7 μg/dl. Blood lead level was inversely related to both pubic hair development and age at menarche, but not breast development, adjusting for race/ethnicity, age, family size, residence in a metropolitan area, poverty-to-income ratio and body mass index. In the three blood lead groups, for example, 60.0%, 51.2% and 44.4% of 10-year-olds, respectively, had reached Tanner stage 2 for pubic hair, and 68.0%, 44.3% and 38.5% of 12-year-olds, respectively, had reached menarche.

In a study of 138 10- to 17-year-old girls from the Akwesasne Mohawk Nation in the USA, Denham et al. (2005) found that, among those with a blood lead level above the median value of 1.2 μg/dl, menarche was reached 10.5 months later than it was among girls with a blood lead level below the median, adjusting for age, socioeconomic status and other pollutants (dichlorodiphenyldichloroethylene, hexachlorobenzene, mirex, mercury).

One cohort study has investigated sexual maturation in boys in relation to lead exposure. Hauser et al. (2008) studied 489 8- to 9-year-old boys in Chapaevsk, Russian Federation. The end-points assessed were physician-assessed testicular volume and genitalia stage. The median blood lead level was 3 μg/dl (interquartile range 2–5 μg/dl). In cross-sectional analyses, adjusting for birth weight, gestational age and age at examination, boys with a blood lead level of 5 μg/dl or greater had reduced odds of having reached genitalia stage 2 (OR 0.6, 95% CI 0.3–0.95, $P = 0.03$). This cohort was followed up ($n = 481$) several years later (Williams et al., 2010), when more boys had entered puberty, with similar findings. After adjusting for baseline body mass index and height, boys with a baseline blood lead level greater than or equal to 5 μg/dl had a reduced risk of pubertal onset based on testicular volume (HR 0.73, 95% CI 0.55–0.97), genitalia staging (HR 0.76, 95% CI 0.59–0.98) and pubic hair staging (HR 0.69, 95% CI 0.44–1.07). The effect sizes corresponded to delays of 6–8 months in the onset of puberty.

(g) Dental health

Lead has been reported to be a risk factor for dental caries among children with blood lead levels less than 10 μg/dl, but the evidence is mixed and lacks consistency across studies in terms of the patterns of associations. Among 24 901 participants ages 2 years and older in NHANES III, blood lead level was evaluated in relation to the number of decayed, filled and missing surfaces (Moss, Lanphear & Auinger, 1999). In all age strata, higher blood lead level was significantly associated with the number of affected surfaces in both deciduous and permanent teeth, adjusting for age, race, poverty-to-income ratio, cigarette exposure, sex, region, parent education, carbohydrate intake, dietary calcium intake and dental

care. For instance, using dental caries as the outcome, among 5- to 17-year-old children, the OR associated with a 5 μg/dl increase in blood lead level was 1.8 (95% CI 1.3–2.5). Among children with blood lead levels in the upper tertile of the distribution (greater than approximately 3 μg/dl), the OR was 1.66 (95% CI 1.1–2.5).

The association between blood lead level and caries was examined in secondary analyses of 543 6- to 10-year-old children participating in the New England Children's Amalgam Trial (Gemmel et al., 2002). The mean blood lead level was 2.3 (SD 1.7) μg/dl. No association between blood lead level and the number of carious surfaces was observed among children from a rural area. An association was observed, however, in the half of the cohort recruited from an urban area (P = 0.005), adjusting for age, sex, family income, ethnicity, maternal education, maternal smoking, dental hygiene habits (frequency of brushing, firmness of brush) and gum chewing. This association was somewhat stronger in deciduous than in permanent teeth. The ranges of both blood lead levels and the numbers of carious tooth surfaces were greater in the urban than in the rural subgroup, which might have made it easier to detect an association. Alternatively, the possibility of residual confounding or the influence of effect modifying factors whose distributions differed across regions cannot be dismissed.

In another set of secondary analyses of 507 8- to 12-year-old children from Lisbon, Portugal, participating in a study of dental amalgam, Martin et al. (2007) reported that blood lead level (mean 4.6 [SD 2.4] μg/dl) was significantly associated with number of carious surfaces, but only among males, and only in primary teeth (adjusting for age, race, IQ and scores on tests of attention, memory and visuomotor function). In contrast, in a study of 292 6- to 11-year-old children in Thailand whose mean blood lead level was 7.2 (SD 1.5) μg/dl, a significant adjusted OR was observed for the number of decayed/filled surfaces (2.4, 95% CI 1.4–4.2), but only in deciduous teeth, not primary teeth (Youravong et al., 2006). Finally, in a retrospective cohort study evaluating blood lead level and the number of decayed, filled and missing surfaces in second and fifth graders, Campbell, Moss & Raubertas (2000) found that children with a mean blood lead level greater than 10 μg/dl in the interval of 18–37 months of age were not at increased risk.

In adults, greater lead exposure has been associated with risk of tooth loss (Arora et al., 2009). In the United States Veterans Administration Normative Aging Study cohort, men in the highest tertile of tibia lead level had an OR of 3.0 (95% CI 1.6–5.8) for having more than nine missing teeth, whereas men in the highest tertile of patella lead level had an OR of 2.4 (95% CI 1.3–4.5) (Arora et al., 2009). Among 4899 men and women 20–56 years of age included in NHANES III (1988–1994), the adjusted prevalence of periodontitis (presence of more than 20% of mesial sites with greater than or equal to 4 mm of attachment loss) was significantly greater among men and women with a blood lead level greater than 7 μg/dl (men: prevalence ratio 1.7, 95% CI 1.0–2.9; women: prevalence ratio 3.8, 95% CI 1.7–8.7) than among men and women with a blood lead level less than 3 μg/dl (Saraiva et al., 2007). Similar findings were reported in smaller studies (Yetkin-Ay et al., 2007; El-Said et al., 2008).

3. ANALYTICAL METHODS

3.1 Determination of lead in food

The analytical methods for the determination of lead are well established. The most common detection techniques are flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), hydride generation atomic fluorescence spectrometry (HG-AFS), inductively coupled plasma optical emission spectrometry (ICP-OES) and, more recently, inductively coupled plasma mass spectrometry (ICP-MS).

The method for the determination of lead by FAAS has been widely used in the past. The method is selective and practically free from interferences. Some common interferences caused by aluminium and iron can be overcome by the addition of ascorbic acid, citric acid or EDTA. The threshold of sensitivity of this technique is around 10 ng/ml. For trace lead quantification in food matrices, preconcentration steps before quantification are usually required. The sensitivity of FAAS is limited by several factors, such as low atomization efficiency and inefficiency of the nebulization process. To overcome this limitation and improve the process of sample introduction, methods such as thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) were developed (Da-Col, Domene & Pereira-Filho, 2009). By using this procedure, total sample introduction, long residence time and good sensitivity can be achieved (Pereira-Filho, Berndt & Arruda, 2002).

ETAAS is a good alternative to FAAS and is widely employed for the determination of the trace elements in biological samples. Preconcentration and separation steps are often employed with this technique before quantification of lead in food. However, several constraints have limited its performance, because the response is often perturbed by multiple physical or chemical reactions in the atomizer, and the limits of detection (LODs) are not always adequate for trace analysis (Sardans, Montes & Peñuelas, 2010). In general, the technique requires the use of a modifier to stabilize lead, allowing its quantification without matrix effect. Various modifiers are used for the determination of lead, among them palladium(II) nitrate/palladium (Daftsis & Zachariadis, 2007), palladium(II) chloride/palladium plus ascorbic acid (Licata et al., 2004), palladium(II) nitrate/palladium plus magnesium nitrate (Tüzen & Soylak, 2005) and ammonium phosphate, Triton X-100 plus monoammonium dihydrogen phosphate (Viñas, Pardo-Martínez & Hernández-Córdoba, 2000). Many improvements have enhanced the use of ETAAS for the determination of traces of lead in biological samples, including advances in atomizer designs, background correction systems, the development of in situ trapping methods, appropriate modifiers and improvements in the light source and detector. Significant enhancement of the technique has been achieved using transversally heated atomizers with platforms, which allow the reduction of LODs. In recent years, the high-resolution continuum source electrothermal atomic absorption spectrometer (HR-CS-ETAAS) has allowed the direct analysis of lead in solid materials with low LODs.

ICP-OES has been widely employed to determine lead in various types of samples. Owing to several spectral interferences, this technique is more useful for

measuring high levels of lead contamination. When lower levels of lead are present, preconcentration steps are necessary. This technique also suffers from interferences and was replaced in the last decade by the use of ICP-MS.

The use of ICP-MS has become more common in food laboratory analysis because of its capability for multielement measurements coupled to low LODs (in the order of nanograms per gram). Additionally, compared with ICP-OES, the technique provides simpler spectral interpretation and isotopic information (Nardi et al., 2009). However, polyatomic interferences resulting from the combination of matrix ions with argon may interfere in this technique; to ensure correct results, some of the interferences must be eliminated or controlled by microwave digestion at high temperatures, whereas others have to be controlled using a mathematical approach (Rocha et al., 2009). The recent use of the dynamic reaction cell technology combined with ICP-MS (DRC-ICP-MS) has allowed the removal of the interferences with a minimum loss of sensitivity. This technology may be considered as a valid alternative to the above-mentioned spectrometric techniques, because it offers various possibilities for the element's determination in different matrices (D'Llio et al., 2008).

Alternative methods, such as stripping voltammetry, have been sporadically employed for the determination of lead in food at the nanogram per gram level (Melucci, Torsi & Locatelli, 2007; Jannat, et al., 2009).

The analytical performances of some techniques for the determination of lead in food are presented in Table 2.

3.1.1 Quality assurance

Certified reference materials (CRMs) have been widely employed in new methods reported in the literature for the determination of lead in food to demonstrate analytical quality assurance.

Julshamn et al. (2007) reported on an interlaboratory method performance (collaborative) study for the determination of lead by ICP-MS after pressure digestion including microwave heating. Thirteen laboratories participated, and the method was tested on a total of seven foodstuffs: carrot purée, fish muscle, mushroom, graham flour, simulated diet, scampi and mussel powder. The elemental concentration of lead (dry matter) ranged from 0.01 to 2.4 mg/kg. The repeatability relative standard deviation and reproducibility relative standard deviation for lead ranged from 3% to 27% and from 8% to 50%, respectively. The study showed that the ICP-MS method is satisfactory as a standard method for the determination of elemental lead in foodstuffs.

A proficiency testing programme for determining lead in seawater shrimp for 97 laboratories worldwide under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation is discussed by Kong, Chan & Wong (2008). Pooled data for lead were normally distributed, with interlaboratory variations of about 35%.

Table 2. Analytical methods for the determination of lead in food

Commodity	*n*	Country (year)	Sample preparation (EF)	Technique	LOD	*n* <LOD	Mean concentration (range)	Reference
Seaweed: *Porphyra*, *Laminaria*	4	France, Japan, Republic of Korea, Spain (2004)	Microwave-assisted digestion (HNO_3 + H_2O_2)	ICP-MS	1.74 ng/g	0	(312–848 ng/g)	Rocha et al. (2009)
Rice, bean, egg, meat, fish, bread, sugar, vegetables, cheese, milk powder, butter, wheat, pear, Brazil nut, coffee, chocolate, biscuit, pasta	18	Brazil	Microwave-assisted digestion (HNO_3 + H_2O_2)	ICP-MS	4 ng/g	2	(ND–104.4 ng/g)	Nardi et al. (2009)
Milk and infant formula	8	Italy	Microwave-assisted digestion (HNO_3 + H_2O_2)	DRC-ICP-MS	0.5 ng/g	0	(3.1–19.2 ng/g)	D'Llio et al. (2008)
Semolina	3	—	Microwave-assisted digestion (HNO_3 + H_2O_2)	ICP-MS	16 ng/l	0	(1.9–12.8 ng/g)	Cubadda & Raggi (2005)
Milk	42	Brazil (2004)	Acid digestion (HNO_3 + HCl)	ETAAS	0.41 ng/ml (LOQ)	4	230 ng/ml (62–476 ng/ml)	Soares et al. (2010)

Table 2 (contd)

Commodity	*n*	Country (year)	Sample preparation (EF)	Technique	LOD	*n* <LOD	Mean concentration (range)	Reference
Non-fat milk powder (CRM), water (CRM)	2	—	Ionic liquid-based single drop microextraction (76)	ETAAS	0.017 ng/ml	—	—	Manzoori, Amjadi & Abdulhassani (2009)
Tomato, pepper, onion	9	Mediterranean countries	Microwave-assisted digestion (HNO_3 + H_2O_2)	ETAAS	0.81 ng/g	0	(12.2–70.6 ng/g)	Bakkali et al. (2009)
Anchovy, spinach, cabbage, onion, dill, parsley, lettuce, tea, rice, salami, chicken	23	Turkey	Coprecipitation with MBT	ETAAS	1.38 ng/g	0	(0.58–73.8 ng/g)	Oymak et al. (2009)
Vegetables (100 varieties)	416	China	Acid digestion (HNO_3 + $HClO_4$ + H_2SO_4)	ETAAS	1 ng/g	—	46 ng/g (<1–655 ng/g)	Song et al. (2009)
Milk	97	Islamic Republic of Iran (2004)	Protein precipitation + acid digestion (HNO_3)	ETAAS	—	—	7.9 ng/ml (1–46 ng/ml)	Tajkarimia et al. (2008)
Konjac flour	7	China	Enzymatic hydrolysis and slurry preparation	ETAAS	27.8 ng/g	0	(96.76–859.91 ng/g)	Chen et al. (2008)
Milk	54	Turkey (2003–2004)	Filtration + centrifugation + microwave-assisted digestion (HNO_3 + H_2O_2)	ETAAS	0.62 ng/ml	0	31.4 ng/g (2.5–313 ng/g)	Sarica & Turker (2007)

Table 2 (contd)

Commodity	*n*	Country (year)	Sample preparation (EF)	Technique	LOD	*n* <LOD	Mean concentration (range)	Reference
Raisins	46	Turkey (2005)	Acid digestion ($HNO_3 + HClO_4 + H_2SO_4$)	ETAAS	6.2 ng/g	—	0.056 µg/g (0.012–0.359 µg/g)	Calisir & Akamn (2007)
Cabbage, wheat, potato, egg, baby food formula, baby food, instant milk, gilthead bream, anchovy, golden grey mullet, trout	14	Slovenia	Microwave-assisted digestion (HNO_3 + HF)	ETAAS	0.2 mg/kg	13	<LOD	Milacic & Kralj (2003)
Mussels							0.85 mg/kg	
Spinach, palmito, crab, shrimps, mussel, sardine, squid	7	Brazil	Cryogenic grinding + slurry preparation	ETAAS	75 ng/g	2	(228–574 ng/g)	Santos et al. (2002)
Infant formula powders	152 (38 brands)	—	Dry ashing	ETAAS	6.4 ng/g	0	(25.7–45.5 ng/g)	Moreno-Rojas et al. (2002)
Wheat flour, corn flour	3	Turkey	Wet digestion ($HNO_3 + H_2O_2$ and membrane filtration of the PAN–Pb complex) (20)	FAAS	3.5 µg/l (blank sample)	0	(4.1–54.7 µg/g)	Soylak et al. (2010)

Table 2 (contd)

Commodity	*n*	Country (year)	Sample preparation (EF)	Technique	LOD	*n* <LOD	Mean concentration (range)	Reference
Tomato, apple, mustard	2	Islamic Republic of Iran	Wet digestion (HNO_3 + H_2O_2; $HClO_4$) and SPE on sodium dodecyl sulfate–coated alumina (63)	FAAS	1.6–2.8 µg/l (blank sample)	0	(0.054–11.6 ng/mg)	Ghaedi et al. (2009)
Coffee, fish, black tea, green tea	4	—	Coprecipitation with zirconium(IV) hydroxide (25)	FAAS	2.5 ng/ml	4	—	Citak, Tuzen & Soylak (2009)
Black tea, black pepper, plant, cocoa powder	4	India	Wet digestion (HNO_3 + $HClO_4$) SPE-C18 membrane disc impregnated with Cyanex 302 (400)	FAAS	1 ng/ml	0	(12.85–49.3 ng/mg)	Karve & Rajgor (2007)
Food supplement	—	Brazil	Microwave-assisted digestion (HNO_3 + H_2O_2)	TS-FF-AAS	6 ng/ml	—	—	Da-Col, Domene & Pereira-Filho (2009)
Seafood (CRM)	—	—	Solid sampling	SS-ZAAS	0.008 ng	—	—	Detcheva & Grobecker (2006)
Guaraná, cabbage	2	Brazil	Acid digestion + SPE (minicolumn of Amberlite XAD-4 modified with DHB) (53)	ICP-OES	0.54 ng/ml	0	(2.7–3.3 ng/mg)	Bezerra et al. (2007)

Table 2 (contd)

Commodity	*n*	Country (year)	Sample preparation (EF)	Technique	LOD	*n* <LOD	Mean concentration (range)	Reference
Whole meal, maize meal, cereal plant meals, cereal plants	5	—	Wet digestion (HCl + HNO_3 + H_2SO_4)	SWASV	0.15–0.103 µg/g	0	(0.349–3.71 µg/g)	Melucci, Torsi & Locatelli (2007)
Infant formula	1	Islamic Republic of Iran	Acid digestion (HNO_3) + dry ashing	DPASV	5 ng/g	0	0.384 mg/kg	Jannat et al. (2009)

CRM, certified reference material; DHB, dihydroxybenzoic acid; DPASV, differential pulse anodic stripping voltammetry; EF, enrichment factor; *n*, number of samples analysed; MBT, 2-mercaptobenzothiazole; ND, not detected; PAN, 1-(2-pyridylazo)-2-naphthol; SPE, solid-phase extraction; SS-ZAAS, solid sampling Zeeman atomic spectrometry; SWASV, square wave anodic stripping voltammetry

3.1.2 Sample preparation

Food samples in general require mineralization and dissolution prior to lead quantification. Sample preparation for food and biological matrices depends on the quantification method and usually begins with matrix pretreatment (acid digestion, slurry, leaching or ashing). Acid digestion with strong acids and oxidants (nitric acid and hydrogen peroxide) is the most employed sample treatment in analysis of food for the determination of lead. Microwave-assisted acid digestion has been extensively employed for this purpose, as it allows the use of large sample masses (1–2 g) under controlled temperature and pressure of the system, which reduces contamination and limits losses of the element during mineralization.

Slurry sampling techniques are also employed, to a lesser extent, but may offer some advantages over microwave-assisted acid digestion, such as time, safety and economy. This technique requires the optimization of particle size, slurry concentration and homogeneity.

Ashing and leaching have been used in only a few studies. Calcination at temperatures above 400 °C may induce losses of lead. In general, ashing methods provide lower analyte recovery when compared with acid digestion methods.

Owing to the very low lead levels in food matrices, insufficient detectability of some techniques and matrix effects, the direct determination of trace amounts of lead is not always reliable, and preliminary preconcentration steps are often required. For this purpose, techniques such as coprecipitation, liquid–liquid extraction, solid-phase extraction, cloud point extraction and on-line coprecipitation using a knotted reactor have been widely employed for lead determination in biological matrices. More recently, in order to reduce amounts of toxic organic solvents, liquid-phase microextraction, single drop microextraction, room temperature ionic liquids and membrane filtration have been proposed (Manzoori, Amjadi & Abdulhassani, 2009; Soylak et al., 2010).

Coprecipitation is one of the most efficient separation techniques for trace heavy metal ions, based on the separation of the collector from the matrix solution. For this purpose, inorganic (aluminium, cerium(IV), erbium, iron(III), magnesium, gallium, samarium and zirconium hydroxides and manganese dioxide) and organic coprecipitants (bismuth diethyldithiocarbamate, ammonium pyrrolidine dithiocarbamate, cobalt tris(pyrrolidine dithioate), sodium diethyldithiocarbamate) have been used as efficient collectors of trace elements (Korn et al., 2006). Coprecipitation has mainly been applied to water analyses.

Separation and concentration procedures using liquid–liquid extraction usually result in high enrichment factors and have been carried out in batch mode and in flow injection or sequential injection systems. The last two have the advantage that all manipulations are carried out automatically, minimizing the risk of sample contamination. To allow the extraction of the lead from an aqueous solution, lead needs to be complexed in a previous step. For this purpose, dithizone and ammonium pyrrolidine dithiocarbamate have been widely employed.

Solid-phase extraction enables the selective removal of trace amounts of metal ions from solutions containing complex matrices with minimal usage of

organic solvents. Several procedures for lead have been reported using various solid supports, such as activated carbon, silica gel, cellulose, Amberlite XAD series resins, Chromosorb resin, Ambersorb resin, polyurethane foam and sodium dodecyl sulfate–coated alumina (Karve & Rajgor, 2007; Ghaedi et al., 2009). Many reagents have been used to load these supports and to retain lead by complexation, among them 2-(2′-thiazolylazo)-*p*-cresol, 2-propylpiperidine-1-carbodithioate, 2-(2-benzothiazolylazo)-2-*p*-cresol, pyrogallol red, 1-(2-pyridylazo)-2-naphthol, dithizone and 2-(5-bromo-2-pyridilazol)-5-diethyl-aminophenol (Korn et al., 2006).

The cloud point extraction procedure offers advantages over conventional liquid–liquid extraction, such as high extraction and preconcentration factors, operational safety due to low surfactant flammability and lower toxicity for the analyst and the environment. The cloud point phenomenon occurs when a non-ionic or amphoteric surfactant above its critical micellar concentration causes the separation of the original solution into two phases when heated at a characteristic temperature called the cloud point temperature. Triton X-114 and PONPE 7.5 have been used as surfactants (Korn et al., 2006).

3.2 Determination of lead in blood

The determination of lead in biological materials, such as blood, urine and tissues, poses several problems, mainly due to the low concentration and the complexity of the sample matrix. In general, the techniques employed for the determination of lead in blood are the same as those described previously for the analysis of lead in food.

Electrothermal absorption spectrometry has been widely employed for blood and clinical analyses. Ashing and atomizing the sample in the presence of a chemical modifier and Zeeman effect background correction are essential for precise direct determination of trace elements in blood fractions. A simultaneous atomic absorption spectrometric (SIMAAS) method for the determination of lead and cadmium was proposed by Kummrow et al. (2008). The method requires a sample volume of 200 μl and presents an LOD of 0.65 ng/ml for lead.

More recently, ICP-MS has proven to be an good alternative method to other analytical techniques that have frequently been applied for this purpose, such as ETAAS, due to its low LODs, wide dynamic range, capability for rapid multielement determination and simple sample pretreatment. Whole blood samples can be analysed directly after simple dilution or decomposition of the organic matrix by ICP-MS. LODs in the picograms per millilitre range can be achieved (Heitland & Köster, 2006).

However, the direct analysis of whole blood after dilution can cause clogging of the sample introduction devices and signal instability in the ICP-MS. Several digestion procedures have been reported, including high-pressure ashing in quartz ampoules and microwave-assisted acid digestion in open or closed vessels. The latter is more frequently used for efficient mineralization and reduced contamination risk.

The volume of the sample for analysis is an important aspect that needs to be considered. It is difficult to acquire blood samples from children through invasive paediatric venipuncture. Furthermore, blood samples must be conserved properly immediately after sampling and stored at low temperatures. To overcome these limitations, the preparation of dried blood spots on filter paper has been proposed as an alternative. H.-F. Hsieh et al. (2009) proposed a method for lead determination in whole blood by laser ablation coupled with ICP-MS, requiring a sample volume of 0.5 μl and with a reported LOD of 0.1–2 ng/ml.

The analytical performance of some methods for the determination of lead in blood is shown in Table 3.

3.2.1 Quality assurance

CRMs have been extensively employed in new methods reported in the literature for the determination of lead in blood to demonstrate analytical quality assurance.

The network of external quality assessment scheme organizers in the field of occupational and environmental laboratory medicine sets standards of performance for laboratories. It aims to develop procedures that permit equivalence of assessment among schemes, so that performance of laboratories taking part in different external quality assessment schemes can be directly compared. The use of a *z*-score clearly demonstrates when a laboratory achieves results that are fit for purpose and allows for comparison of performance among schemes. The network organized a study in 2005, with 420 participants, for the determination of lead in blood. The analytical techniques employed were ETAAS, ICP-MS and others, representing 85%, 13% and 2%, respectively. For a target concentration of 2 μmol/l (416 ng/ml) blood lead, a typical between-laboratory precision (coefficient of variation) in the range of 10–16% was established. The quality specification that was proposed based on the desirable total allowable error for lead in blood was ±40 μg/l or ±10%, whichever is the greater value (Taylor et al., 2006).

4. SAMPLING PROTOCOLS

In recent years, considerable attention has been directed towards improving and ensuring the quality of analytical data on contaminants in foods. Whether the data are used for assessing risk from exposure (food surveillance), for food control (regulatory monitoring) or for monitoring standards for trading purposes, it is critical that contaminants be identified correctly and that quantitative data be reliable. Sampling should be based on the use of appropriate sampling techniques, sampling plans and testing methodology. Data collected should be sufficient for the statistical analyses required. Information on appropriate sampling procedures should be supplied.

Sampling plans to be implemented either by food control authorities or by commercial entities (self-inspection performed by producers and/or traders) are described in the general guidelines for sampling for food, provided in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (FAO/WHO, 2004a).

Table 3. Analytical methods for the determination of lead in blood

Country (year)	*n*	Sample amount	Sample preparation (EF)	Technique	LOD (ng/ml)	Mean concentration (range)	Reference
Brazil (2007)	92 (mothers)	100 µl	Acid digestion (HNO_3)	ICP-MS	0.2	2.7 ng/ml (median) (1.0–5.5 ng/ml)	Koyashiki et al. (2010)
—	CRM	0.5 µl	Sample is loaded onto the surface of a hydrophobic filter membrane	LA-ICP-MS	0.1–2	—	H.-F. Hsieh et al. (2009)
South Africa (1995)	Schoolchildren (8–10 years)	1 ml	Wet digestion ($HNO_3 + H_2O_2$)	ICP-MS	0.72	56.4 ng/ml	Bazzi, Nriagu & Linder (2008)
Belgium (2003)	1679 adolescents	500 µl	Wet digestion ($HNO_3 + H_2O_2$)	HR-ICP-MS	2	21.7 ng/ml[a] (9.9–45.4 ng/ml) ($P < 0.0001$)	Schroijen et al. (2008)
Germany (2005)	130	500 µl	Dilution (Triton X-100 + NH_4OH)	DRC-ICP-MS	0.008	22 ng/ml (5–83 ng/ml)	Heitland & Köster (2006)
USA	934 (African American communities)	16 µl	Dilution with HNO_3	ICP-MS	15	27 ng/ml (<15–200 ng/ml)	Nriagu et al. (2006)
—	—	—	Microwave-assisted acid digestion	SF-ICP-MS	0.32	—	Bocca et al. (2005)
Sweden	31	1 ml	Microwave digestion	ICP-SMS	0.085	17 ng/ml (4–43 ng/ml)	Rodushkin, Ödman & Branth (1999)

Table 3 (contd)

Country (year)	*n*	Sample amount	Sample preparation (EF)	Technique	LOD (ng/ml)	Mean concentration (range)	Reference
—	—	—	Microwave digestion (2.71)	ETAAS	0.83	—	Olmedo et al. (2010)
Sweden	5	30 µl	Drop-to-drop microextraction assisted with ultrasonication	ETAAS	0.08	35.6 ng/ml (15.5–50.5 ng/ml)	Shrivas & Patel (2010)
Brazil (2007)	40	200 µl	Protein precipitation and dilution	ETAAS	0.65	25.1 ng/ml (9.3–56.7 ng/ml)	Kummrow et al. (2008)
—	CRM	50–100 µl	Direct sampling of the punched blood filter paper	ETAAS	2.5	—	Resano et al. (2007)
—	—	5 ml	Wet digestion (HNO_3)	ETAAS	16–19 ng/g pellet 45–152 ng/g supernatant	—	Daftsis & Zachariadis (2007)
—	CRM	2 ml	Microwave-assisted digestion	ETAAS (colloidal Pd modifier)	1.2	—	Viitak & Volynsky (2006)
Germany (2000)	238 (children) 213 (mothers)	—	—	ETAAS	1.3	Children 31.5 ng/ml (6–86 ng/ml) Mothers 26.6 ng/ml (2–91 ng/ml)	Wilhelm et al. (2005)
Egypt	93 (28–40 years)	1 ml	Oxidation with $KMnO_4$	ETAAS	0.95	124 ng/ml (65–175 ng/ml)	Mortada et al. (2002)

Table 3 (contd)

Country (year)	*n*	Sample amount	Sample preparation (EF)	Technique	LOD (ng/ml)	Mean concentration (range)	Reference
Germany (2002–2003)	430 (children, aged about 10 years)	—	—	FAAS	12.5 (LOQ)	22.2 ng/ml (42 samples <LOQ)	Link et al. (2007)
Italy (2004)	110	—	Wet digestion (HNO_3)	ICP-OES	0.32	39.5 ng/ml	Alimonti et al. (2005)
—	CRM	50 µl	Wet digestion and then online resin column preconcentration	HG-AFS	0.004	—	Wan, Xu & Wang (2006)
China	5	3 ml	Wet digestion (HNO_3)	CL	5	21.1 ng/ml (5.3–44.3 ng/ml)	Qu et al. (2008)

CL, chemiluminescence; CRM, certified reference material; EF, enrichment factor; HR-ICP-MS, high-resolution inductively coupled plasma mass spectrometry; ICP-SMS, double focusing sector field inductively coupled plasma mass spectrometry; LA-ICP-MS, laser ablation coupled with inductively coupled plasma mass spectrometry; LOQ, limit of quantification; *n*, number of samples analysed; SF-ICP-MS, sector field inductively coupled plasma mass spectrometry

[a] Geometric mean.

5. PREVENTION AND CONTROL

Lead contamination of food arises mainly from the environment (air and soil) and from food processing (lead paint and lead-containing equipment), food handling and food packaging (lead-soldered cans, coloured plastic bags and wrapping papers, lead-glazed ceramic, lead-containing metal vessels). Atmospheric lead from industrial pollution or leaded gasoline can contaminate food through deposition on agricultural crop plants. Water is also a source of lead contamination of food.

There have been worldwide efforts to reduce lead exposure from food, focusing on implementing standards for allowable lead levels in food and food additives; ending the use of lead-soldered cans; controlling lead levels in water; reducing leaching from lead-containing vessels or restricting their use for decorative purposes; and identifying and reacting to additional sources of lead contamination in foods or dietary supplements. Although not targeted specifically at food, efforts to reduce environmental sources of lead, including restrictions on industrial emissions and restricted use of leaded gasoline, have also contributed to declining lead levels in food (FAO/WHO, 2004b).

For the prevention and control of lead in food, good agricultural and manufacturing practices should be followed. The main source of lead in vegetables arises from atmospheric lead deposition. During processing, maximum removal of surface lead from plants should be practised, for example, by thoroughly washing vegetables, particularly leafy vegetables; removing the outer leaves of leafy vegetables; and peeling root vegetables, where appropriate. The transfer of lead from soil to crop tissues is generally low; the bioconcentration factor is in the range of 0.001–0.5, depending on plant species, environmental conditions and experimental setup (Chamberlain, 1983).

Food processors should ensure that the water supply for food processing complies with maximum limits for lead established by national or local authorities. Food processors should choose foods and food ingredients, including ingredients used for dietary supplements, that have the lowest lead levels possible.

6. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

The Committee, at its present meeting, reviewed data on lead occurrence in different food commodities received from seven countries—namely, Australia, Brazil, China, France, Germany, Singapore and the USA—and from the European Food Safety Authority (EFSA), covering data from Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Norway, Poland, Romania, Spain, Sweden and three commercial operators (EFSA, 2010). The Committee conducted a literature search and identified published literature for five additional countries: Egypt, India, Lebanon, New Zealand and Nigeria.

The data submitted by France and Germany were included in the assessment report of EFSA (2010). In order to avoid duplicating the data in this analysis, the individual data submitted from both countries were not separately considered in the assessment of the current meeting.

The total number of analytical results (single or composite samples) was 110 899, with 84.9% coming from Europe, 7.6% from the USA, 1.9% from Latin

America, 3.1% from Asia and 2.5% from the Pacific region. No data were received from Africa.

The Committee noted that the occurrence data reported by the countries were, in general, obtained using validated analytical methods.

The data submitted for each country are summarized below. The occurrence data obtained by the Committee from the published literature are summarized in section 7.

6.1 Australia

Food Standards Australia New Zealand (FSANZ) submitted lead data collected in the 23rd Australian Total Diet Study (TDS) conducted in 2008–2009. The data submitted were not in the Global Environment Monitoring System – Food Contaminant Monitoring and Assessment Programme (GEMS/Food) format. The analytical method used for the determination of lead was ICP-MS, and the LOD and limit of quantification (LOQ) were reported as the same value, varying in the range of 0.0001–0.005 mg/kg, according to the food commodity. Lead concentrations in different food commodities (570 individual samples) were reported (Table 4). The highest lead value of 0.248 mg/kg was determined in a food in the baked goods category (a “fancy bread”). Sixteen per cent of the samples analysed presented a lead concentration below the LOD of the method.

Data from the 20th and 19th Australian TDSs were also submitted (FSANZ, 2001, 2003). For these data, the analytical methods used were not reported. The weighted mean lead levels for the food commodities are presented in Tables 5 and 6 for the 20th and 19th Australian TDSs, respectively.

6.2 Brazil

Brazil submitted data on lead levels in three meat categories: meat muscles (beef and pork), poultry muscles (chicken and turkey) and kidneys (beef, chicken and turkey). The data were provided from industry, and the samples were collected during the 2002–2009 period. The results were aggregated and reported in 38 groups. No information was provided about the analytical method used for the quantification of lead. The LOD and LOQ were reported as 0.005 mg/kg and 0.010 mg/kg, respectively. In total, 2163 samples were analysed (616 pork and beef muscles; 1368 poultry muscles; 179 kidneys); the lead concentrations were below the LOQ of the method in 91% of these samples. The maximum weighted mean lead concentrations were in meat muscles, poultry muscles and kidney, at 0.345 mg/kg, 0.270 mg/kg and 0.140 mg/kg, respectively.

6.3 China

Data from two different sources were provided by China. Results from China’s 2007 TDS (China, 2010) included lead levels in 12 food composites from 12 provinces. The mean lead levels were as follows: cereals, 0.015 mg/kg; legumes, 0.060 mg/kg; potatoes, 0.064 mg/kg; meat, 0.038 mg/kg; eggs, 0.039 mg/kg; aquatic foods, 0.075 mg/kg; milk, 0.003 mg/kg; vegetables, 0.037 mg/kg; fruits, 0.033 mg/kg; sugar, 0.004 mg/kg; beverages and water, 0.001 mg/kg; and alcoholic beverages, 0.002 mg/kg.

Table 4. Lead concentration in different food commodities from Australia (23rd Australian TDS)

Food category	*n*	*n* <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	24	2	0.008	0.022
Wheat (including breads)	16	1	0.009	0.020
Rice	4	1	0.003	0.007
Baked goods including fancy bread	20	0	0.027	0.248
Oats	4	0	0.003	0.003
Roots and tubers	12	0	0.004	0.011
Pulses + legumes	4	0	0.005	0.007
Fruits	80	20	0.003	0.031
Dried fruit	12	0	0.016	0.030
Fruit juices	12	0	0.013	0.032
Vegetables including juices	118	21	0.013	0.072
Meat muscle	36	0	0.010	0.057
Poultry muscle	20	0	0.005	0.017
Poultry liver	4	0	0.007	0.008
Eggs	10	3	0.002	0.039
Finfish	18	0	0.005	0.010
Shellfish	8	0	0.010	0.019
Dairy products	42	13	0.004	0.056
Nuts and oilseeds	8	1	0.004	0.007
Animal fats	4	2	0.002	0.008
Vegetable oils and fats	8	7	0.0001	0.001
Stimulants	12	1	0.015	0.101
Sugar and honey	8	4	0.026	0.095
Spices	8	0	0.002	0.005
Alcoholic beverages	20	3	0.007	0.018
Miscellanous	50	15	0.009	0.143
Total samples	570	94	—	—

Table 5. Lead concentration in different food commodities from Australia (20th Australian TDS)

Food category	*n*	*n* <LOD	Weighted mean (maximum) lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	48	33	0.006 (0.05)
Wheat (including most breads)	46	31	0.009 (0.06)
Rice	9	9	<LOD
Baked goods (including fancy breads)	30	22	0.004 (0.02)
Oats	9	9	<LOD
Roots and tubers	28	26	0.001 (0.01)
Pulses + legumes	9	9	<LOD
Fruits	144	142	0.0003 (0.01)
Dried fruit	9	0	0.038 (0.06)
Fruit juices	28	28	<LOD
Vegetables including juices	202	177	0.002 (0.05)
Meat muscle	133	113	0.004 (0.17)
Poultry muscle	21	20	0.001 (0.01)
Poultry liver	21	19	0.002 (0.04)
Eggs	28	26	0.001 (0.01)
Finfish	51	48	0.001 (0.02)
Shellfish	21	13	0.189 (0.05)
Dairy products	67	58	0.003 (0.04)
Nuts and oilseeds	18	18	<LOD
Vegetable oils and fats	28	28	<LOD
Stimulants	9	9	<LOD
Sugar and honey	9	9	<LOD
Spices	21	21	<LOD
Alcoholic beverages	21	0	0.018 (0.06)
Total samples	1010	868	—

Table 6. Lead concentration in different food commodities from Australia (19th Australian TDS)

Food category	*n*	Weighted mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	45	0.0142	0.290
Wheat (including breads)	48	0.010	0.010
Rice	18	0.005	0.019
Baked goods	27	0.007	0.050
Oats	9	0.003	0.007
Roots and tubers	27	0.001	0.022
Pulses + legumes	27	0.012	0.050
Fruits	168	0.004	0.040
Dried fruit	9	0.020	0.050
Fruit juices	27	<LOD	0.006
Vegetables including juices	198	0.003	0.030
Organ meats	48	0.106	0.630
Meat muscle	8331	0.002	0.037
Minced meats	69	0.003	0.078
Eggs	27	<LOD	0.007
Finfish	30	0.003	0.007
Shellfish	30	0.147	1.100
Dairy products	84	0.008	0.050
Nuts and oilseeds	18	0.003	0.014
Vegetable oils and fats	18	<LOD	<LOD
Stimulants	9	0.003	0.010
Sugar and honey	9	0.079	0.110
Spices	21	<LOD	<LOD
Alcoholic beverages	21	<LOD	<LOD
Miscellaneous	27	0.004	0.020
Total samples	9345	—	—

Table 7. Lead concentration in different food commodities from China

Food category	*n*	*n* <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Fruits	1929	643	0.075	3.73
Leeks and onions	326	64	0.104	2.72
Total	2255	707	—	—

Individual results were also submitted by China from its monitoring programme using the GEMS/Food format and comprising the 2000–2006 period. In total, 2255 samples of fruits and vegetables were analysed; 707 of the samples presented a quantifiable result (above the LOQ of the method). The analytical method used was not reported. The LOQs of the method were in the range of 0.0005–0.05 mg/kg. The LOD was reported as the same value as the LOQ. The highest lead concentration (3.73 mg/kg) was reported for a sample of pome fruit collected in 2000. The mean and maximum lead concentrations and number of samples analysed are reported in Table 7.

6.4 Europe

EFSA carried out an assessment on lead in food, including the risks to humans from dietary exposure to lead (EFSA, 2010). The work was conducted by the Scientific Panel on Contaminants in the Food Chain at the request of the European Commission.

The Committee at the present meeting decided that the summary results from the EFSA report would be used in the current assessment.

EFSA received a total of 139 423 results from food testing, of which 97.9% were from 14 member states (Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Poland, Romania, Spain and Sweden) and Norway and 2.1% from three commercial operators.

Approximately 94 000 results covered the period from 2003 to 2009 and were suitable for calculating lead concentrations in 15 major food categories (Table 8). Germany was the major contributor, providing 44% of the concentration data, followed by France (15%), the Czech Republic (9.7%) and Romania (9.6%).

The analytical methods used to perform the determination of lead were graphite furnace atomic absorption spectrometry (GFAAS) (24.3% of the analyses), followed by ICP-MS (9.8% of the analyses). For 52.5% of the samples analysed, no information was provided on the analytical method used, apart from detection and quantification limits. The median LODs for non-specified atomic absorption spectrometry, GFAAS and ICP-MS were 0.006 mg/kg, 0.01 mg/kg and 0.003 mg/kg, respectively.

Table 8. Lead concentration in different food commodities from Europe

Food category	*n*	% of samples <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
All cereal and cereal products	4 774	56.6	0.0286	7.120
Sugar and sugar products including chocolate	1 794	63.6	0.0339	4.100
Fat	518	76.7	0.0387	7.300
All vegetables, nuts and pulses	11 011	52.3	0.0733	16.20
Starchy root and potatoes	1 059	47.8	0.0223	1.321
Fruits	3 915	61.4	0.0137	3.700
Juices, soft drinks and bottled water	3 565	69.7	0.0047	0.660
Coffee, tea, cocoa	655	36.2	0.222	6.210
Alcoholic beverages	2 228	36.2	0.0216	5.800
All meat and meat products and offal	40 301	52.3	0.2534	867.0
All fish and seafood	11 453	68.7	0.0543	4.060
Eggs	615	88.6	0.0052	0.205
Milk and dairy products	3 210	80.6	0.0089	4.550
Miscellaneous/ special dietary products	4 923	42.8	0.3652	155.0
Tap water	4 087	38.2	0.0052	1.950
Total	94 108	—	—	—

6.5 Singapore

In total, 1009 samples were collected during 2008. Lead was determined in 437 samples. The analytical method used for the quantification of lead was not reported. The LOD and LOQ were 0.09 mg/kg and 0.3 mg/kg, respectively. Fifty-seven per cent of the samples presented a lead content lower than the LOD. The highest lead levels were reported for three cocoa powder samples (45.4, 35.4 and 25.3 mg/kg). Lead concentrations in different food commodities are presented in Table 9 (Singapore, 2010).

Table 9. Lead levels in different food commodities from Singapore

Food category	*n*	*n* <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	5	5	<LOD	<LOD
Baked goods	8	7	0.018	0.14
Fruit juices	5	4	0.032	0.16
Vegetables including juices	66	20	0.402	1.97
Mushrooms/fungus	113	28	0.616	10.1
Molluscs	8	4	0.074	0.19
Finfish	9	0	0.224	0.45
Shellfish	2	0	0.125	0.14
Seaweed	101	38	0.180	1.44
Cocoa products except for cocoa butter	206	44	0.692	45.4
Cocoa butter	34	34	<LOD	<LOD
Nuts and oilseeds	3	3	<LOD	<LOD
Animal fats	59	59	<LOD	<LOD
Vegetable oils and fats	197	196	0.001	0.1
Stimulants	40	0	1.029	3.9
Sugar and honey	66	61	0.019	0.72
Spices	27	11	0.107	0.44
Alcoholic beverages	2	2	<LOD	<LOD
Miscellaneous	58	55	0.004	0.16
Total	1009	572	—	—

6.6 United States of America

The USA published data on selected food items in its TDS comprising the period from 1999 to 2008. In total, 4841 samples were analysed using GFAAS. The LOD and the LOQ of the method were in the range of 0.004–0.02 mg/kg and 0.01–0.06 mg/kg, respectively. Eighty-three per cent of the samples presented a lead content lower than the LOD of the method. The highest lead content of 0.18 mg/kg was reported for a shrimp sample collected in 2006.

Lead levels in different food commodities from the TDS (1999–2008) are presented in Table 10 (USFDA, 2010).

Table 10. Lead levels in different food commodities from the USA (TDS 1999–2008)

Food category	*n*	*n* <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	93	90	0.0004	0.017
Wheat (including breads)	366	311	0.002	0.026
Rice	39	39	<LOD	<LOD
Baked goods	39	28	0.004	0.019
Oats	39	38	0.0004	0.014
Roots and tubers	117	104	0.001	0.019
Pulses + legumes	156	146	0.001	0.013
Fruits	769	565	0.003	0.046
Dried fruit	54	34	0.006	0.032
Fruit juices	361	223	0.003	0.029
Vegetables including juices	1302	1055	0.003	0.136
Meat muscle	483	465	0.001	0.054
Organ meats	39	18	0.013	0.049
Poultry muscle	102	99	0.0004	0.014
Eggs	93	93	<LOD	<LOD
Finfish	102	96	0.001	0.013
Shellfish	39	23	0.012	0.180
Dairy products	288	269	0.001	0.033
Nuts and oilseeds	117	114	0.001	0.033
Animal fats	39	38	0.001	0.029
Vegetable oils and fats	63	61	0.001	0.021
Stimulants	39	36	0.0004	0.007
Sugar and honey	39	39	<LOD	<LOD
Miscellaneous	63	51	0.002	0.021
Total	4841	4035	—	—

The USA also provided non-TDS monitoring/surveillance data from the period 1999–2006 (Table 11) (USFDA, 2010). In total, 3633 samples were analysed using four different analytical methods: FAAS, GFAAS, ICP-OES and ICP-MS. The highest lead content of 74 mg/kg was reported for a fruit juice sample collected in 2000. Fifty-six per cent of all samples analysed presented a lead concentration lower than the LOD of the method.

Table 11. Lead levels in different food commodities from the USA (non-TDS 1999–2006)

Food category	*n*	*n* <LOD	Mean lead concentration (mg/kg)	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	35	27	0.013	0.123
Rice	15	11	0.004	0.021
Baked goods	79	49	0.232	16.50
Oats	2	2	<LOD	<LOD
Pulses + legumes	24	16	0.006	0.063
Fruits	425	238	0.130	28.86
Dried fruit	198	90	0.046	1.336
Fruit juices	428	220	0.352	74
Vegetables including juices	277	158	0.300	27.56
Mushrooms and other fungi	18	9	0.224	3.670
Seaweed	1	0	0.100	0.1
Dairy products	130	113	0.013	0.290
Nuts and oilseeds	20	15	0.024	0.299
Sugar and honey including candy	25	10	0.082	0.750
Meat muscle	319	285	0.013	1.359
Organ meats	319	124	0.047	1.237
Poultry muscle	200	152	0.003	0.075
Mollusc	3	0	0.065	0.102
Finfish	446	288	0.009	0.233
Shellfish	665	211	0.075	11.80
Miscellaneous	4	3	0.012	0.046
Total	3633	2021	—	—

7. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

7.1 Introduction

Human exposure to lead can occur via food, water, air, soil and dust. Lead is found in all categories of food, including meat, milk, fruits and vegetables, cocoa and cocoa products and processed foods. Water can also contain lead, depending upon the source and the water system characteristics. Although non-dietary sources are potentially important sources of exposure, the Committee generally did not have data to estimate exposure via non-dietary routes and thus did not evaluate the relative contributions of non-dietary sources of exposure.

7.2 Considerations in determining dietary exposure to lead

The sources of lead in food may include soil remaining in or on the food, atmospheric deposition, water, contact with lead-containing processing equipment and packaging. Therefore, it is important to estimate lead levels in food that is as close as possible to the form of the food that is consumed. Levels in raw agricultural commodities do not necessarily reflect levels in foods as they are consumed.

The guidelines for conducting exposure assessments for contaminants in foods (http://www.who.int/ipcs/food/principles/en/index1.html) recommend that regional dietary exposure estimates should be calculated using regional average contaminant concentration data and the GEMS/Food consumption cluster diets (http://www.who.int/foodsafety/chem/gems/en/index1.html). The Committee considered estimates from the TDS or similar studies to be the most relevant in the case of lead, as lead may be introduced during cooking and processing. In contrast, the WHO GEMS/Food consumption cluster diets cannot distinguish the form of the food that is consumed. Therefore, the Committee focused on exposure assessments in which the data were collected on foods as they are consumed and did not use the GEMS/Food data to calculate exposure to lead for this evaluation.

Lead occurrence data were submitted by seven countries. All but one of those countries provided a national exposure assessment using the submitted data in combination with national food consumption data. Most of the exposure assessments were conducted using TDS data that took into consideration the effects of processing/cooking. EFSA provided data for 14 countries and used those data to estimate exposures for 19 countries. Cooking and processing were considered where possible. In addition to the submitted studies, the Committee conducted a literature search and identified several additional national exposure assessments that also took into account cooking and processing.

The Committee included estimates of children's exposure wherever possible. Most of the submitted TDSs estimated children's exposure. The GEMS/Food consumption cluster diets do not include estimates of children's consumption. For those countries that did not report children's exposure, the Committee assumed that children's exposure would be 2–3 times that of the entire population and included those values in this report.

7.3 Assessment of dietary exposure to lead

Dietary exposure estimates were available to the Committee for 29 countries. Each region/country made its own decisions as to the appropriate matching of food lead levels to food consumption data as well as the treatment of samples without detectable lead levels. Estimates of dietary exposure for individual countries are presented below. EFSA conducted assessments for 19 European countries, and those are presented together. In addition, results from the United Kingdom and France TDSs are presented as part of the Committee's evaluation of trends in dietary exposure to lead.

Exposure assessments for each country are presented below.

7.3.1 Australia

FSANZ conducted exposure assessments for Australian[1] consumers using the 19th and 20th Australian TDSs and provided those assessments to the Committee. Although there are more recent Australian TDSs, exposure assessments are not yet available for lead. Therefore, the Committee relied on the 19th and 20th TDSs for evaluating exposures. The 19th Australian TDS was published in 2001 (FSANZ, 2001), with samples collected and analysed in 1998–1999. The 20th Australian TDS was published in 2003 (FSANZ, 2003), with samples collected and analysed in 2000–2001. FSANZ reported a range of exposures based on mean food consumption and either the lower- or upper-bound median concentrations of lead in the analysed foods. The lower end of the range of reported exposures in the Australian TDSs assumed that results less than the limit of reporting (LOR) are equal to zero (lower-bound approach), and the upper end of the range assumed that results less than the LOR are the same as the LOR (upper-bound approach). The LOQ of the method was reported to be 0.0005–0.001 mg/kg food. The food consumption data used in estimating exposures for both the 19th and 20th Australian TDSs were from the 1995 National Nutrition Survey. For infants, estimated dietary exposures are based on a constructed infant diet. The resulting Australian dietary exposures to lead are summarized in Table 12.

The highest estimated exposures (per unit body weight) were for toddlers (2 years), whose mean exposures were 1.19–1.92 µg/kg bw per day in the 19th Australian TDS and 0.03–0.93 µg/kg bw per day in the 20th Australian TDS.

7.3.2 Brazil

Brazil provided lead levels for meat only. Lead levels in beef and poultry muscle meat and kidney from beef and poultry were provided. The Committee decided that it would be misleading to estimate dietary exposure from a single food category; thus, no estimate was undertaken.

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[1] New Zealand also conducts a TDS, which is published separately and is summarized under New Zealand.

Table 12. Australian dietary exposures to lead (based on the 19th and 20th Australian TDSs and the 1995 National Nutrition Survey)

Population group	Dietary exposure (µg/kg bw per day)		Average body weight (kg)
	19th Australian TDS	20th Australian TDS	
Adult males 25–34 years	0.42–0.73	0.06–0.40	82
Adult females 25–34 years	0.27–0.56	0.02–0.35	66
Boys 12 years	0.70–1.01	0.02–0.43	49
Girls 12 years	0.59–0.84	0.01–0.34	52
Toddlers 2 years	1.19–1.92	0.03–0.93	14
Infants 9 months	0.57–1.50	0.01–1.2	9.2

7.3.3 Canada

Health Canada has repeated its TDS over six separate periods since 1969. The most recent TDS was started in 2005 (Canadian TDS, 1993–2010). The data were available to the Committee from the Canadian TDS web site (Health Canada, 2010).

Each TDS is conducted in several major Canadian cities over the span of the survey period, normally one city each year. For each city, each individual food item tested (there are about 210 individual food items for the current Canadian TDS) is purchased from three to four different supermarkets. Food samples are prepared and processed as they "would be consumed" in the average household kitchen (i.e. raw meats are cooked; fresh vegetables are cooked or properly peeled, trimmed or otherwise cleaned for serving, if not cooked). These processed foods are then mixed according to each category to make composites (there are over 140 different food composites in the current study). All food composites are analysed for the presence of toxic and nutritionally important chemicals. These concentrations are then combined with food intake information (estimates of how much Canadians consume of each food group) to provide estimates of the dietary exposures to these chemicals for Canadians for 16 different age/sex groups.

The results for the 2002 and 1993–1996 surveys are presented in Table 13.

In 2002, mean lead exposures for the total population were 0.11 µg/kg bw per day. Children 1–4 years of age were the subgroup with the highest mean lead exposure, 0.27 µg/kg bw per day.

In previous TDSs, lead exposure was higher. In 1993–1996, lead exposure for the total population was 0.19 µg/kg bw per day. Between 1993–1996 and 2002, exposure declined about 40% from 0.19 µg/kg bw per day (Table 13).

Table 13. Average dietary exposures of lead for Canadians in different age/sex groups for TDSs in 2002 (Vancouver) and in 1993–1996 (Montreal)

Population subgroup	Dietary exposure (µg/kg bw per day)	
	2002 (Vancouver)	1993–1996 (Montreal)
0–1 month, male + female	0.20	0.45
2–3 months, male + female	0.26	0.54
4–6 months, male + female	0.19	0.42
7–9 months, male + female	0.22	0.45
10–12 months, male + female	0.24	0.50
1–4 years, male + female	0.27	0.49
5–11 years, male + female	0.21	0.36
12–19 years, male	0.15	0.25
12–19 years, female	0.12	0.19
20–39 years, male	0.13	0.22
20–39 years, female	0.11	0.18
40–64 years, male	0.12	0.20
40–64 years, female	0.10	0.17
65+ years, male	0.09	0.16
65+ years, female	0.08	0.14
All ages, male + female	0.11	0.19

7.3.4 Chile

No data on Chilean lead exposures were submitted to the Committee, but a published study was available (Muñoz et al., 2005). In that study, dietary exposure to lead by the population of Santiago, Chile, was determined using the Chilean TDS. The most frequently consumed food products were included in the basket. Prior to analysis, the foods were prepared according to typical Chilean procedures and then grouped into 17 food categories according to their chemical characteristics and analysed. The LOD for lead was 50 µg/kg dry weight for solid samples and 1.6 µg/l for liquid samples. The estimated dietary exposure to lead was 210 µg/day (3 µg/kg bw per day for a 63 kg individual). The authors acknowledged that these exposures were quite high and that they were due to high lead levels in foods rather than from high amounts of food consumption. Milk and milk products, fruits, breads and sugars contributed most to the dietary exposure.

Children's exposures were not reported. The Committee assumed that children would have 2–3 times the exposure of adults per unit body weight and that the range of Chilean children's exposures would be 6–9 µg/kg bw per day.

Table 14. Chinese consumer lead exposures by food category (2007 Chinese TDS)

Food category	Mean exposure (μg/day)	SD	Mean exposure (μg/kg bw per day)[a]	% of total
Cereals	16.44	20.87	0.26	34
Legumes	4.11	5.72	0.07	9
Potatoes	5.7	12.74	0.09	8
Meat	3.32	8.26	0.05	7
Eggs	1.74	3.03	0.03	2
Aquatic foods	3.06	5.51	0.05	9
Milk	0.08	0.07	0.00	0
Vegetables	16.85	34.36	0.27	21
Fruits	2.57	5.94	0.04	8
Sugar	0.01	0.02	0.00	0
Beverages and water	0.58	0.41	0.01	2
Alcoholic beverages	0.02	0.02	0.00	0
Total	54.48	62.61	0.86	100

[a] Assuming a body weight of 63 kg.

7.3.5 China

China submitted two exposure assessments based on the results of the 2007 and the 2000 Chinese TDSs (Wang et al., 2009).

The 2007 Chinese TDS estimated exposure for the total population. The overall exposure to lead for Chinese consumers was estimated to be 55 μg/day (0.87 μg/kg bw day, assuming a mean body weight of 63 kg). The food categories making the largest contributions were cereals (34%) and vegetables (21%). The contributions to exposure from these and other food categories are presented in Table 14.

In the 2000 Chinese TDS (Wang, 2009), the exposures for various age groups were determined. The results are presented in Table 15. When exposures are presented on a microgram per day basis, adults have the highest exposures. If data are adjusted by body weight, children 2–7 years of age have estimated exposures of 3.1 μg/kg bw per day (mean) and 8.2 μg/kg bw per day (upper 97.5th percentile).

Table 15. Lead dietary exposures of different age/sex population groups in China (2000 Chinese TDS)

Age/sex	Body weight (kg)	Mean exposure ± SD (µg/day)	97.5th percentile exposure (µg/day)	Mean exposure (µg/kg bw per day)
2–7 years, male + female	17.9	54.9 ± 37.5	146.8	3.1
8–12 years, male + female	33.1	78.0 ± 54.0	207.9	2.4
13–19 years, male	56.4	99.0 ± 59.2	250.5	1.8
13–19 years, female	50	92.1 ± 56.7	230	1.8
20–50 years, male	63	112.7 ± 83.5	308.3	1.8
20–50 years, female	56	101.2 ± 68.9	279.7	1.8
51–65 years, male	65	102.9 ± 56.5	228.4	1.6
51–65 years, female	58	92.8 ± 51.8	253.3	1.6
>65 years, male	59.5	102.0 ± 65.2	250.9	1.7
>65 years, female	52	79.4 ± 49.8	202.8	1.5

7.3.6 Egypt

No data were submitted to the Committee for Egypt. The Committee obtained a published report of a market basket survey that contained levels of lead in various fruits and vegetables sold in Egyptian markets. Atomic absorption spectrometry was used to estimate and evaluate the levels of lead (Radwan & Salama, 2006). The authors reported dietary exposures of 21 µg/day from fruits, 30 µg/day from vegetables and 1 µg/day from potatoes. Total exposure was not estimated. Assuming a body weight of 68 kg, total exposure from these categories would be 0.74 µg/kg bw per day.

7.3.7 Europe

EFSA released an assessment of lead exposure on 18 March 2010. Some European countries also conduct exposure assessments using their own TDSs. For example, the United Kingdom has conducted exposure assessments using the United Kingdom TDS since 1980.

(a) EFSA

The Committee reviewed the EFSA report and included the results in the present evaluation of European consumer exposures to lead.

EFSA conducted a detailed evaluation of the submitted data and created a single database of occurrences of lead in foods, which was then used to estimate lead exposure in 19 countries. Some data were determined by the study authors to be outliers and were excluded. The foods were grouped into categories. The number of test results for each food in a category was not proportional to the food's relative contribution to the diet. Therefore, sampling adjustment factors were developed to allow the determination of a weighted lead level for each food category.

For the purposes of exposure assessment, EFSA assumed that the lead levels were the same in all countries and that only the diet varied. EFSA combined the occurrence data with consumption information obtained from the EFSA Concise European Food Consumption Database. According to the EFSA report, individual food consumption data that were available in the Concise European Food Consumption Database were used for the lead exposure analysis. This provides a more accurate estimate, in that individual body weights are used for the calculations. Only aggregated food consumption statistics for this database are presented on the EFSA web site (http://www.efsa.europa.eu/en/datex/datexfooddb.htm). The individual data were not available to the Committee.

EFSA divided the food consumption data into 15 broad food groups and some subgroups, giving a total of 28 separate groups. Individual data on sex, age and body weight were included.

EFSA determined lead dietary exposure for average adult consumers in 19 European countries—Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Iceland, Ireland, Italy, the Netherlands, Norway, Poland, Slovakia and Sweden—by combining the lead levels in the analysed foods with information on consumption from the EFSA Concise European Food Consumption Database.

EFSA recognized the variation in the methods used to collect and analyse the data as well as the need to consider the impact of assumptions regarding censored data. Thus, EFSA estimated exposures using several different assumptions and methods. For example, mean exposures were estimated with two different assumptions regarding residue levels in samples without detectable lead (lower-bound estimates assumed a zero concentration for samples without detectable lead, and upper-bound estimates assumed that lead was present at the limits of the analytical method). Two different exposure assessment methods were also used: 1) a deterministic approach and 2) a probabilistic approach. The probabilistic assessment was conducted twice, using lower-bound and upper-bound values for the non-quantifiable samples. EFSA concluded that estimates of exposure were similar for assessments that used probabilistic and deterministic methods. Therefore, only the results of the deterministic approach were reported by EFSA (upper- and lower-bound deterministic estimates). The Committee reviewed the estimates of dietary exposure to identify the most useful information to include in

this analysis. In order to facilitate cross-country comparisons, the Committee selected the results of analyses that were as similar as possible to those that were available for other countries (e.g. lower-bound estimates).

The Committee selected the ranges that estimate the lower bound for the country with the lowest average exposure and the upper bound for the country with the highest average exposure. The range was 0.36–1.24 μg/kg bw per day for consumers with mean dietary exposures and 0.73–2.43 μg/kg bw per day for consumers with high dietary exposures, respectively.

EFSA estimated mean and 95th percentile lead dietary exposures for each country using the standardized European-wide distribution of lead occurrence in foods in combination with national estimates of food consumption. Mean and 95th percentile lead dietary exposures were determined for each country's whole population and subgroups of the population. In a separate analysis, EFSA estimated exposures for children and infants by combining the results of multiple surveys of exposure.

EFSA estimated children's exposures based on a compilation of several different surveys of children's exposure to lead. Mean exposures for children aged 1–3 years ranged from 1.10 to 3.10 μg/kg bw per day based on lower-bound and upper-bound assumptions, respectively. Estimates for children aged 1–3 years with high dietary exposure ranged from 1.71 to 5.51 μg/kg bw per day. The corresponding exposures for children aged 4–7 years are 0.80–2.61 μg/kg bw per day based on lower-bound and upper-bound assumptions, respectively, for the mean and 1.30–4.4 μg/kg bw per day for children with high dietary exposure.

EFSA also concluded that "the available evidence for women of child-bearing age and vegetarians does not indicate a dietary exposure that is different from that of the general adult population".

EFSA estimated exposures for breastfed infants to be 0.21 μg/kg bw per day on average and 0.32 μg/kg bw per day for consumers with high dietary exposure. EFSA noted that the lead levels in breast milk were very variable.

EFSA also estimated exposures for infants fed infant formula and reported average exposures from 0.27 to 0.63 μg/kg bw per day, based on lower-bound and upper-bound assumptions, respectively. For infants with high dietary exposure, lead exposures ranged from 0.40 to 0.94 μg/kg bw per day.

Lead exposures for vegetarians from the adult population were reported to be similar to those of other consumers. EFSA estimated exposures to be 1.98–2.44 μg/kg bw per day for consumers of game and 0.81–1.27 μg/kg bw per day for consumers of offal.

EFSA evaluated the contribution of different food categories to exposure and noted that it varied widely between countries. EFSA reported that

> the largest contributors to the calculated overall lead exposure are vegetables, nuts and pulses contributing 19 % to the lower bound and 14 % to the upper bound estimates. Cereals and cereal products contributed 13 % to the lower bound and 14 % to the upper bound. For the lower bound miscellaneous products and food for special uses

contributed 12 %, starchy roots and potatoes 8%, meat and meat products 8 %, alcoholic beverages 7 % and milk and dairy products 6 %. For the upper bound the contributions were: juices, soft drinks and bottled water (11 %), alcoholic beverages (9%) meat and meat products including offal (9 %), milk and dairy products (8 %), miscellaneous products and food for special uses (7 %) and starchy roots and potatoes (6 %).

Additional detail is available in the EFSA study report (EFSA, 2010).

(b) European TDSs

France and the United Kingdom submitted TDS data to the Committee.

(i) France

In addition to the results presented by EFSA, the Committee reviewed the results of a lead exposure assessment conducted by France based on the French TDS (Leblanc et al., 2005), which provided information about trends in dietary exposure to lead. The estimated average daily dietary exposure of the French population to lead was 18 μg for adults aged 15 years or more (0.26 μg/kg bw per day for a 70 kg person) and 13 μg for children aged 3–14 (0.43 μg/kg bw per day for a 30 kg child).

The 97.5th percentile exposure for adults was 3.6 μg/kg bw per week (0.51 μg/kg bw per day), and for children, 6.4 μg/kg bw per week (0.91 μg/kg bw per day).

The food groups contributing most to exposure of the population were bread, rusk, soups, vegetables, fruits, drinking-water, non-alcoholic beverages, alcoholic beverages, and sugars and confectionery.

The study authors noted that “compared to existing French data, the Pb exposures have declined by a factor of 2 to 4”. No information was available as to the time frame for these declines.

(ii) United Kingdom

The Committee also reviewed the United Kingdom exposure assessments based on its TDS since 1980. The United Kingdom TDS allowed the Committee to evaluate trends in lead levels in the diet over time. Based on the 2006 TDS, the population dietary exposure to lead was 0.006 mg/day. In the 2006 TDS, the major contributors to the population dietary exposure were the beverages, bread and other vegetables groups. Dietary exposures of the general United Kingdom population in the 1980 TDS were estimated to be 0.12 mg/day. Lead exposure declined by 95% from 1980 to 2006.

7.3.8 India

No occurrence data were submitted for India. The Committee obtained a published study of lead exposures that was conducted from a TDS-like market basket study in Mumbai, India, in 1991–1994 (Tripathi et al., 1997). Lead levels were estimated in air particulates, water and food samples collected from different suburbs in Mumbai. The data were used to estimate exposure via inhalation and ingestion. In the study, food samples were purchased from different grocery stores

situated in different suburbs. The foods were selected to represent the food and liquids consumed during a 24 h period by the adult population. The samples were divided into seven groups: cereals (rice, wheat and Jowar); pulses (red gram, black gram and green gram); vegetables (potato, tomato, carrot, brinjal, cauliflower, beans, cabbage and ladies finger); leafy vegetables (amaranth, spinach and fenugreek); milk; meat; and fruits. The concentrations of lead and cadmium in all these samples were estimated by the differential pulse anodic stripping voltammetric (DPASV) technique using PARC Model 174 A Polarographic Analyser. Approximately 1000 ml drinking-water samples were collected from 13 houses located in different suburbs of Mumbai.

The concentration of lead in water varied between 0.6 and 2.6 µg/l, with a geometric mean of 1.2 µg/l and a geometric standard deviation of 1.73 µg/l (Table 16).

The total dietary lead exposure was reported to be 25.1 µg/day (Table 17).

Table 16. Mean concentrations of lead in food in India

Food	No. of samples	Geometric mean lead concentration (µg/kg)
Cereal	15	18.2
Pulses	13	253.3
Leafy vegetables	11	100.4
Other vegetables	32	4.1
Milk and milk products	4	1.6
Meat	6	57.0
Fruits	7	7.4
Water	13	1.2

Table 17. Food consumption and dietary lead exposure of Mumbai adults

Name of food	Food consumption (g/day)	Dietary lead exposure (µg/day)
Cereal	445	8.1
Pulses	5.5	13.9
Leafy vegetables	17	1.7
Other vegetables	88	0.4
Milk and milk products	113	0.2
Meat	14	0.8
Fruits	18	0.02
Total	—	25.1

The study also measured levels of lead in air and estimated uptake by humans. The total intake of lead through air, water and food was reported to be 30 μg/day. The diet was reported to account for about 60% of total exposure to lead.

7.3.9 Lebanon

No occurrence data were submitted for Lebanon. The Committee obtained a published study of lead exposures in adult urban populations of Lebanon (Nasreddine et al., 2006). According to the report, the exposure assessment was performed using the TDS approach, as recommended by WHO. Five “total diets” were collected during 2003–2004. The concentrations in food were expressed in milligrams of lead per kilogram fresh matter, and lead exposures were expressed in milligrams per day per person. For foods containing levels of lead below the LOQ, a value equal to half the LOQ was assigned and used for calculation purposes. The LOD for lead was 2 μg/kg. Average and maximal consumer exposures to lead were calculated. Using these data in conjunction with a TDS, the mean and maximal daily dietary exposures per person per day from “total diet” food groups were determined. The mean exposure was estimated to be 18.5 μg/day, and the maximum exposure was 29.6 μg/day. Water contributed the most to exposure. The foods contributing most to exposure were bread and toast, fruits, pizza and pies, and vegetables (raw and cooked).

The study did not estimate exposure for children or other subgroups of the population. The Committee estimated exposure for children by assuming that children’s exposures would be 2–3 times the adult exposures (37–55.5 μg/day).

7.3.10 New Zealand

No occurrence data were submitted for New Zealand. The Committee obtained the New Zealand TDS for 2003–2004 (NZFSA, 2005; Vannoort & Thomson, 2005), which provided dietary exposure estimates for lead. In that study, the New Zealand TDS sampled 121 different foods, 110 of which represented at least 70% of the most commonly consumed food items for the majority of New Zealanders. The foods were analysed for lead. Fourteen-day simulated typical diets using these 121 foods were derived mainly from food frequency and 24 h diet recall data from the 1997 National Nutrition Study for adults 15+ years of age and the 2002 Children’s Nutrition Study for 5- to 14-year-olds (both commissioned by the New Zealand Ministry of Health). Data from recent studies were used to simulate typical diets for children younger than 5 years of age. The simulated typical diets were established for the following eight age/sex groups: 25+ years, male; 25+ years, female; 19–24 years, young male; 11–14 years, boy; 11–14 years, girl; 5–6 years, child; 1–3 years, toddler; and 6–12 months, infant.

Approximately 4440 different food samples that were intended to be typical of what was available at the point of sale were obtained. All foods were bought at two different times of the year to provide a measure of seasonal variation. Most of these were composited to provide a total of 968 different food samples for elemental analyses. The foods in the 2003–2004 New Zealand TDS were prepared ready for consumption, prior to analysis.

The estimates of dietary exposure were calculated by using the mean concentration of lead in the food samples and the model diets. The estimated exposures were 0.13 µg/kg bw per day (0.91 µg/kg bw per week) for a young male and 0.34 µg/kg bw per day (2.4 µg/kg bw per week) for an infant.

Most food groups contributed to dietary lead exposure for all age/sex groups. Grains contributed 24–27% of dietary lead for adults and 36–39% for children. Chicken, eggs, fish and meat contributed 12–16%, and takeaways contributed 9–24%, of adult dietary lead; the corresponding percentages for children were 7–12% and 10–15%. The main food groups contributing to weekly dietary exposure to lead for infants are grain (18%), chicken, eggs, fish and meat (4%), takeaways (6%), fruit (18%) and infant formula and weaning foods (38%).

7.3.11 Nigeria

No occurrence data were submitted for Nigeria. The Committee obtained a published study (Maduabuchi et al., 2006) that estimated the levels of lead in some foods (canned and non-canned beverages, paediatric syrups, fish, spinach, fluted pumpkin, root crop [type not specified] and cocoa yam). Only very limited information is available about the study. There is no information about the analytical methods used, LODs or numbers of samples. The authors reported lead concentrations of 2–7.3 µg/l in canned beverages and 1–92 µg/l in non-canned beverages. No further details were presented about the types of beverages, either canned or non-canned. Several paediatric syrups were also analysed. One syrup was reported to contain lead at 90 µg/l. One fish (*Ethmaliosa timbriata*) was reported to contain lead at a concentration of 2400 µg/kg. No further details are available about the study.

No dietary exposure assessment was provided. Given the limited information about the data and the limited numbers of foods, the Committee could not conduct an assessment.

7.3.12 Singapore

No exposure assessment was provided by Singapore. The Committee decided that it would not be reliable to use the GEMS/Food consumption cluster diets to estimate exposure, as many of the sampled foods do not have consumption estimates in the consumption cluster diets; conversely, many foods that are commonly eaten were not analysed for lead in the Singapore study (see section 6.5 above).

7.3.13 United States of America

Estimates of dietary exposure to lead were provided based on the analytical results of the USA TDS samples collected from 2004 through 2008 and food consumption data collected in the 2003–2006 NHANES. The TDS is an ongoing monitoring programme in which about 280 foods and beverages representing all major components of the average American diet are collected and analysed for various contaminants and nutrients. TDS samples are collected 4 times each year. Samples are collected from grocery stores and fast food restaurants in three

different cities. The foods are prepared table-ready (i.e. as they would be consumed), and the three samples of each TDS food are then composited before analysis.

Consumption data from the NHANES 2003–2006 Dietary Interview were used to calculate daily dietary exposures to lead. During the 2003–2006 NHANES, consumption records were collected on 2 non-consecutive days for approximately 16 800 individuals. Survey participants reported detailed information about the types and amounts of foods consumed on those days; in all, approximately 6000 different foods and beverages were reported in the survey.

For calculating dietary exposures, lead analytical results for TDS foods were linked to the 2003–2006 NHANES consumption data by mapping TDS foods to NHANES food codes that were most similar in composition. As an example, the TDS food “white bread” was linked to all NHANES codes for yeast breads and rolls made from white flour. As another example, the TDS food “applesauce” was linked to NHANES codes for applesauce as well as other cooked apple products. This approach assumes that, based on the similarity of their ingredients, the analytical result for a TDS food would be an acceptable surrogate for the NHANES foods to which it is mapped. Exposure to lead was estimated via Monte Carlo simulations. For each instance of food consumption as reported by a survey participant, a value for lead was randomly selected from the distribution of analytical results for TDS samples collected from 2004 to 2008. Total daily exposures were calculated for each of 14 age/sex subgroups and the total population of the USA; self-reported body weights of survey participants were used to convert exposure estimates to a per kilogram of body weight basis.

Table 18 summarizes the results for the mean and 90th percentile exposures to lead for consumers in the USA.

7.3.14 Comparisons between countries

The Committee selected a representative dietary exposure value for each country in order to allow comparisons across countries and across regions for the total and/or adult population (Table 19) and for children (Table 20). Unfortunately, estimates of exposure for the same population were not always available for every country. In order to improve comparability, the Committee adjusted some data by standard body weight assumptions if the study reported only dietary exposure per day. For the total/adult population, mean exposures ranged from 0.02 to 3 μg/kg bw per day, depending on the country and also on the assumptions made in conducting the assessments. Children’s exposures ranged from 0.03 to 9 μg/kg bw per day, again depending on the country, age of the children and assumptions made in conducting the assessments.

Lead is widely distributed in food. Lead was reported in all but one of the food categories for which data were submitted for this evaluation. The relative contribution of a food category to dietary exposure to lead depends upon the level in the food (both the quantities and the frequency of occurrence) and the amount of the food that is consumed. Among the data submitted, foods with the highest frequency of detection were shellfish (especially bivalves), cocoa products, organ

Table 18. Consumer dietary exposure to lead in the USA (USA 2004–2008 TDSs)

Population group	Dietary lead exposure (µg/kg bw per day)	
	Mean	90th percentile
6–11 months, male + female	0.13	0.30
2 years, male + female	0.11	0.23
6 years, male + female	0.06	0.13
10 years, male + female	0.04	0.09
14–16 years, female	0.02	0.05
14–16 years, male	0.02	0.05
25–30 years, female	0.03	0.06
25–30 years, male	0.02	0.06
40–45 years, female	0.03	0.06
40–45 years, male	0.03	0.06
60–65 years, female	0.02	0.05
60–65 years, male	0.02	0.06
70+ years, female	0.03	0.06
70+ years, male	0.02	0.05
Total, male + female	0.03	0.08

meats, tea, roots and tubers, seaweed and mushrooms/fungi. Foods with the highest amounts of residues were found within every category. No single food or food category always had high or low levels of lead. Levels greater than 1 mg/kg were reported by some countries in wild game, some meat and meat products (especially organ meats and offal), cereals and cereal products, fruits and vegetables. Some countries identified water as having low but frequently detected concentrations of lead. Food consumption, a third determinant of exposure, also varied widely and was considered in all of the assessments. The assignment of values to samples without detectable lead has important effects. The most important contributors to overall dietary exposure were reported by some countries. Foods contributing most to exposure are noted for each country in the sections above.

7.4 Relative contribution of diet to total lead exposure

The relative contribution of diet to total lead exposure is not well known but will probably vary depending upon locale and the contribution from non-dietary sources. Estimates from EFSA suggest that at least half of children's exposure may be due to non-dietary sources of exposure and that soil and dust are major contributors to the non-dietary exposures. The diet was reported to contribute 60% of lead exposure for adults in Mumbai, India.

Table 19. National lead dietary exposure estimates for total/adult population

Country/region	Population group	Exposure (μg/kg bw per day)	
		Mean	High
Australia	Adult males 25–34 years	0.06–0.40[a]	—
	Adult females 25–34 years	0.02–0.35[a]	—
Canada	All (2002 study)	0.11[b]	—
Chile	Adults in Santiago	3[c]	—
China	Adults	0.9[d]	1.8 (97.5th percentile)
Egypt	All (exposures measured for selected crops only)	0.74	—
Europe	Adults (individual estimates by country)	0.36–1.24[e]	0.73–2.43 (95th percentile)
India	Adults in Mumbai	0.44[c]	—
Lebanon	All	0.27[f]	0.43
New Zealand	Adult males	0.13[g]	—
USA	All	0.03[h]	0.08 (90th percentile)

[a] The lower end of the range of reported exposures assumed that results less than the LOR are equal to zero, and the upper end of the range assumed that results less than the LOR are the same as the LOR (data from the 20th TDS).
[b] LOD/LOQ not provided; mean values were specified for all but a few foods.
[c] Assuming a body weight of 68 kg.
[d] From the 2007 study and assuming a body weight of 63 kg.
[e] Range between country with lowest mean exposure and country with highest mean exposure. For lowest mean exposure values, results less than the LOQ were set to zero; for highest mean exposure values, results less than the LOQ were set equal to the LOQ.
[f] Assuming a body weight of 68 kg; foods with concentrations less than the LOQ were assigned a concentration of ½ LOQ.
[g] Samples with concentrations less than the LOD were assigned a concentration of ½ LOD.
[h] Samples with concentrations less than the LOQ were assigned a concentration of zero.

7.5 Temporal changes in lead exposures

Lead levels in foods have declined over time. The estimated values depend upon the population, the time frame and, to some extent, the methods for lead analysis. The Committee had access to data from five countries (Canada, France, New Zealand, the United Kingdom and the USA) that allowed the trends in lead exposure to be estimated.

Table 20. National lead dietary exposure estimates for children

Country/region	Age	Exposure (µg/kg bw per day)	
		Mean	High
Australia	2 years	0.03–0.93[a]	
Canada	4 years	0.19[b]	
	2–3 years	0.26[b]	
Chile	Children	6–9[c]	
China	2–7 years	3.1	8.2 (97.5th percentile)
Europe	1–3 years	1.10–3.10[d]	1 year: 2.1–5.5 (95th percentile)[e] 3 years: 1.7–5.2 (95th percentile)
	4–7 years	0.80–2.61[d]	4 years: 1.5–4.4 (95th percentile) 7 years: 1.4–4.4 (95th percentile)
India	Children	0.9–1.3[c]	
New Zealand	Infants	0.34[f]	
	1–3 years	0.31[f]	
USA	6–11 months	0.13[g]	0.3 (90th percentile)
	2 years	0.11[g]	0.2 (90th percentile)

[a] The lower end of the range of reported exposures assumed that results less than the LOR are equal to zero, and the upper end of the range assumed that results less than the LOR are the same as the LOR.
[b] LOD/LOQ not provided; mean values were specified for all but a few foods.
[c] Assuming that children have 2–3 times the adult exposure per unit body weight, respectively.
[d] Means for the country with the lowest exposure and the country with the highest exposure. Lowest mean exposure (lower bound) calculated with results less than the LOQ assigned zero; highest mean exposure (upper bound) calculated with results less than the LOQ set at the LOQ.
[e] Children's estimates for consumers with high exposure are based on EFSA's combination of estimates from multiple surveys (depending upon the age group, 8–13 surveys were combined).
[f] Samples with concentrations less than the LOD were assigned a concentration of ½ LOD.
[g] Samples with concentrations less than the LOQ were assigned a concentration of zero.

New Zealand summarized changes in dietary exposure to lead since 1982 in its 2003–2004 TDS report. Lead exposures for 19- to 24-year-old males were 3.6 µg/kg bw per day in 1982 and 0.13 µg/kg bw per day in 2003–2004. This represents a decline of approximately 75%.

Dietary exposures for the general population in the United Kingdom have declined approximately 95% between 1980 and 2006. Exposures were estimated to be 0.12 mg/day in the 1980 TDS and 0.006 mg/day in the 2006 TDS.

Canada and France have reported a 50% decline in exposure to lead over the past 10–15 years.

The USA reported declines in lead exposure for all age groups (M. Bolger, personal communication, 2010). Teenage males (14–16 years) showed the greatest decline, from 70 μg/day in 1976 to 3.45 μg/day in 2000.

8. DOSE–RESPONSE ANALYSIS

8.1 Identification of key data for risk assessment

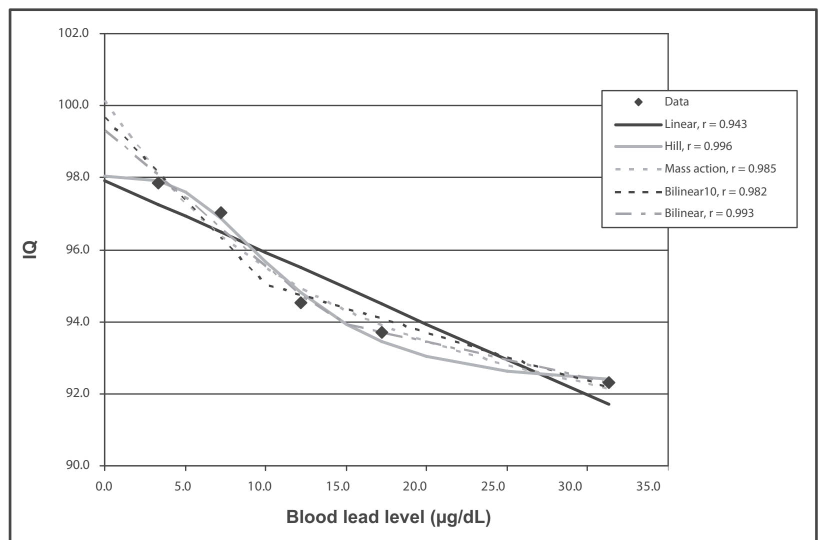
8.1.1 Pivotal data from biochemical and toxicological studies

The data from biochemical and toxicological studies in experimental animals provide support for the plausibility of associations reported in epidemiological studies. However, in view of the large numbers of studies providing quantitative data on dose–response relationships in humans, the studies in experimental animals are not considered to be pivotal for the dose–response analysis.

8.1.2 Pivotal data from human clinical/epidemiological studies

Exposure to lead has been shown to be associated with a wide range of effects, including various neurological effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, reduced sexual maturation and effects on dental health. EFSA (2010) identified three effects as providing pivotal data for its risk assessment: 1) developmental neurotoxicity in young children, as measured by decreased IQ score; 2) cardiovascular effects in adults, measured by increased systolic blood pressure; and 3) increased prevalence of chronic kidney disease in adults, as measured by a decrease in GFR. For each of these end-points, EFSA calculated a BMD and its lower 95th percentile confidence limit (BMDL). The lowest BMD and BMDL were for a decrease in cognitive ability by 1 IQ point, which could be adverse at the population level. The Committee agreed that the neurodevelopmental effects of lead occurred at lower blood lead concentrations than the other effects and therefore considered these to be the pivotal data in its assessment for children. For adults, the Committee concluded that the pivotal data were for the lead-associated increase in systolic blood pressure, as this was associated with the lowest blood lead concentrations and with the greatest and most consistent weight of evidence.

Figure 1. Five models fit to central estimates from Lanphear et al. (2005)



8.2 General modelling considerations

8.2.1 Selection of data

As it is the most recent and most comprehensive meta-analysis available, the results of Lanphear et al. (2005) were used as the basis for a dose–response model. This meta-analysis is based on the same studies used in the EFSA (2010) analysis. Although four different measures of dose were examined as part of the meta-analysis, concurrent blood lead level as the measure of exposure was used for dose–response modelling because it showed the highest correlation with changes in IQ. The mean values for each of the five dose groups are shown in Figure 1.

8.2.2 Selection of mathematical model

Six different models were initially considered. The first four have a linear form, whereas the last two are sigmoidal:

1) *Linear*: The change in IQ from the zero intercept is proportional to dose. This model has two adjustable parameters: intercept and slope. The linear model is equivalent to the hockey stick model with a threshold of zero.
2) *Hockey stick*: The change in IQ above a threshold is proportional to the dose above the threshold. This model has three adjustable parameters: intercept, threshold and slope. The hockey stick model is equivalent to a bilinear model with a low-dose slope of 0.
3) *Bilinear10*: High- and low-dose slopes, with an inflection point at a lead concentration of 10 μg/dl. Although 10 μg/dl has traditionally been used as a

cut-off level to distinguish between high and low exposures to lead, there is no empirical support for the use of this value as an inflection point. This model was included because it is one of the models used in the EFSA analysis (i.e. the piecewise linear model). The bilinear10 has three adjustable parameters: intercept, low-dose slope and high-dose slope.

4) *Bilinear*: High- and low-dose slopes, with an inflection point as an additional parameter. This model has four adjustable parameters: intercept, low-dose slope, high-dose slope and the inflection point.
5) *Mass action*: A simple sigmoidal model that is theoretically based on reversible ligand–receptor interaction. This model has three adjustable parameters: intercept, maximum effect (amplitude) and median effective dose (ED_{50}). Mass action is equivalent to the Hill model with the power parameter equal to one.
6) *Hill*: This model is a more complex sigmoidal model that is theoretically based on reversible ligand interaction with multiple binding sites. From a curve-fitting point of view, the inclusion of an additional power parameter makes the slope of the model more flexible. This model has four adjustable parameters: intercept, maximum effect (amplitude), ED_{50} and power.

8.3 Dose–response modelling

8.3.1 Blood lead and IQ in children

All six models were fit to the central estimates using least-squares regression. The results for five of the models are shown in Figure 1 above. The hockey stick model provided the same fit with the same slope parameter as the linear model. CIs were estimated with a 1000-iteration bootstrap analysis, in which each model was successively fit to a set of dose–response pairs generated by random sampling from the CIs from the Lanphear et al. (2005) meta-analysis.

Table 21 compares the blood lead levels associated with a change of 1 IQ point for each model with estimates from the previous evaluation by the Committee and the EFSA evaluation. It may be noted that while the central (median) estimates from the present analysis are quite similar to those presented by EFSA (Budtz-Jørgensen, 2010), the CIs are wider. This difference is attributable to the wider CIs present in the estimates from the Lanphear et al. (2005) meta-analysis. Similarly, the estimates from the 2000 evaluation by the Committee (Annex 1, reference *144*) do not have CIs, as they are based on a meta-analysis (Schwartz, 1993) that had no representation of uncertainty. The slight differences in central estimates are presumably attributable to the fact that the Schwartz (1993) meta-analysis does not include some of the newer studies used by Lanphear et al. (2005) and Budtz-Jørgensen (2010).

8.3.2 Dietary lead exposure and IQ

The relationship between dietary lead exposure and blood lead level was assumed to be linear with a slope somewhere between 0.052 and 0.16 µg/dl per 1 µg/day of lead exposure. The high end of this range comes from a USEPA (1986) analysis of infants consuming lead-contaminated drinking-water in Scotland. This estimate underlies the USEPA lead model used by EFSA (2010). The low end of the range comes from the 2000 reanalysis by the Committee (Annex 1, reference

Table 21. Blood lead levels associated with a decrease of 1 IQ point in children

Model	Blood lead level (μg/dl) associated with a decrease of 1 IQ point		
	FAO/WHO (2000)[a]	EFSA (2010): BMD (BMDL)	Present analysis: Central estimate (CI)
Linear	2.6	5.6 (4.1)[b]	5.1 (2.8–25)
Hockey stick	7.6	—	5.1 (2.8–25)
Mass action	—	—	1.4 (0.1–9)
Hill	6.9	—	8.5 (0.7–27)
Bilinear10	—	1.8 (1.2)	2.3 (0.9–19)
Bilinear	—	—	2.1 (0.8–17)

[a] Annex 1, reference *144*.
[b] From Budtz-Jørgensen (2010).

144) of the same data with an intercept parameter included that presumably accounts for other sources of lead exposure.

Since the Hill and bilinear models provided the best fit and have the characteristics of the other models, they were chosen for characterization of the dose–response relationship. Each of these two models is superimposed over the Lanphear et al. (2005) meta-analysis results in Figures 2 and 3, respectively. In order to integrate model uncertainty with the uncertainty inherent in the meta-analysis, a dose–response function was also developed with combined output from both the Hill and bilinear models, with each model considered to be equiprobable (i.e. the outputs from the 1000-iteration bootstrap analysis for each model were combined to produce a set of 2000 estimates). This function is shown in Figure 4. It may be noted that the uncertainty associated with this function is greater at low doses. This is attributable to the contribution of model uncertainty in addition to the uncertainty from the meta-analysis. In particular, the bilinear model tends to yield higher estimated impacts on IQ at low doses compared with the Hill model. Dietary exposures associated with specific decreases in IQ are presented in Table 22.

However, it should be noted that these estimates presume that there are no other exposures to lead. As dietary exposure is less than exposure from air, water, soil and paint for many people in the world, this assumption is often not correct. This is particularly important for the Hill model, where impacts on IQ are greatest when lead exposure is close to the ED_{50}. So, although a few micrograms per day may have a negligible impact on IQ without other exposures, such a dietary exposure may be a concern when other lead exposures push total exposure to the steeper part of the curve. As the bilinear model is linear at low doses, the impact of a given dietary exposure will be about the same regardless of what the other exposures are. Because the bilinear model may provide a better estimate when other exposures are unknown or highly variable, estimates using the bilinear model only are presented in Table 23.

Figure 2. Hill model bootstrap analysis, superimposed over Lanphear et al. (2005)

Note: The data points and associated CIs are derived from the the Lanphear et al. (2005) meta-analysis. The thin line and shaded area represent a log-linear model and associated CIs, which are also from the Lanphear et al. (2005) meta-analysis. The thicker line plots the Hill model from the current analysis, with associated CIs given by the dotted lines.

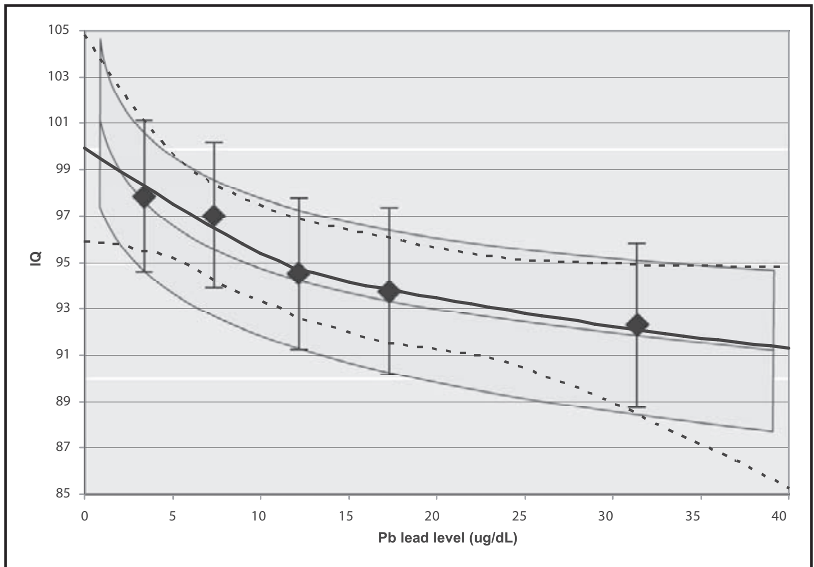
8.3.3 Blood lead and blood pressure in adults

For adults, increased systolic blood pressure was selected as the most sensitive end-point. A linear slope relating increases in systolic blood pressure as a function of blood lead level was derived by averaging the estimates from four different studies (Table 24). This resulted in a median of 0.28 mmHg (0.037 kPa) per 1 µg/dl, with an SD of 0.15 mmHg (0.02 kPa) per 1 µg/dl (5th to 95th percentiles 0.03–0.53 mmHg [0.004–0.071 kPa] per 1 µg/dl).

8.3.4 Dietary lead exposure and blood pressure in adults

Blood lead levels were converted to dietary exposures using the range of values previously used by the Committee for adults (blood lead level of 0.023–0.07 µg/dl per 1 µg/day of dietary lead exposure). Dietary exposure corresponding to an increase in systolic blood pressure of 1 mmHg (0.1333 kPa) was estimated to be 80 (5th to 95th percentiles 34–1700) µg/day, or about 1.3 (5th to 95th percentiles 0.6–28) µg/kg bw per day, assuming a body weight of 60 kg. As the relationship is linear, the changes in blood pressure associated with other dietary exposures are proportional.

Figure 3. Bilinear model bootstrap analysis, superimposed over Lanphear et al. (2005)



Note: The data points and associated CIs are derived from the Lanphear et al. (2005) meta-analysis. The thin line and shaded area represent a log-linear model and associated CIs, which are also from the Lanphear et al. (2005) meta-analysis. The thicker line plots the bilinear model from the current analysis, with associated CIs given by the dotted lines.

9. COMMENTS

9.1 Absorption, distribution, metabolism and excretion

Absorption of lead from the gastrointestinal tract is influenced by physiological factors (e.g. age, fasting, calcium and iron status, pregnancy) and the physicochemical characteristics of the ingested material. Absorption is higher in children than in adults and is lower in the presence of food. Absorbed lead is transferred to soft tissues, including liver and kidney, and to bone tissue, where it accumulates with age. Under certain conditions, such as pregnancy and osteoporosis, bone resorption can result in increased concentrations of lead in blood. Lead readily crosses the placenta and is transferred into breast milk. In humans, the half-life of lead is approximately 30 days in blood and 10–30 years in bone. Urine and faeces are the major routes of excretion. Lead binds to thiol groups and other ligands in proteins. Its toxicity has been attributed to inhibition of enzymes (e.g. those involved in haem synthesis) and to interference with calcium, magnesium and zinc homeostasis.

Figure 4. Dose–response function from pooled bilinear and Hill models

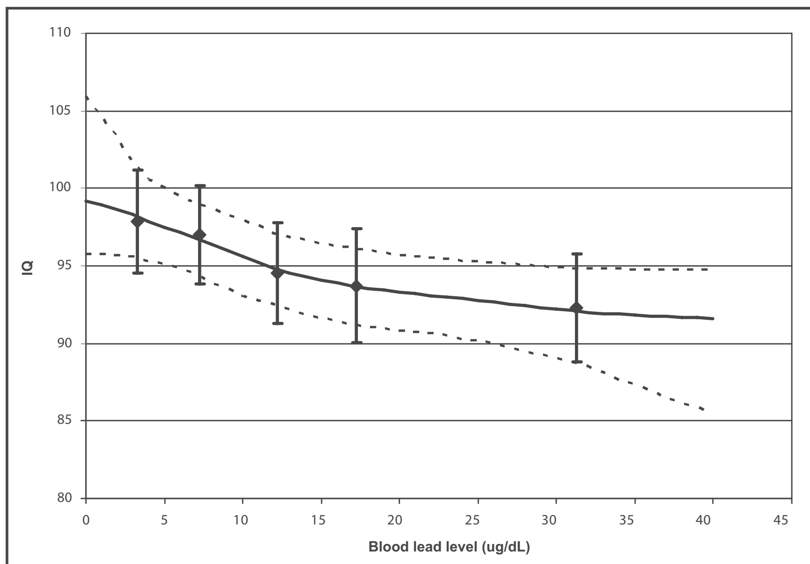


Table 22. Estimated dietary lead exposures associated with decreases in IQ in children using the combined outputs of the bilinear and Hill models

IQ decrease in children	Dietary exposure (µg/day)[a]	Dietary exposure for 20 kg child (µg/kg bw per day)[a]
0.5	17 (2–194)	0.8 (0.1–9.7)
1	30 (4–208)	1.5 (0.2–10.4)
1.5	40 (5–224)	2.0 (0.3–11.2)
2	48 (7–241)	2.4 (0.4–12.0)
2.5	55 (9–261)	2.8 (0.4–13.1)
3	63 (11–296)	3.1 (0.5–14.8)

[a] Median estimate with 5th to 95th percentile CI in parentheses.

9.2 Toxicological data

The acute toxicity of lead is low. Chronic oral exposure of experimental animals to inorganic lead has effects on multiple organs, including kidney and liver, and systems, including the cardiovascular, haematological, immune, reproductive and nervous systems. IARC has concluded that there is sufficient evidence for the carcinogenicity of inorganic lead compounds in experimental animals, causing renal and brain tumours, and that the evidence for the carcinogenicity of organic lead

Table 23. Estimated dietary exposures associated with IQ impacts using the bilinear model only[a]

IQ decrease in children	Dietary exposure (μg/day)[a]	Dietary exposure for 20 kg child (μg/kg bw per day)[a]
0.5	6 (2–124)	0.3 (0.1–6.2)
1	12 (4–145)	0.6 (0.2–7.2)
1.5	19 (6–170)	0.9 (0.3–8.5)
2	25 (8–193)	1.3 (0.4–9.7)
2.5	31 (9–217)	1.6 (0.5–10.9)
3	38 (11–237)	1.9 (0.6–11.8)

[a] Median estimate with 5th to 95th percentile CI in parentheses.

Table 24. Slope estimates relating blood lead level to systolic blood pressure

Reference	Slope estimate (mmHg[a] per 1 μg/dl)	
	Median estimate	Standard deviation
Glenn et al. (2003)	0.25	0.12
Vupputuri et al. (2003)	0.47	0.20
Nash et al. (2003)	0.32	0.19
Glenn et al. (2006)	0.09	0.05
Average	0.28	0.15

[a] 1 mmHg = 0.1333 kPa.

compounds is inadequate. The results of genotoxicity studies and the inhibition of DNA repair suggest a non-DNA-reactive mode of action for the carcinogenicity of lead.

9.3 Observations in humans

There is an extensive body of literature on epidemiological studies of lead. Blood is the tissue used most frequently to estimate exposure to lead, and blood lead levels generally reflect exposure in recent months. However, if the level of exposure is relatively stable, then blood lead level is a good indicator of exposure over the longer term. Longitudinal surveys in some countries have shown substantial reductions in population blood lead levels in recent decades. Programmes such as those that have eliminated the use of leaded petrol are considered to be an important factor, resulting in an average reduction of 39% in mean blood lead level over the 5-year period following implementation. Reductions

in population blood lead levels in some countries have also been associated with the discontinued use of lead solder in food cans.

Exposure to lead has been shown to be associated with a wide range of effects, including various neurological and behavioural effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, delayed sexual maturation and impaired dental health. IARC concluded that there is *sufficient evidence* in animals but only *limited evidence* in humans for the carcinogenicity of inorganic lead and that inorganic lead compounds are *probably carcinogenic* to humans (group 2A). More recent studies do not indicate that any revision to the IARC conclusions is required.

For children, the weight of evidence is greatest, and evidence across studies is most consistent, for an association of blood lead levels with impaired neuro-development, specifically reduction of IQ. Moreover, this effect has generally been associated with lower blood lead concentrations than those associated with the effects observed in other organ systems. Although the estimated IQ decrease per microgram of lead per decilitre of blood is small when viewed as the impact on an individual child (6.9 points over the range of 2.4–30 µg/dl), the decrement is considered to be important when interpreted as a reduction in population IQ. For example, if the mean IQ were reduced by 3 points, from 100 to 97, while the standard deviation and other characteristics of the distribution remained the same, there would be an 8% increase in the number of individuals with a score below 100. Moreover, there would be a 57% increase in the number of individuals with an IQ score below 70 (2 standard deviations below the expected population mean, commonly considered to be the cut-off for identifying individuals with an intellectual disability) and a 40% reduction in the number of individuals with an IQ score greater than 130 (considered to be the cut-off for identifying individuals with a “very superior” IQ). Furthermore, the Committee noted that a lead-associated reduction in IQ may be regarded as a marker for many other neurodevelopmental effects for which the evidence is not as robust but which have been observed in children at approximately the same blood lead levels (e.g. ADHD, reading deficit, executive dysfunction, fine motor deficit).

For adults, the adverse effect for which the weight of evidence is greatest and most consistent is a lead-associated increase in blood pressure. As with the lead-associated reduction in IQ, the increase is small when viewed as the effect on an individual’s blood pressure, but important when viewed as a shift in the distribution of blood pressure within a population. Increased blood pressure is associated with increased risk of cardiovascular mortality. In a meta-analysis of 61 prospective studies involving more than 1 million adults, increased blood pressure was associated with age-specific increased mortality rates for ischaemic heart disease and stroke, and the proportional difference in risk associated with a given absolute difference in blood pressure was similar at all blood pressures above 115 mmHg (15 kPa) systolic or 75 mmHg (10 kPa) diastolic.

9.4 Analytical methods for the determination of lead in food and blood

The analytical methods for the determination of lead in food are well established. The techniques of choice are ETAAS and ICP-MS. To a minor extent, FAAS and ICP-OES are used. In the last decade, many technical improvements have been made to ETAAS, such as the design of the atomizer, background correction systems and improvement in the light source and detector. These have allowed the determination of lead in food at the low microgram per kilogram level. ICP-MS is increasingly used in food laboratories owing to its capability to perform multi-element measurements in a wide variety of food matrices. In addition, the use of DRC-ICP-MS has allowed the removal of interferences with a minimum loss of sensitivity, while lowering the LOQs for lead, to allow the determination of lead in food at levels lower than 0.1 µg/kg.

The determination of lead in blood has been carried out using mainly ETAAS or ICP-MS. The methods are well established, and the LODs at the 0.1 ng/ml level are adequate to quantify lead in blood. Sample preparation is simple, but advances can be made in reducing the volume of sample required for analyses. One novel technique is the use of laser ablation coupled with ICP-MS, which requires a sample volume of less than 1 µl of whole blood for the quantification of lead.

The sample preparation procedure used most frequently for the determination of lead in food is acid digestion in the presence of strong oxidants in open or closed vessels. Microwave-assisted acid digestion has been extensively employed, which allows the use of large sample masses (1–2 g) under controlled temperature and pressure of the system, reducing contamination and avoiding losses of the element during mineralization.

Lead data for different food commodities submitted and evaluated at this meeting were almost all obtained by validated analytical methods or generated by accredited laboratories. The LODs and LOQs depend on the food matrix and the analytical technique employed. Analytical methods with poor LODs (>0.01 mg/kg) may erroneously lead to the conclusion that there is no lead present in the food.

As an example, Australia used a more sensitive analytical method for its 23rd TDS than previously used in its 19th and 20th TDSs. This resulted in a significant increase in the percentage of samples with detectable lead. However, more sensitive methods require greater resources and may limit the numbers of samples that can be analysed. Therefore, an appropriate balance in number of samples that can be analysed and the sensitivity of the method will be required in the planning of surveillance programmes.

9.5 Sampling protocols

General guidance for sampling for foods is described in the Codex Alimentarius Commission guidelines CAC/GL 50-2004 (FAO/WHO, 2004a).

9.6 Prevention and control

There have been widespread efforts to reduce lead exposure from food, focusing on implementing standards for lead levels in food, water and food additives;

ending the use of lead-soldered cans; regulating the use of lead in paint and petrol; controlling lead levels in water; reducing leaching from lead-containing vessels; and identifying and reacting to additional sources of lead contamination in foods or dietary supplements. Dust on foods should be removed before processing and/or consumption. For the prevention and control of lead in foods, good agricultural and manufacturing practices should be followed.

9.7 Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed data on lead occurrence in different food commodities received from seven countries—Australia, Brazil, China, France, Germany, Singapore and the USA. In addition, EFSA submitted data from Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Norway, Poland, Romania, Spain and Sweden and three commercial operators. The data from France and Germany were included in the assessment report of EFSA. In order to avoid duplicating the data in this analysis, the individual data submitted from both countries were not separately considered in the assessment of the current meeting.

The total number of analytical results (single or composite samples) was 110 899, with 84.9% coming from Europe, 7.6% from the USA, 1.9% from Latin America, 3.1% from Asia and 2.5% from the Pacific region. No data were received from Africa.

A summary of the available occurrence data submitted for this meeting by food category is presented in Table 25. The weighted mean is provided for each food category and for the range of means across countries. The means were weighted to adjust for different numbers of samples for foods within a category from different countries. All but one food category contained at least some foods with detectable lead levels. Maximum lead concentrations were determined for each category. However, some data sets, such as the Chinese TDS, provided mean lead concentrations only, so it was not possible to determine maximum concentrations for every data set. Thus, data contributing high concentrations to the country mean could not be included in the evaluation of the maximum concentrations. Each category contains a number of foods with similar characteristics (e.g. baked goods, muscle meats). The miscellaneous category includes beverages, food supplements, infant formula, tap and bottled water and other foods for special dietary purposes, as well as foods that did not fit in other categories. Within the miscellaneous category, generally the highest reported concentrations were for foods for special dietary uses and not for beverages. Infant formula essentially contained no detectable lead. EFSA reported that breast milk contained highly variable levels of lead. Sugar and sugar products and animal and vegetable fats rarely contained detectable levels of lead. Food categories with the highest frequency of detectable lead include meat, especially offal, organ meats and wild game, shellfish (particularly bivalves), cocoa, tea, cereal grains and cereal products, and vegetables.

Table 25. Summary of lead occurrence data submitted for this meeting

Food category	*n*	Weighted mean lead concentration (mg/kg)[a]	Range of national mean concentrations (mg/kg)[b]	Maximum lead concentration (mg/kg)
Cereals/grains not included elsewhere and mixed grains	5 027	0.009	<LOD–0.029	7.12
Wheat (including breads)	506	0.005	<LOD–0.009	0.040
Rice	85	0.002	<LOD–0.004	0.021
Baked goods including "fancy breads"	203	0.047	0.001–0.23	16.5
Oats	63	0.001	<LOD–0.003	0.050
Roots and tubers	1 255	0.007	0.001–0.065	1.32
Pulses + legumes	326	0.004	<LOD–0.060	0.063
Fruits	7 480	0.030	<LOD–0.13	28.9
Dried fruit	282	0.086	0.006-0.34	1.34
Fruit juices	4 426	0.058	<LOD–0.35	74
Vegetables including juices	13 402	0.101	<LOD–0.40	27.6
Eggs	785	0.008	<LOD–0.039	0.21
All seafood (EFSA only)	11 453	0.054	—	4.06
Snails	11	0.069	0.065–0.074	0.19
Finfish	656	0.040	<LOD–0.22	0.45
Shellfish	765	0.070	0.010–0.19	11.80
Aquatic animals (China only)	12	0.015	—	—
Dairy foods	3 833	0.006	0.001–0.013	4.55
Nuts and oilseeds	184	0.005	<LOD–0.024	0.30
Animal fats	102	0.001	<LOD–0.002	0.029
Vegetable oils and fats	832	0.007	<LOD–0.039	7.30
Stimulants (coffee, tea, cocoas)[c]	764	0.211	<LOD–1.03	6.21

Table 25 (contd)

Food category	*n*	Weighted mean lead concentration (mg/kg)[a]	Range of national mean concentrations (mg/kg)[b]	Maximum lead concentration (mg/kg)
Sugar and honey	1 962	0.032	<LOD–0.082	4.10
Spices	86	0.027	<LOD–0.11	0.44
Alcoholic beverages	2 304	0.070	<LOD–0.38	5.80
Cocoa & chocolate & products[c]	206	0.692	<LOD–0.69	45.4
Cocoa butter	34	<LOD	<LOD	<LOD
Muscle meat excluding poultry	1 817	0.047	0.0001–0.013	1.36
Meat not included elsewhere	131	0.420	0.22–0.25	10.10
Organ meats except kidney	102	0.140	0.10–0.18	1.44
Muscle meat and poultry combined	40 313	0.134	0.004–0.25	867
Muscle minced	69	0.001	0.001	0.078
Kidney	537	0.067	0.013–0.14	1.24
Muscle poultry	1 589	0.098	0.003–0.021	0.075
Offal	73	0.018	0.006–0.042	0.008
Miscellaneous	9 224	0.035	<LOD–0.20	155
Total	110 899	—	—	—

[a] The means were weighted to adjust for different numbers of samples for foods within a category.
[b] Range includes means from the 2007 Chinese TDS and the 20th Australian TDS; maximum lead values were not available from the Chinese TDS and the 20th Australian TDS.
[c] In some cases, cocoas were included in a stimulants category, and in others, they were separately categorized.

9.8 Food consumption and dietary exposure assessment

The Committee obtained estimates of exposure to lead based on TDSs for nine countries (Australia, Canada, Chile, China, France, Lebanon, New Zealand, the United Kingdom and the USA) or from other evaluations that had considered levels in foods as consumed (Egypt, India and EFSA). EFSA conducted assessments for 19 European countries, and those are presented together.

The guidelines for conducting exposure assessments for contaminants in foods recommend that dietary exposure estimates should be calculated using regional average contaminant concentration data and the GEMS/Food consumption cluster diets. The WHO GEMS/Food consumption cluster diets contain limited information on the forms of the foods that are considered. Dietary exposure estimates were available to the Committee for 28 countries, mostly based on food as consumed. Lead is taken up from soil into food crops, and the sources of lead in food may also include soil remaining in or on the food, atmospheric deposition, water, contact with lead-containing processing equipment and packaging. It is important to estimate lead levels in food that is as close as possible to the form of the food that is consumed, as levels in raw agricultural commodities do not necessarily reflect levels in foods as they are consumed. The Committee concluded that the submitted data reflected lead exposures in foods as consumed and were more appropriate than the GEMS/Food consumption cluster diets for use in lead assessments. Limited information was available describing lead levels in foods or estimating dietary exposures in developing countries.

The Committee included estimates of children's exposure wherever possible. The GEMS/Food consumption cluster diets do not include estimates of children's consumption. Estimates of children's exposure were available for 19 European countries (in the EFSA assessment) and for Australia, Canada, China, New Zealand and the USA. Where exposure assessments were available for the adult population but not for children, the Committee assumed that children's exposure would be 2–3 times that of the general population on a body weight basis, based on the general observation that children consume 2–3 times more food than adults relative to their body weight, and included those values in this report.

Estimates of dietary exposure for individual countries are presented below. Each region/country made its own decisions as to the appropriate matching of food lead levels to food consumption data and also in the treatment of samples without detectable lead levels.

The Committee selected a representative dietary exposure value for each country in order to allow comparisons across countries and across regions for the total/adult population (see Table 19 in section 7.3.14 above) and for children (see Table 20 in section 7.3.14 above). Unfortunately, estimates for the same population subgroup were not always available. In particular, estimates were provided for different age groups by different countries. The Committee selected subgroups that were as similar as possible for comparison purposes. In order to improve comparability, the Committee adjusted some data by standard body weight assumptions. For the total/adult population, mean exposures ranged from 0.02 to 3 μg/kg bw per day (see Table 19). Some of the countries also provided estimates of high exposure for consumers. The definition of a consumer with high exposure ranged from the 90th to 97.5th percentile for the population, depending on the country. The estimated high exposures ranged from 0.06 to 2.43 μg/kg bw per day (see Table 19). Children's mean exposures ranged from 0.03 to 9 μg/kg bw per day (see Table 20). Some countries also provided estimates of high exposures for children. The definition of a consumer with high exposure ranged from the 90th to 97.5th percentile exposures for children. The estimated exposures for children who

were defined by the country as consumers with high exposure ranged from 0.2 to 8.2 µg/kg bw per day (see Table 20).

9.8.1 Food category contributions to exposure

The most important contributors to overall dietary exposure were reported by some countries. EFSA evaluated the categories of foods contributing most to exposure and reported large differences between countries. EFSA reported that

> the largest contributors to the calculated overall lead exposure are vegetables, nuts and pulses contributing 19 % to the lower bound and 14 % to the upper bound estimates. Cereals and cereal products contributed 13 % to the lower bound and 14 % to the upper bound. For the lower bound miscellaneous products and food for special uses contributed 12 %, starchy roots and potatoes 8%, meat and meat products 8 %, alcoholic beverages 7 % and milk and dairy products 6 %. For the upper bound the contributions were: juices, soft drinks and bottled water (11 %), alcoholic beverages (9%) meat and meat products including offal (9 %), milk and dairy products (8 %), miscellaneous products and food for special uses (7 %) and starchy roots and potatoes (6 %).

Milk and milk products, fruits, breads and sugars contributed most to the dietary exposure in a published Chilean TDS. In the 2007 Chinese TDS, the food categories making the largest contributions were cereals (34%) and vegetables (21%). The Lebanese TDS included water and food, water contributing the most to exposure. The foods contributing most to Lebanese exposure were bread and toast, fruits, pizza and pies, and vegetables (raw and cooked). In the New Zealand TDS, grains contributed 24–27% of dietary lead for adults and 36–39% for children. Chicken, eggs, fish and meat contributed 12–16% of adult dietary lead, and takeaways contributed 9–24%; for children, the corresponding contributions were 7–12% and 10–15%. New Zealand also identified the main food groups contributing to weekly dietary exposure to lead for infants: grains (18%), chicken, eggs, fish and meat (4%), takeaways (6%), fruit (18%) and infant formula and weaning foods (38%).

The relative contribution of diet to total lead exposure is not well known but will probably vary depending upon locale and the contribution from non-dietary sources. Estimates from EFSA suggest that at least half of children's exposure may be due to non-dietary sources of exposure and that soil and dust are major contributors to the non-dietary exposures.

9.8.2 Temporal changes in estimates of dietary exposure to lead since the 1980s

Lead levels in foods have declined over time in many developed countries. The Committee had access to data from five countries (Canada, France, New Zealand, the United Kingdom and the USA) that allowed the trends in lead exposure to be estimated. New Zealand reported changes in dietary exposure to lead since 1982 in its 2003–2004 TDS report. Lead exposure estimates for 19- to 24-year-old males were 3.6 µg/kg bw per day in 1982 and 0.13 µg/kg bw per day in 2003–2004. This represents an apparent decline in exposure to lead of approximately 75%. Dietary exposure estimates for the general population in the United Kingdom declined by approximately 95% between 1980 and 2006, from 0.12 mg/day

estimated in the 1980 TDS to 0.006 mg/day in the 2006 TDS. Canada and France have also reported a 50% decline in exposure to lead over the past 10–15 years. The USA reported declines in lead exposure for all age groups, with the greatest decline in teenage males (from 70 µg/day in 1976 to 3.45 µg/day in 2000). During the time periods reported by these countries, there were changes in the food supply that likely contributed to actual declines in dietary exposures. However, some of the apparent decline in exposure may actually be due to improved sensitivity of the analytical methods and the corresponding selection of less conservative values for those samples without detectable levels of lead.

9.9 Dose–response analysis

The dose–response modelling for blood lead levels and children's IQ is based on estimates in the Lanphear et al. (2005) pooled analysis, which includes several newer studies that were not included in the meta-analysis used by the Committee at its fifty-third meeting (Annex 1, reference *143*). The Lanphear et al. (2005) analysis included 1333 children enrolled in seven longitudinal cohort studies conducted in the USA, Mexico, Kosovo and Australia, who were followed from birth or early infancy to 5–10 years of age. In this analysis, use of a log-linear model produced an estimated IQ decline of 6.9 points in concurrent blood lead level over a range of 2.4–30 µg/dl. The slope of the inverse association between IQ and concurrent blood lead level was steeper among children with a maximum observed (at any time point) blood lead level below 7.5 µg/dl than it was among children with a maximum blood lead level of 7.5 µg/dl or higher. After initial consideration of six different dose–response models, the bilinear and Hill models were selected for use in characterizing the dose–response relationship between blood lead level and IQ because they provided the best fit.

The relationship between blood lead levels and dietary exposure to lead was estimated to be between 0.052 and 0.16 µg/dl of lead in blood per 1 µg/day of dietary lead exposure. This range was based on toxicokinetic analyses of data on Scottish infants exposed to lead in drinking-water. These analyses were used by the Committee previously.

Dietary exposures associated with a range of decreases in IQ (i.e. 0.5–3 IQ points) were calculated by combining the dose–response models with the toxicokinetic data, using a Monte Carlo simulation. The resulting CIs reflect the uncertainties in both the dose–response modelling of blood lead levels and the extrapolation to dietary exposure. When the outputs from the Monte Carlo simulation of the alternative bilinear and Hill models were combined, the chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 30 µg of lead per day, with a 5th to 95th percentile CI ranging from 4 to 208 µg/day (see Table 22 in section 8.3.2 above). This is equivalent to 1.5 µg/kg bw per day (5th to 95th percentiles 0.2–10.4 µg/kg bw per day) for a 20 kg child.

Although the combined outputs of the bilinear and Hill models provide a more complete accounting of the uncertainties associated with the dose–response relationship of lead and IQ, the bilinear model may be more useful in circumstances where other, non-dietary exposures are highly variable or unknown, because the

incremental effect of any given lead source/exposure is theoretically independent of other exposures (i.e. the impact of a given dietary exposure will be about the same, regardless of other exposures). Using the bilinear model alone, the chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 12 µg/day, with a 5th to 95th percentile CI ranging from 4 to 145 µg/day (see Table 23 in section 8.3.2 above). This is equivalent to 0.6 µg/kg bw per day (5th to 95th percentiles 0.2–7.2 µg/kg bw per day) for a 20 kg child. The Committee decided to use the results of the bilinear model in its evaluation because it represents a more conservative approach at low doses and allows non-dietary sources of exposure to be considered independently. However, application of the results of the combined model outputs might be more appropriate in situations where non-dietary exposure is minimal.

For adults, increased systolic blood pressure was selected as the most sensitive end-point. A linear slope relating increases in systolic blood pressure as a function of blood lead level was derived by averaging the estimates from four different studies: 0.28 mmHg (0.037 kPa) per 1 µg/dl (5th to 95th percentiles 0.03–0.53 mmHg [0.004–0.071 kPa] per µg/dl). Blood lead levels were converted to dietary exposures using the range of values previously used by the Committee for adults (blood lead level 0.023–0.07 µg/dl per 1 µg/day of dietary lead exposure). Dietary exposure corresponding to an increase in systolic blood pressure of 1 mmHg (0.1333 kPa) was estimated to be 80 (5th to 95th percentiles 34–1700) µg/day, or about 1.3 (5th to 95th percentiles 0.6–28) µg/kg bw per day. As the relationship is linear, the increases in blood pressure associated with other dietary exposures are proportional. Published studies used by WHO in estimating the global burden of disease attributable to lead indicate that relative risks of ischaemic heart disease and cerebrovascular stroke associated with small increases in blood pressure (0.4–3.7 mmHg [0.053–0.49 kPa] systolic blood pressure) have been estimated to be in the range of 1.01–1.4, with higher relative risks at younger ages.

10. EVALUATION

Exposure to lead is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes. Impaired neurodevelopment in children is generally associated with lower blood lead concentrations than the other effects, the weight of evidence is greater for neurodevelopmental effects than for other health effects and the results across studies are more consistent than those for other effects. For adults, the adverse effect associated with lowest blood lead concentrations for which the weight of evidence is greatest and most consistent is a lead-associated increase in systolic blood pressure. Therefore, the Committee concluded that the effects on neuro-development and systolic blood pressure provided the appropriate bases for dose–response analyses.

Based on the dose–response analyses, the Committee estimated that the previously established PTWI of 25 µg/kg bw is associated with a decrease of at least 3 IQ points in children and an increase in systolic blood pressure of approximately

3 mmHg (0.4 kPa) in adults. These changes are important when viewed as a shift in the distribution of IQ or blood pressure within a population. The Committee therefore concluded that the PTWI could no longer be considered health protective, and it was withdrawn.

Because the dose–response analyses do not provide any indication of a threshold for the key effects of lead, the Committee concluded that it was not possible to establish a new PTWI that would be considered to be health protective. The dose–response analyses conducted by the Committee should be used to identify the magnitude of effect associated with identified levels of dietary lead exposure in different populations.

The Committee reaffirmed that because of the neurodevelopmental effects, fetuses, infants and children are the subgroups that are most sensitive to lead. The mean dietary exposure estimates for children aged about 1–4 years range from 0.03 to 9 µg/kg bw per day. The health impact at the lower end of this range is considered negligible by the Committee, because it is below the exposure level of 0.3 µg/kg bw per day calculated to be associated with a population decrease of 0.5 IQ point. The higher end of the exposure range is higher than the level of 1.9 µg/kg bw per day calculated to be associated with a population decrease of 3 IQ points, which is deemed by the Committee to be a concern. For adults, the mean dietary lead exposure estimates range from 0.02 to 3 µg/kg bw per day. The lower end of this range (0.02 µg/kg bw per day) is considerably below the exposure level of 1.2 µg/kg bw per day calculated by the Committee to be associated with a population increase in systolic blood pressure of 1 mmHg (0.1333 kPa). The Committee considered that any health risk that would be expected to occur at this exposure level is negligible. At the higher end of the range (3 µg/kg bw per day), a population increase of approximately 2 mmHg (0.3 kPa) in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated, in a large meta-analysis, with modest increases in the risks of ischaemic heart disease and cerebrovascular stroke.

The Committee considered this to be of some concern, but less than that for the neurodevelopmental effects observed in children.

The Committee stressed that these estimates are based on dietary exposure (mainly food) and that other sources of exposure to lead also need to be considered. The Committee concluded that, in populations with prolonged dietary exposures to lead that are at the higher end of the ranges identified above, measures should be taken to identify major contributing sources and foods and, if appropriate, to identify methods of reducing dietary exposure that are commensurate with the level of risk reduction.

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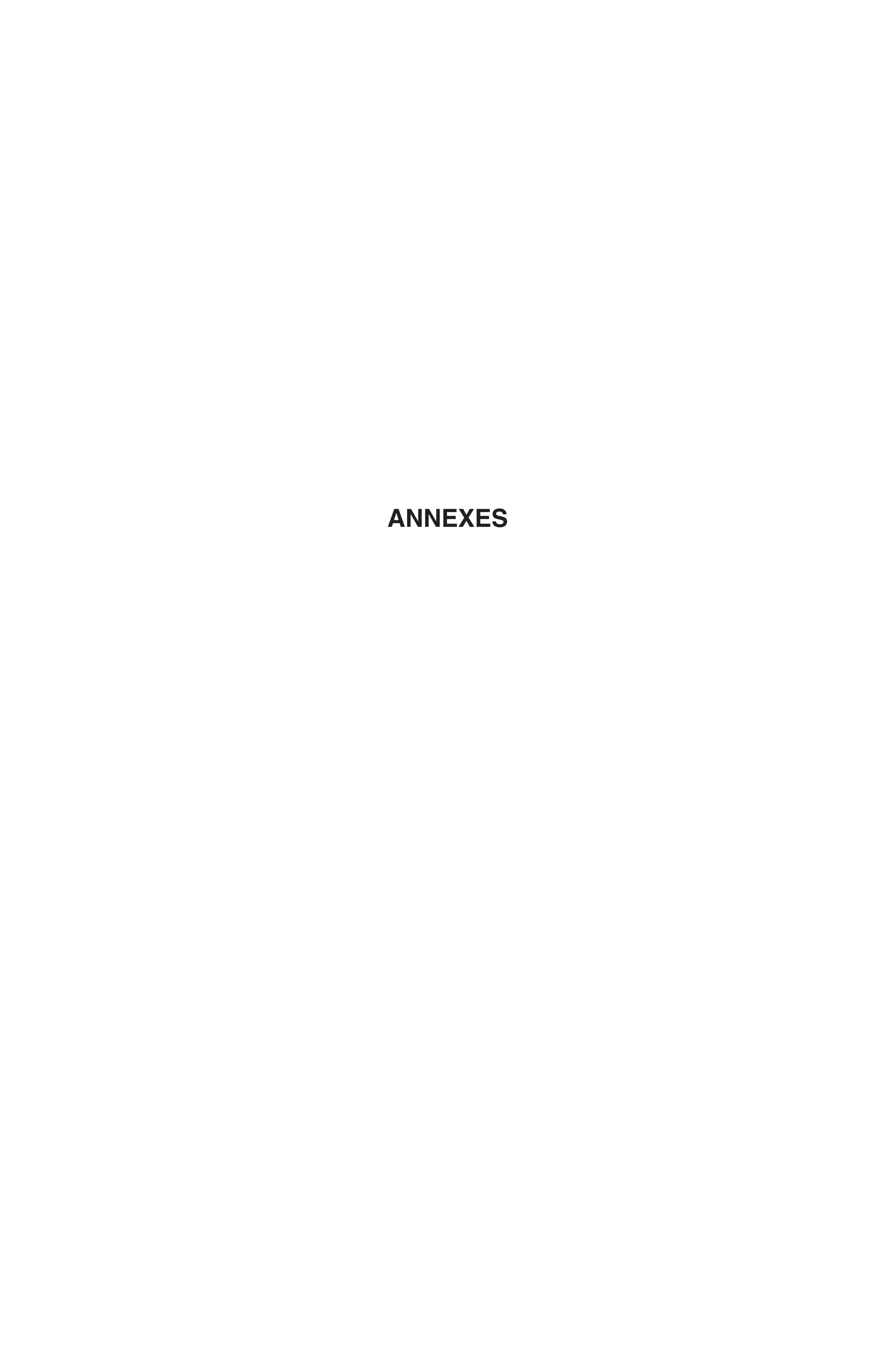

ANNEXES

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 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 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.
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 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; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
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 Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
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.
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.
22. E*valuation 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 Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
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.
25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
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 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.
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.
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 Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, 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 Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some 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.
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 Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

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, 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 Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
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 Meetings 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 antioxidant, 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.
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.
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.* WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
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.* WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
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.* WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.
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.* WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
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.* WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41, 1988.
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.* WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
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.
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ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

α1MG	α_1-microglobulin
β2MG	β_2-microglobulin
ADHD	attention deficit hyperactivity disorder
ADI	acceptable daily intake
ALA	aminolaevulinic acid
ALAD	δ-aminolaevulinic acid dehydratase
ALAS	δ-aminolaevulinic acid synthase
ALS	amyotrophic lateral sclerosis
ATPase	adenosine triphosphatase
BIFF-AAS	beam injection flame furnace atomic absorption spectrometry
BMD	benchmark dose
BMD_x	benchmark dose for an x% response
BMDL	95% lower confidence limit on the benchmark dose
$BMDL_x$	95% lower confidence limit on the benchmark dose for an x% response
BMI	body mass index
BMR	benchmark response
bw	body weight
CAS	Chemical Abstracts Service
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	confidence interval
CL	chemiluminescence
CRM	certified reference material
CTX	serum crosslinked C-telopeptide of type I collagen
CYP	cytochrome P450
DC_{50}	concentration at which a test material would induce deoxyribonucleic acid damage in 50% of the cells
DHB	dihydroxybenzoic acid
DMT1	divalent metal transporter 1
DNA	deoxyribonucleic acid
DPASV	differential pulse anodic stripping voltammetry
DRC-ICP-MS	dynamic reaction cell technology combined with inductively coupled plasma mass spectrometry
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
DTH	delayed-type hypersensitivity
EC_{50}	median effective concentration
ED_{50}	median effective dose
EDTA	ethylenediaminetetraacetic acid
EF	enrichment factor

EFSA	European Food Safety Authority
ETAAS	electrothermal (graphite furnace and Zeeman furnace) atomic absorption spectrometry
F	female; filial generation (e.g. F_0, F_1)
FAAS	flame atomic absorption spectrometry
FAO	Food and Agriculture Organization of the United Nations
FOB	functional observational battery
FSANZ	Food Standards Australia New Zealand
GABA	γ-aminobutyric acid
GD	gestational day
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GFAAS	graphite furnace atomic absorption spectrometry
GFR	glomerular filtration rate
GLP	good laboratory practice
GSD	geometric standard deviation
HG-AFS	hydride generation atomic fluorescence spectrometry
HGPRT	hypoxanthine–guanine–phosphoribosyl transferase
HR	hazard ratio
HR-CS-ETAAS	high-resolution continuum source electrothermal atomic absorption spectrometry
HR-ICP-MS	high-resolution inductively coupled plasma mass spectrometry
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ICP-SMS	double focusing sector field inductively coupled plasma mass spectrometry
IQ	intelligence quotient
IUCLID	International Uniform Chemical Information Database
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_i	inhibition constant
LA-ICP-MS	laser ablation coupled with inductively coupled plasma mass spectrometry
LC_{50}	median lethal concentration
LC-PDA-MS	liquid chromatography–photodiode array–mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
LOR	limit of reporting
M	male

MBT	2-mercaptobenzothiazole
ML	maximum level
MSDI	maximized survey-derived intake
MTP1	metal transporter protein 1
NA	not applicable; not available
NAG	*N*-acetyl-β-D-glucosaminidase
ND	no data reported; not detected; non-detects; not determined
NFS	not further specified
NHANES	National Health and Nutrition Examination Survey (USA)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NR	not required for evaluation; not reported
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
OSCAR	**Os**teoporosis—**Ca**dmium as a **R**isk Factor
P	parental generation (e.g. P_1)
PAN	1-(2-pyridylazo)-2-naphthol
PBPK	physiologically based pharmacokinetic
pHC	protein heterogeneous in charge
PON1	paraoxanase
ppm	parts per million
PROT	protein
PTMI	provisional tolerable monthly intake
PTWI	provisional tolerable weekly intake
QA	quality assurance
r	correlation coefficient
R	recovery
RBP	retinol-binding protein
rpm	revolutions per minute
RR	relative risk
S9	9000 × *g* supernatant from rat liver
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SF-ICP-MS	sector field inductively coupled plasma mass spectrometry
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
SIMAAS	simultaneous atomic absorption spectrometry
SPE	solid-phase extraction
SPET	single portion exposure technique
SS-ZAAS	solid sampling Zeeman atomic absorption spectrometry

SWASV	square wave anodic stripping voltammetry
T_3	triiodothyronine
T_4	thyroxine
TDS	total diet study
Th	T helper
TK	thymidine kinase
T_{max}	time to maximum concentration
TS-FF-AAS	thermospray flame furnace atomic absorption spectrometry
TSH	thyroid stimulating hormone
U	units
UDS	unscheduled DNA synthesis
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USP	United States Pharmacopeia
UV	ultraviolet
UVC	ultraviolet C
WHO	World Health Organization
ZF-AAS	Zeeman furnace atomic absorption spectrometry

ANNEX 3

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 8–17 June 2010

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ANNEX 4

TOLERABLE INTAKES, OTHER TOXICOLOGICAL INFORMATION AND INFORMATION ON SPECIFICATIONS

FOOD ADDITIVES CONSIDERED FOR SPECIFICATIONS ONLY

Food additive	Specifications[a]
Activated carbon	R
Annatto extract (oil-processed bixin)	W
Cassia gum	R
Indigotine	R
Steviol glycosides	R
Sucrose esters of fatty acids	R
Sucrose monoesters of lauric, palmitic or stearic acid	N, T
Titanium dioxide	R

[a] N, new specifications; R, existing specifications revised; T, tentative specifications; W, existing specifications withdrawn.

FLAVOURING AGENTS EVALUATED BY THE PROCEDURE FOR THE SAFETY EVALUATION OF FLAVOURING AGENTS[1]

A. Alicyclic ketones, secondary alcohols and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Cyclohexanone diethyl ketal	2051	N	No safety concern
3,3,5-Trimethylcyclohexyl acetate	2053	N	No safety concern

[1] The flavouring agent 2-aminoacetophenone (No. 2043) was on the agenda to be evaluated in the group of aromatic substituted secondary alcohols, ketones and related esters. Although the compound fulfils some of the structural requirements for this group, the main toxicologically relevant structural feature is the amino group; hence, the compound was not evaluated and should be evaluated in the future in the group of aliphatic and aromatic amines and amides. The flavouring agent (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) was on the agenda to be evaluated in the group of benzyl derivatives. However, as this compound did not meet the structural requirements for this group, the compound was not evaluated at this meeting.

A. (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
2-(trans-2-Pentenyl)cyclopentanone	2049	N	No safety concern
2-Cyclopentylcyclopentanone	2050	N	No safety concern
2-Cyclohexenone	2052	N	No safety concern
2,6,6-Trimethyl-2-hydroxycyclohexanone	2054	N	No safety concern
Cyclotene propionate	2055	N	No safety concern
Cyclotene butyrate	2056	N	No safety concern
4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (mixture of isomers)	2057	N	No safety concern
4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (mixture of isomers)	2058	N	No safety concern
Structural class III			
(−)-8,9-Dehydrotheaspirone	2059	N	No safety concern
(±)-2,6,10,10-Tetramethyl-1-oxaspiro[4.5] deca-2,6-dien-8-one	2060	N	No safety concern

[a] N, new specifications.

B. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
cis-4-(2,2,3-Trimethylcyclopentyl) butanoic acid	1899	N	No safety concern
Mixture of 2,4-, 3,5- and 3,6-Dimethyl-3-cyclohexenylcarbaldehyde	1900	N	No safety concern
(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	1902	N	No safety concern
d-Limonen-10-ol	1903	N	No safety concern
p-Menthan-7-ol	1904	N	No safety concern

B (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
p-Menth-1-en-9-ol	1905	N	No safety concern
1,3-*p*-Menthadien-7-al	1906	N	No safety concern
Structural class II			
Methyl dihydrojasmonate	1898	N	No safety concern
cis- and *trans*-2-Heptylcyclopropanecarboxylic acid	1907	N	No safety concern
(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl)cyclopropanecarbaldehyde	1908	N	No safety concern
Structural class III		N	
Perillaldehyde propyleneglycol acetal	1901	N	No safety concern

[a] N, new specifications.

C. Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones

Flavouring agent	No.	Specifications[a]	Conclusion based oncurrent estimated dietary exposure
Structural class II			
3-Methyl-2,4-nonedione	2032	N	No safety concern
Mixture of 3-Hydroxy-5-methyl-2-hexanone and 2-Hydroxy-5-methyl-3-hexanone	2034	N	No safety concern
3-Hydroxy-2-octanone	2035	N	No safety concern
2,3-Octanedione	2036	N	No safety concern
4,5-Octanedione	2037	N	No safety concern
(±)-2-Hydroxypiperitone	2038	N	No safety concern
Structural class III			
Acetoin propyleneglycol ketal	2033	N	No safety concern
1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	2039	N	No safety concern

[a] N, new specifications.

D. Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Dimethylbenzyl carbinyl crotonate	2025	N	No safety concern
Dimethylbenzyl carbinyl hexanoate	2026	N	No safety concern
Caryophyllene alcohol	2027	N	No safety concern
Cubebol	2028	N	No safety concern
(−)-Sclareol	2029	N	No safety concern
(+)-Cedrol	2030	N	No safety concern
α-Bisabolol	2031	N	No safety concern

[a] N, new specifications.

E. Aliphatic and aromatic amines and amides

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Choline chloride	2003	N	No safety concern
3-(Methylthio)propylamine	2004	N	No safety concern
Structural class III			
N-Ethyl-2,2-diisopropylbutanamide	2005	N	Additional data required to complete evaluation
Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide	2006	N	No safety concern
(±)-*N*-Lactoyl tyramine	2007	N	Additional data required to complete evaluation
N-(2-(Pyridin-2-yl)ethyl)-3-p-menthanecarboxamide	2008	N	No safety concern
N-p-Benzeneacetonitrile menthanecarboxamide	2009	N	No safety concern
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	N	Additional data required to complete evaluation
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	N	Additional data required to complete evaluation

[a] N, new specifications.

F. Aliphatic lactones

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
5-Pentyl-3H-furan-2-one	1989	N	No safety concern
5-Hydroxy-4-methylhexanoic acid δ-lactone	1990	N	No safety concern
Isoambrettolide	1991	N	No safety concern
7-Decen-4-olide	1992	N	No safety concern
9-Decen-5-olide	1993	N	No safety concern
8-Decen-5-olide	1994	N	No safety concern
Orin lactone	1995	N	No safety concern
9-Dodecen-5-olide	1996	N	No safety concern
9-Tetradecen-5-olide	1997	N	No safety concern
γ-Octadecalactone	1998	N	No safety concern
δ-Octadecalactone	1999	N	No safety concern
Structural class III			
4-Hydroxy-2-butenoic acid γ-lactone	2000	N	No safety concern
2-Nonenoic acid γ-lactone	2001	N	No safety concern
4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	2002	N	No safety concern

[a] N, new specifications.

G. Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Hydroxyacetone	1945	N	No safety concern
Propyl pyruvate	1946	N	No safety concern
Methyl 3-hydroxybutyrate	1947	N	No safety concern
Dodecyl lactate	1948	N	No safety concern
(±)-Ethyl 3-hydroxy-2-methylbutyrate	1949	N	No safety concern
Hexadecyl lactate	1950	N	No safety concern

G (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Methyl 3-acetoxy-2-methylbutyrate	1951	N	No safety concern
1-Hydroxy-4-methyl-2-pentanone	1952	N	No safety concern
Ethyl 2-acetylhexanoate	1953	N	No safety concern
3-Isopropenyl-6-oxoheptanoic acid	1954	N	No safety concern
Ethyl 3-hydroxyoctanoate	1955	N	No safety concern
Methyl 3-acetoxyoctanoate	1956	N	No safety concern
5-Oxooctanoic acid	1957	N	No safety concern
Ethyl 2-acetyloctanoate	1958	N	No safety concern
Ethyl 5-acetoxyoctanoate	1959	N	No safety concern
5-Oxodecanoic acid	1960	N	No safety concern
Ethyl 5-oxodecanoate	1961	N	No safety concern
Ethyl 5-hydroxydecanoate	1962	N	No safety concern
5-Oxododecanoic acid	1963	N	No safety concern
Dimethyl adipate	1964	N	No safety concern
Dipropyl adipate	1965	N	No safety concern
Diisopropyl adipate	1966	N	No safety concern
Diisobutyl adipate	1967	N	No safety concern
Dioctyl adipate	1968	N	No safety concern
Methyl levulinate	1970	N	No safety concern
Propyl levulinate	1971	N	No safety concern
Isoamyl levulinate	1972	N	No safety concern
cis-3-Hexenyl acetoacetate	1974	N	No safety concern
Propyleneglycol diacetate	1976	N	No safety concern
Mixture of 6-(5-Decenoyloxy)decanoic acid and 6-(6-Decenoyloxy)decanoic acid	1977	N	No safety concern
Propyleneglycol dipropionate	1978	N	No safety concern
Propyleneglycol monobutyrate (mixture of isomers)	1979	N	No safety concern
Propyleneglycol dibutyrate	1980	N	No safety concern
Propyleneglycol mono-2-methylbutyrate (mixture of isomers)	1981	N	No safety concern

G (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Propyleneglycol di-2-methylbutyrate	1982	N	No safety concern
Propyleneglycol monohexanoate (mixture of isomers)	1983	N	No safety concern
Propyleneglycol dihexanoate	1984	N	No safety concern
Propyleneglycol dioctanoate	1985	N	No safety concern
2-Oxo-3-ethyl-4-butanolide	1986	N	No safety concern
Ethyl 5-hydroxyoctanoate	1987	N	No safety concern
Structural class III			
Ethyl acetoacetate ethyleneglycol ketal	1969	N	No safety concern
Ethyl levulinate propyleneglycol ketal	1973	N	Additional data required to complete evaluation
Hydroxycitronellal propyleneglycol acetal	1975	N	No safety concern
Mixture of Isopropylideneglyceryl 5-hydroxyoctanoate and δ-Decalactone (No. 232)	1988	N	Additional data required to complete evaluation

[a] N, new specifications.

H. Aliphatic secondary alcohols, ketones and related esters and acetals

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
(±)-Octan-3-yl formate	2070	N	No safety concern
2-Pentyl 2-methylpentanoate	2072	N	No safety concern
3-Octyl butyrate	2073	N	No safety concern
Structural class II			
(*R*)-(−)-1-Octen-3-ol	2071	N	No safety concern
2-Decanone	2074	N	No safety concern
Structural class III			
6-Methyl-5-hepten-2-one propyleneglycol acetal	2075	N	No safety concern
2-Nonanone propyleneglycol acetal	2076	N	No safety concern

[a] N, new specifications.

I. Aromatic substituted secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
4-Hydroxyacetophenone	2040	N	No safety concern
3-Hydroxy-4-phenylbutan-2-one	2041	N	No safety concern
2-Methoxyacetophenone	2042	N	No safety concern
2-Methylacetophenone	2044	N	No safety concern
2-Hydroxy-5-methylacetophenone	2045	N	No safety concern
Dihydrogalangal acetate	2046	N	Additional data required to complete evaluation
2,3,3-Trimethylindan-1-one	2047	N	No safety concern
Structural class III			
4-(3,4-Methylenedioxyphenyl)-2-butanone	2048	N	No safety concern

[a] N, new specifications.

J. Benzyl derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
Benzyl hexanoate	2061	N	No safety concern
o-Anisaldehyde	2062	N	No safety concern
Prenyl benzoate	2063	N	No safety concern
Benzyl levulinate	2064	N	No safety concern
4-Methylbenzyl alcohol	2065	N	No safety concern
Benzyl nonanoate	2066	N	No safety concern
Structural class II			
2-Ethylhexyl benzoate	2068	N	No safety concern
Structural class III			
4-Methylbenzaldehyde propyleneglycol acetal	2067	N	No safety concern

[a] N, new specifications.

K. Phenol and phenol derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
4-Propenylphenol	2012	N	No safety concern
2,4,6-Trimethylphenol	2013	N	No safety concern
Sodium 3-methoxy-4-hydroxycinnamate	2014	N	No safety concern
Guaicol butyrate	2015	N	No safety concern
Guaicol isobutyrate	2016	N	No safety concern
Guaicol propionate	2017	N	No safety concern
4-(2-Propenyl)phenyl-β-D-glucopyranoside	2018	N	No safety concern
Phenyl butyrate	2019	N	No safety concern
Hydroxy(4-hydroxy-3-methoxyphenyl)acetic acid	2020	N	No safety concern
Structural class II			
1-(4-Hydroxy-3-methoxyphenyl)-decan-3-one	2021	N	No safety concern
Structural class III			
3-(4-Hydroxy-phenyl)-1-(2,4,6-trihydroxy-phenyl)-propan-1-one	2022	N	No safety concern
Magnolol	2023	N	No safety concern
5,7-Dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chroman-4-one	2024	N	No safety concern

[a] N, new specifications.

L. Simple aliphatic and aromatic sulfides and thiols

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Subgroup i: Simple sulfides			
Structural class I			
Methyl octyl sulfide	1909	N	No safety concern
Methyl 1-propenyl sulfide	1910	N	No safety concern
Di-(1-propenyl)-sulfide (mixture of isomers)	1911	N	No safety concern

L (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class III			
Butanal dibenzyl thioacetal	1939	N	Additional data required to complete evaluation
Subgroup ii: Acyclic sulfides with oxidized side-chains			
Structural class I			
Ethyl 2-hydroxyethyl sulfide	1912	N	No safety concern
2-(Methylthio)ethyl acetate	1913	N	No safety concern
Ethyl 3-(methylthio)-(2*Z*)-propenoate	1915	N	No safety concern
Ethyl 3-(methylthio)-(2*E*)-propenoate	1916	N	No safety concern
Ethyl 3-(methylthio)-2-propenoate (mixture of isomers)	1917	N	No safety concern
4-Methyl-2-(methylthiomethyl)-2-pentenal	1918	N	No safety concern
4-Methyl-2-(methylthiomethyl)-2-hexenal	1919	N	No safety concern
5-Methyl-2-(methylthiomethyl)-2-hexenal	1920	N	No safety concern
Butyl β-(methylthio)acrylate	1921	N	No safety concern
Ethyl 3-(ethylthio)butyrate	1922	N	No safety concern
Methional diethyl acetal	1940	N	No safety concern
3-(Methylthio)propyl hexanoate	1941	N	Additional data required to complete evaluation
Structural class III			
1-(3-(Methylthio)-butyryl)-2,6,6-trimethylcyclohexene	1942	N	No safety concern
Subgroup iii: Cyclic sulfides			
Structural class II			
2-Oxothiolane	1923	N	No safety concern
Structural class III			
(±)-*cis*- and *trans*-2-Pentyl-4-propyl-1,3-oxathiane	1943	N	Additional data required to complete evaluation
2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	1944	N	Additional data required to complete evaluation
Subgroup iv: Simple thiols			
Structural class I			

L (contd)

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Dodecanethiol	1924	N	No safety concern
Subgroup v: Thiols with oxidized side-chains			
Structural class I			
2-Hydroxyethanethiol	1925	N	No safety concern
4-Mercapto-4-methyl-2-hexanone	1926	N	No safety concern
3-Mercapto-3-methylbutyl isovalerate	1927	N	No safety concern
(±)-Ethyl 3-mercapto-2-methylbutanoate	1928	N	No safety concern
3-Mercaptohexanal	1929	N	No safety concern
3-Mercaptopropionic acid	1936	N	No safety concern
2-Ethylhexyl 3-mercaptopropionate	1938	N	No safety concern
Structural class III			
3-(Methylthio)propyl mercaptoacetate	1914	N	Additional data required to complete evaluation
Subgroup vii: Simple disulfides			
Structural class I			
Diisoamyl disulfide	1930	N	No safety concern
Butyl propyl disulfide	1932	N	No safety concern
di-*sec*-Butyl disulfide	1933	N	No safety concern
Structural class III			
Bis(2-methylphenyl) disulfide	1931	N	Additional data required to complete evaluation
Methyl 2-methylphenyl disulfide	1935	N	No safety concern
Subgroup ix: Trisulfides			
Structural class I			
Diisoamyl trisulfide	1934	N	No safety concern
Subgroup xi: Thioesters			
Structural class I			
Methyl isobutanethioate	1937	N	No safety concern

[a] N, new specifications.

FLAVOURING AGENTS CONSIDERED FOR SPECIFICATIONS ONLY

No.	Flavouring agent	Specifications[a]
439	4-Carvomenthenol	R
952	5,6,7,8-Tetrahydroquinoxaline	R

[a] R, revised specifications.

CONTAMINANTS EVALUATED TOXICOLOGICALLY

Cadmium

Since cadmium was last considered by the Committee, there have been a number of new epidemiological studies that have reported cadmium-related biomarkers in urine following environmental exposure. The Committee noted that a large meta-analysis of studies that measured the dose–response relationship between the excretion of β_2-microglobulin and cadmium in urine was available. As the apparent half-life of cadmium in human kidneys is about 15 years, steady state would be achieved after 45–60 years of exposure. Therefore, data relating β_2-microglobulin excretion in urine to cadmium excretion in urine for individuals who are 50 years of age and older provided the most reliable basis on which to determine a critical concentration of cadmium in the urine. An analysis of the group mean data from individuals who were 50 years of age and older showed that the urinary excretion of less than 5.24 (confidence interval 4.94–5.57) μg of cadmium per gram creatinine was not associated with an increased excretion of β_2-microglobulin. Higher urinary cadmium levels were associated with a steep increase in β_2-microglobulin excretion.

To determine a corresponding dietary exposure that would result in a urinary cadmium concentration at the breakpoint of 5.24 (confidence interval 4.94–5.57) μg of cadmium per gram creatinine, a one-compartment toxicokinetic model was used. The lower bound of the 5th population percentile dietary cadmium exposure that equates to the breakpoint was estimated to be 0.8 μg/kg body weight per day or 25 μg/kg body weight per month.

The Committee noted that the existing health-based guidance value for cadmium was expressed on a weekly basis (provisional tolerable weekly intake, or PTWI), but, owing to cadmium's exceptionally long half-life, considered that a monthly value was more appropriate. **The Committee therefore withdrew the PTWI of 7 μg/kg body weight.**

In view of the long half-life of cadmium, daily ingestion in food has a small or even a negligible effect on overall exposure. In order to assess long- or short-term risks to health due to cadmium exposure, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the

tolerable intake as a monthly value in the form of a provisional tolerable monthly intake (PTMI). **The Committee established a PTMI of 25 µg/kg body weight.**

The estimates of exposure to cadmium through the diet for all age groups, including consumers with high exposure and subgroups with special dietary habits (e.g. vegetarians), examined by the Committee at this meeting are below the PTMI.

Lead

Exposure to lead is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes. For children, impaired neurodevelopment is generally associated with lower blood lead concentrations than the other effects, the weight of evidence is greater for neurodevelopmental effects than for other health effects, and the results across studies are more consistent than those for other effects. For adults, the adverse effect associated with lowest blood lead concentrations for which the weight of evidence is greatest and most consistent is a lead-associated increase in systolic blood pressure. Therefore, the Committee concluded that the effects on neurodevelopment and increase in systolic blood pressure provided the appropriate bases for dose–response analyses.

Based on the dose–response analyses, the Committee estimated that the previously established PTWI of 25 µg/kg body weight is associated with a decrease of at least 3 intelligence quotient (IQ) points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults. These changes are important when viewed as a shift in the distribution of IQ or blood pressure within a population. **The Committee therefore concluded that the PTWI could no longer be considered health protective and withdrew it.**

Because the dose–response analyses do not provide any indication of a threshold for the key effects of lead, the Committee concluded that it was not possible to establish a new PTWI that would be considered to be health protective. The dose–response analyses conducted by the Committee should be used to identify the magnitude of effect associated with identified levels of dietary lead exposure in different populations.

The Committee reaffirmed that because of the neurodevelopmental effects, fetuses, infants and children are the subgroups that are most sensitive to lead. The estimated mean dietary exposures of children aged about 1–4 years range from 0.03 to 9 µg/kg body weight per day. The health impact at the lower end of this range (0.03 µg/kg body weight per day) is considered negligible by the Committee, because it is below the exposure level of 0.3 µg/kg body weight per day calculated to be associated with a population decrease of 0.5 IQ point. The higher end of the exposure range (9 µg/kg body weight per day) is higher than the level of 1.9 µg/kg body weight per day calculated to be associated with a population decrease of 3 IQ points, which is deemed by the Committee to be a concern. For adults, the mean dietary lead exposure estimates range from 0.02 to 3.0 µg/kg body weight per day. The lower end of this range (0.02 µg/kg body weight per day) is considerably below the exposure level of 1.2 µg/kg body weight per day calculated by the Committee

to be associated with a population increase in systolic blood pressure of 1 mmHg (0.1 kPa). The Committee considered that any health risk that would be expected to occur at this exposure level is negligible. At the higher end of the range (3.0 µg/kg body weight per day), a population increase of approximately 2 mmHg (0.3 kPa) in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated, in a large meta-analysis, with modest increases in the risks of ischaemic heart disease and cerebrovascular stroke. The Committee considered this to be of some concern, but less than that for the neurodevelopmental effects observed in children.

The Committee stressed that these estimates are based on dietary exposure (mainly food) and that other sources of exposure to lead also need to be considered.

The Committee concluded that, in populations with prolonged dietary exposures to lead that are in the higher end of the ranges identified above, measures should be taken to identify major contributing sources and foods and, if appropriate, to identify methods of reducing dietary exposure that are commensurate with the level of risk reduction.

ANNEX 5

SUMMARY OF THE SAFETY EVALUATION OF SECONDARY COMPONENTS FOR FLAVOURING AGENTS WITH MINIMUM ASSAY VALUES OF LESS THAN 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Alicyclic ketones, secondary alcohols and related esters				
2053	3,3,5-Trimethylcyclohexyl acetate	90	6–7% 3,3,5-trimethylcyclohexanol	3,3,5-Trimethylcyclohexanol (No. 1099) was evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*) and found to be of no safety concern at estimated dietary exposures as a flavouring agent.
2055	Cyclotene propionate	92	4–5% cyclotene	Cyclotene (No. 418) was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and was concluded to be of no safety concern at estimated dietary exposures as a flavouring agent.
Alicyclic primary alcohols, aldehydes, acids and related esters				
1898	Methyl dihydrojasmonate	85	9–11% methyl epi-dihydrojasmonate	Methyl epi-dihydrojasmonate is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1901	Perillaldehyde propyleneglycol acetal	91	3–4% perillaldehyde; 2–3% propylene glycol	Perillaldehyde (No. 973) and propylene glycol are metabolites of the primary substance and are considered not to present a safety concern at current estimated dietary exposures.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1902	(±)-*cis*- and *trans*-1,2-Dihydroperillaldehyde	80	10–11% *trans*-4-isopropyl-cyclohexane-1-carboxaldehyde; 4–5% *cis*-4-isopropyl-cyclohexane-1-carboxaldehyde; 1–2% 4-isopropenyl-cyclohex-1-enecarboxaldehyde	*trans*-4-Isopropyl-cyclohexane-1-carboxaldehyde, *cis*-4-isopropyl-cyclohexane-1-carboxaldehyde and 4-isopropenyl-cyclohex-1-enecarboxaldehyde are expected to share the same metabolic fate as the primary substance, i.e. oxidation of the aldehyde to the carboxylic acid, followed by glucuronic acid conjugation. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
1906	1,3-*p*-Menthadien-7-al	91	5–6% cumin aldehyde	Cumin aldehyde (No. 868) was evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.
1908	(±)-*cis*- and *trans*-2-Methyl-2-(4-methyl-3-pentenyl) cyclopropanecarbaldehyde	90	5–10% [2-methyl-2-(4-methylpent-3-en-1-yl)-cyclopropyl]methanol	[2-Methyl-2-(4-methylpent-3-en-1-yl)-cyclopropyl]methanol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. oxidation to the carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances				
2027	Caryophyllene alcohol	92	3–6% dihydrocloven-9-ol	Dihydrocloven-9-ol is expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
2031	α-Bisabolol	93	1–2% β-bisabolol	β-Bisabolol is expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic and aromatic amines and amides				
2007	(±)-*N*-Lactoyl tyramine	90	2–3% lactic acid; 2–3% ethyl lactate	Lactic acid (No. 930) and ethyl lactate (No. 931) were evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and were concluded to be of no safety concern at estimated dietary exposures as flavouring agents.
2009	*N*-*p*-Benzeneacetonitrile menthanecarboxamide	94	2–5% *N*-*p*-benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*); neo-isomer	*N*-*p*-Benzeneacetonitrile menthanecarboxamide, (1*R*, 3*S*, 4*S*) is expected to share the same metabolic fate as the primary substance, i.e. oxidation followed by elimination. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Aliphatic lactones				
2002	4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	93	1–2% 3,4-dimethyl 5-ketobutanoic acid γ-lactone	3,4-Dimethyl 5-ketobutanoic acid γ-lactone is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups				
1948	Dodecyl lactate	88	10% dodecanol	Dodecanol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1950	Hexadecyl lactate	88	15% 1-hexadecanol	1-Hexadecanol (No. 114) was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.
1962	Ethyl 5-hydroxydecanoate	56	40–42% δ-decalactone	δ-Decalactone (No. 232) was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1974	*cis*-3-Hexenyl acetoacetate	93	2–3% *cis*-3-hexenol	*cis*-3-Hexenol is a metabolite of the primary substance and is expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1979	Propyleneglycol monobutyrate	88	6–10% propyleneglycol dibutyrate	Propyleneglycol dibutyrate (No. 1980) was evaluated at the current meeting and was considered not to present a safety concern at current estimated dietary exposures to the flavouring agent.
1987	Ethyl 5-hydroxyoctanoate	50	5–6% ethanol; 17–18% 1,5-octanolide; 21–24% 5-hydroxydecanoic acid and ethyl-5-hydroxyoctanoate ester	Ethanol (No. 41) was evaluated by the Committee at its forty-sixth meeting (Annex 1, reference *122*) and was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent. 1,5-Octanolide, 5-hydroxydecanoic acid and ethyl-5-hydroxyoctanoate ester are metabolites of the primary substance and expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1988	Mixture of Isopropylideneglyceryl 5-hydroxydecanoate and δ-Decalactone (No. 232)	73	The mixture contains 25% isopropylideneglyceryl 5-hydroxydecanoate and 47–49% δ-decalactone (No. 232); other components are 22–24% 2,2-dimethyl-1,3-dioxolane-4-methanol and 1–5% 2-propyl 5-hydroxydecanoate	Isopropylideneglyceryl 5-hydroxydecanoate, δ-decalactone (No. 232), 2,2-dimethyl-1,3-dioxolane-4-methanol and 2-propyl 5-hydroxydecanoate are expected to share the same metabolic fate, i.e. hydrolysis to the corresponding acid and alcohol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aliphatic secondary alcohols, ketones and related esters and acetals				
2075	6-Methyl-5-hepten-2-one propyleneglycol acetal	88	7–9% 6-methyl-6-hepten-2-one propyleneglycol acetal	6-Methyl-6-hepten-2-one propyleneglycol acetal is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding ketone and diol, followed by complete metabolism in the fatty acid pathway or tricarboxylic acid cycle. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
Aromatic substituted secondary alcohols, ketones and related esters				
2041	3-Hydroxy-4-phenylbutan-2-one	93	3–5% 4-hydroxy-4-phenylbutan-2-one	4-Hydroxy-4-phenylbutan-2-one is expected to share the same metabolic fate as the primary substance, i.e. reduction of the ketone to the corresponding secondary alcohol, followed by formation of the glucuronic acid conjugate and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Phenol and phenol derivatives				
2014	Sodium 3-methoxy-4-hydroxycinnamate	93	2–5% vanillin	An acceptable daily intake (ADI) of 0–10 mg/kg bw was established for vanillin by the Committee at its eleventh meeting (Annex, reference 14). At the fifty-seventh meeting of the Committee, when vanillin (No. 889) was evaluated using the Procedure, vanillin was concluded to be of no safety concern at estimated dietary exposures to the flavouring agent, and the ADI was maintained (Annex 1, reference *154*).
2023	Magnolol	92	3–7% honokiol; 1–2% eudesmol	Honokiol and eudesmol are expected to share the same metabolic fate as the primary substance, i.e. formation of the glucuronic acid conjugate and elimination in the urine. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
Simple aliphatic and aromatic sulfides and thiols				
1915	Ethyl 3-(methylthio)-(2*Z*)-propenoate	88	7–9% ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916)	Ethyl 3-(methylthio)-(2*E*)-propenoate (No. 1916) is expected to share the same metabolic fate as the primary substance, i.e. oxidation of the sulfur to the corresponding sulfoxide or sulfone in addition to ester hydrolysis to the corresponding alcohol and carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

(contd)

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
1916	Ethyl 3-(methylthio)-(2*E*)-propenoate	81	14–16% ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915)	Ethyl 3-(methylthio)-(2*Z*)-propenoate (No. 1915) is expected to share the same metabolic fate as the primary substance, i.e. oxidation of the sulfur to the corresponding sulfoxide or sulfone in addition to ester hydrolysis to the corresponding alcohol and carboxylic acid, followed by glucuronic acid conjugation. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.
1932	Butyl propyl disulfide	51	24–25% dipropyl disulfide; 23–24% dibutyl disulfide	Dipropyl disulfide and dibutyl disulfide are expected to share the same metabolic fate as the primary substance, i.e. reduction of the disulfide, followed by formation of mixed disulfides with glutathione and cysteine. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.
1944	2-Pentenyl-4-propyl-1,3-oxathiane (mixture of isomers)	88	5–8% 2-[(2*E*)-pent-2-en-1-yl]-4-propyl-1,3-oxathiane; 2–3% 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane	2-[(2*E*)-Pent-2-en-1-yl]-4-propyl-1,3-oxathiane and 2-[(1*Z*)-pent-1-en-1-yl]-4-propyl-1,3-oxathiane are expected to share the same metabolic fate as the primary substance, i.e. oxidization to the sulfoxide and sulfone and hydrolysis to the thioalcohol, which may undergo further oxidation, alkylation or conjugation. They do not present a safety concern at current estimated dietary exposures to the flavouring agent.

This volume contains monographs prepared at the seventy-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Geneva, Switzerland, from 8 to 17 June 2010.

The toxicological monographs in this volume summarize the safety data on two contaminants in food: cadmium and lead.

Monographs on nine groups of related flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents are also included.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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